Review

Guidance from above: common cues direct distinct signaling outcomes in vascular and neural patterning

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The nervous and vascular systems are both exquisitely branched and complicated systems and their proper development requires careful guidance of nerves and vessels. The recent realization that common ligand-receptor pairs are used in guiding the patterning of both systems has prompted the question of whether similar signaling pathways are used in both systems. This review highlights recent progress in our understanding of the similarities and differences in the intracellular signaling mechanisms downstream of semaphorins, ephrins and vascular endothelial growth factor in neurons and endothelial cells during neural and vascular development. We present evidence that similar intracellular signaling principles underlying cytoskeletal regulation are used to control neural and vascular guidance, although the specific molecules used in neurons and endothelial cells are often different.

Introduction

The development and proper function of the nervous and vascular systems are essential for the survival of all higher organisms. Interestingly, these two systems share many similarities. Developmentally, they are the first specific tissue systems to appear in embryonic development and both continue to dynamically remodel throughout life. Anatomically, they are both highly branched and complicated networks; however, both are remarkably reproducible from one individual to another. Functionally, they both extend to every part of the organism and either coordinate function or provide oxygen and nutrients. They also regulate each other's function; for example, arteries supply neurons with oxygenated blood and nerves control blood-vessel dilation and contraction. In addition, in the periphery, blood vessels and nerves often run in parallel [1].

This similarity also extends to the cellular level. Neurons explore their environment and find targets using growth cones (dynamic palmate structures at the leading edge of the growing axon that can sense and react to environmental cues). Similarly, vessels use specialized tip cells, which are located at the front of navigating blood vessels and are morphologically and functionally similar to the axonal growth cone [2]. Actin filaments and microtubules are the major cytoskeletal components that determine the structure and motility of growth cones and tip cells, thereby controlling axon guidance and endothelial cell migration (Box 1).

Recently, it has become clear that neuronal and vascular morphogenesis is also tightly interwoven at the molecular level. Thus far, four major axon-guidance-molecule families (ephrins, semaphorins, slits and netrins), which are widely expressed by multiple cell types and exert diverse biological functions, have been implicated in angiogenesis [1,3–6]. In addition, vascular endothelial growth factor (VEGF), a well-known angiogenic factor, has been shown to participate in the function of the nervous system. This raises the question of whether these molecules use the same downstream signaling pathways to exert their effects on neurons and endothelial cells to control axon and vascular guidance. This review highlights recent progress in our understanding of the similarities and differences in the intracellular signaling mechanisms used by semaphorins, ephrins and VEGF in neurons and endothelial cells. Currently, less is known about the intracellular signaling of slits and netrins in endothelial cells; for a discussion of their dual role in axon and vascular guidance, see Refs [1,3–5].

Semaphorins

The semaphorins are the largest family of guidance molecules containing both secreted molecules capable of long-range diffusion and membrane-bound proteins that function as short-range guidance cues [7] (Box 2). Great progress has been made recently on semaphorin downstream signaling in neurons and endothelial cells. Here, we discuss two semaphorins, the transmembrane Sema4D and the secreted Sema3A, and compare their signaling mechanisms in the nervous and vascular systems. Short descriptions of the proteins mentioned in each section can be found in Table 1.

Sema4D signaling in the nervous and vascular systems

Sema4D binds to Plexin-B1, inducing growth-cone collapse and preventing axon outgrowth in cultured embryonic day (E) 18.5 hippocampal neurons [8–10], an effect that is blocked in neurons from *Plexin-B1* knockout mice [11]. In the vascular system, Sema4D increases endothelial cell migration [12] and is possibly involved in tumor-induced angiogenesis [13]. *Sema4D* or *Plexin-B1* knockout mice display defects in the ability of cancer cells to generate tumor masses and metastases [14], and a detailed *in vivo*

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Box 1. Cytoskeletal reorganization in neuronal growth-cone dynamics, dendritic spine morphogenesis and endothelial tip cell motility

Actin filaments are cytoskeletal components that determine the structure and motility of growth cones and tip cells and are organized into two distinct morphological elements: (i) dense, finger-like projections called filopodia; and (ii) loosely interwoven, veil-like structures termed lamellipodia. In addition, microtubules are the other major structural element of growth cones and tip cells. Microtubules dynamically extend and retract along the filopodial actin filaments.

Cytoskeletal reorganization is the central event for axon guidance, dendritic spine morphogenesis and endothelial cell migration. The leading edge of the growth cone senses the attractive or repulsive cues, which, through various signaling pathways, changes the balance between the polymerization of actin at its barbed ends and the retrograde flow of the entire filament. In the case of chemoattraction, the polymerization of actin drives the filopodia and lamellipodia in the direction of attractive cues. Once the initial filopodial contact is established, microtubules follow and form stable bundles in the axon shaft. Whether the function of microtubules is only to stabilize the extending axons is still controversial. In the case of chemorepulsion, repulsive signals initiate the cytoskeletal reconfigurations necessary for redirecting the axonal path by inducing the focal loss of actin bundles followed by the focal loss of dynamic microtubules [88]. Like growth cones, tip cells are highly dynamic structures that use lamellipodia and filopodia to detect the extracellular environment and lead the growing vessel in the correct direction [2]. The movement of these protrusions is driven by actin polymerization in the direction of attractive cues. For example, Netrin receptor Unc5b is expressed in the endothelial tip cells. *Unc5b* knockout mice exhibit excess filopodial extension. Exposure of tips cells to Netrin-1 induces rapid filopodial retraction and backward movement of the cells [89]. In addition, endothelial cells can be stabilized by adherence to the ECM or to adjacent cells. These adhesions serve as traction sites as the cell moves forward over them and are disassembled at rear of the cell, enabling it to detach [2,90]. Thus, to understand the guidance mechanisms behind growth-cone and tip cell movement, it is important to determine how guidance cues are transduced and how they affect actin and microtubule dynamics and organization. Dendritic spines are specialized post-synaptic structures that emanate from the dendritic shaft. They are formed from dendritic filopodia, which are small, actin-rich protrusions that are rapidly formed and retract from the dendritic shaft. Similarly to axons, actin rearrangements are crucial for this rapid filopodial formation and retraction. Another process that has been seen in dendritic filopodia is actin treadmilling, where actin fibers are disassembled at the dendritic shaft and polymerize at the tip of the filopodia. Long, thin filopodia are remodeled into shorter and stubbier mature spines in development, a process that could involve the nucleation and branching of actin. Rho family GTPases have been found to link intracellular signals to actin dynamics within the spine and depending on which family members are activated, spines can retract, elongate or change shape.

histological analysis of the vascular and nervous systems in both knockout mice is warranted.

The small GTPase RhoA is a central player in Sema4D–Plexin-B1 signaling in both neurons and endothelial cells. Small GTPases are proteins that participate in many cellular processes. They are present in an active GTP-bound form and an inactive GDP-bound form. Two types of proteins regulate their function; GTPase-activating proteins (GAPs) stimulate hydrolysis of GTP into GDP, thus inactivating the GTPase, whereas guanine-nucleotide-exchange factors (GEFs) replace GDP with GTP, thus activating the GTPase. In both cultured hippocampal

Box 2. Background of the semaphorin, ephrin and VEGF families of proteins

The semaphorins

The semaphorins are the largest family of guidance molecules, comprising eight classes with a total of 29 members and containing both secreted molecules capable of long-range diffusion and membrane-bound proteins that function as short-range guidance cues [7]. Semaphorins signal through multimeric receptor complexes. Secreted class 3 semaphorins usually signal through a holoreceptor composed of the ligand-binding subunit Neuropilin (Npn) and the signal-transducing subunit Plexin-A receptors to induce growth-cone guidance, with the exception of Sema3E, which can signal through a Plexin-D alone [40]. Most of the membrane-bound semaphorins can signal directly through plexins. Plexins make up a family of large transmembrane proteins. Plexin-A was first identified as the *Drosophila* class I and II semaphorin receptor, but plexins are now understood to be crucial receptors for many other members of the semaphorin family. Semaphorins were initially implicated as axon guidance cues but are now known to have broader roles in morphogenesis, including vascular development and tumor angiogenesis [7,42,43].

Ephrins

There are two families of ephrins: the ephrin-A family is GPI-linked and comprises five members, whereas ephrin-Bs are transmembrane and the family is made up of three proteins. Their corresponding Eph receptors, the nine EphA receptors and five EphB receptors, are receptor tyrosine kinases. With very few exceptions, ephrin-As bind promiscuously to EphA receptors, whereas the ephrin-Bs bind to EphB receptors. Among the domains present on the Eph receptors are an extracellular ephrin-binding domain and intracellular kinase- and PDZ-binding domains. Interestingly, because ephrin ligands are either membrane-tethered or transmembrane proteins, Eph–ephrin signaling can be bidirectional. When the signaling proceeds through the Eph receptor, this is termed 'forward' signaling. When the signaling is mediated through the ephrin ligand, this is referred to as 'reverse' signaling. Although Eph–ephrin signaling was first described as being important in axon repulsion, ephrins have been implicated in a wide variety of biological processes such as cell survival, proliferation and migration. For a review of ephrin function, see Ref. [91].

The VEGF family

The VEGF family comprises five classes, VEGF-A, -B, -C, -D and placental growth factor (PLGF). Alternative splicing of *VEGF-A* generates three different sized proteins: $VEGF_{121}$, $VEGF_{165}$ and $VEGF_{180}$, all of which have different receptors and functions. $VEGF_{165}$ has been shown to be most important for many aspects of embryonic development and will be the variant referred to throughout this review. VEGF has a crucial role during the development of the vascular system through its tyrosine kinase receptors: VEGFR1 (also called Flt1), which regulates monocyte migration; VEGFR2 (also called Flk1), which regulates many aspects of endothelial cell biology such as migration, proliferation and permeability; and VEGFR3, which is important for the development of the lymphatic system. In addition to these receptors, the VEGFR2 co-receptor Neuropilin-1 (Npn-1) binds both $VEGF_{121}$ and $VEGF_{165}$ [35,39]. Endothelial tip cells migrate towards higher concentrations of VEGF, whereas the endothelial stalk cells that follow the tip cells proliferate in response to high VEGF concentrations [2]. Several recent studies have also revealed the role of VEGF-regulated Dll4–Notch signaling in suppressing endothelial tip cell formation. During vessel sprouting, VEGF induces the expression of the Notch ligand Delta-like ligand (Dll)4. Higher Dll4 expression in the leading tip cells activates Notch in the adjacent stalk cells and suppressing their formation into tip cells. This results in the proper ratio between tip and stalk cells required for correct sprouting and branching patterns. In addition, Dll4–Notch signaling provides a negative-feedback loop to prevent overexuberant angiogenic sprouting, thereby promoting the timely formation of a well-differentiated vascular network [92].

Table 1. Description of the signaling molecules involved in semaphorin, ephrin and VEGF pathways[a]

Abbreviated name	Full name	Function
Akt	Protein kinase B	General protein kinase. Signals downstream of PI3K to mediate the effects of various growth factors
Cdk5	Cyclin-dependent kinase 5	A serine/threonine kinase; initially identified for its involvement in cell-cycle progression. Most studied functions were in nervous system
Cofilin		Actin-binding protein, which disassembles actin filament
CRMP2	Collapsin-response-mediator protein2	Tubulin-binding protein, which stimulates microtubule assembly; necessary for Sema3 signaling and subsequent remodeling of the cytoskeleton
CXCR4	Chemokine receptor 4	A receptor for SDF-1
ECM	Extracellular matrix	The extracellular part of animal tissue that usually provides structural support to the cells in addition to performing various other important functions
Ephexin		A RhoGEF that activates Rho by converting Rho–GDP to Rho–GTP
ErbB2	Human epidermal growth factor receptor 2	Receptor tyrosine kinase
ERM	Ezrin–radixin–moesin (ERM) proteins	A class of proteins that mediates linkage between the plasma membrane and the F-actin cytoskeleton and regulates filopodia
FAK	Focal adhesion kinase	A focal adhesion-associated protein kinase involved in cellular adhesion and spreading processes
FARP2	FERM, RhoGEF and pleckstrin domain-containing protein 2	A Rac1GEF that activates Rac1 by converting Rac1-GDP to Rac-GTP
Fer	far (fps [fes]-related) tyrosine kinase	A member of the FPS (FES) family of non-transmembrane receptor tyrosine kinases. It regulates cell–cell adhesion and mediates signaling from the cell surface to the cytoskeleton via growth factor receptors. The fps (fes) proto-oncogene encodes a structurally unique member of the non-receptor protein-tyrosine-kinase (PTK) family
Fes	c-fes/fps protein	Fes has tyrosine-specific protein kinase activity that is required for the maintenance of cellular transformation
Fyn		A member of the Src family of non-receptor tyrosine kinases
Girdin	(also known as) Girders of actin filament	Actin-binding protein
Grb4		SH2–SH3-domain-containing adaptor protein
GRIP1	Glutamate receptor interacting protein 1	An adaptor protein containing seven PDZ domains
GSK3	Glycogen synthase kinase 3	Serine/threonine protein kinase. The phosphorylation of target proteins by GSK-3 usually inhibits them
Kalirin		A RhoGEF that activates Rho by converting Rho–GDP to Rho–GTP
L1	L1 cell adhesion molecule	Cell adhesion molecule with an important role in the development of the nervous system
LIMK	LIM motif-containing protein kinase	A protein kinase that regulates actin filament dynamics. It phosphorylates and inactivates the actin-binding and depolymerizing factor Cofilin, thereby stabilizing the actin cytoskeleton
Met	Mesenchymal-epithelial transition factor	Receptor for hepatocyte growth factor and scatter factor. Has a tyrosine-protein kinase activity
Nck1/2	Non-catalytic region of tyrosine kinase adaptor protein 1 (and 2)	SH2–SH3-domain-containing adaptor protein
Npn-1	Neuropilin 1	Transmembrane co-receptor for both vascular endothelial growth factor and semaphorin family members. Npn-1 has versatile roles in angiogenesis, axon guidance, cell survival, migration and invasion
p75-NTR	p75 neurotrophin receptor	A low-affinity receptor for neurotrophins with wide-ranging roles in nervous system development and tumor growth
PAK	p21 protein (Cdc42/Rac)-activated kinase	Activated by Cdc42 and Rac1. Involved in dissolution of stress fibers and reorganization of the cytoskeleton
PDZ–Rho GEF	PDZ-domain-containing Rho-guanine nucleotide exchange factor	A RhoGEF that activates Rho by converting Rho–GDP to Rho–GTP, and also contains a PDZ domain
PI3K	Phosphoinositide Kinase-3	PI3Ks have been linked to an extraordinarily diverse group of cellular functions. Many of these functions relate to the ability of class I PI3Ks to activate protein kinase B (Akt)
PIPKIγ661	Type-I phosphatidylinositol phosphate kinase γ661	An enzyme that generates PI4,5P2, targeted to and regulating focal adhesions
PLC-γ	Phospholipase-c γ	Catalyzes the formation of inositol 1,4,5-trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate, which then have multiple roles in intracellular signaling events
PTEN	Phosphatase and tensin homolog	Antagonizes the PI3K–AKT signaling pathway by dephosphorylating phosphoinositides and thereby modulating cell-cycle progression and cell survival
Pyk2	FAK-related proline-rich tyrosine kinase 2	A non-receptor tyrosine kinase
Rac1 GEF	Rac1-guanine nucleotide exchange factor	A positive regulator of Rac1 that converts inactive Rac1–GDP to active Rac1–GTP
ROCK	Rho-kinase	A serine/threonine-specific protein kinase activated by GTP-bound RhoA
R-Ras GAP	R-Ras GTPase-activating proteins	Negative regulator of Ras that converts active Ras–GTP to inactive Ras–GDP
SDF-1	Stromal derived factor 1	A chemoattractant chemokine involved in cell migration
Shb	Src homology 2-domain-containing transforming protein B	Adaptor protein
Tiam1	T-cell lymphoma and metastasis 1	A Rac1 GEF that activates Rac1 by converting Rac1–GDP to Rac1–GTP
TSAd	(Also known as) SH2 domain protein 2a	Adaptor protein
α2-chimaerin		A Rac GAP that inactivates Rac by converting Rac–GTP to Rac–GDP

[a]Abbreviations: fes, feline sarcoma; fps, Fujinami poultry sarcoma.

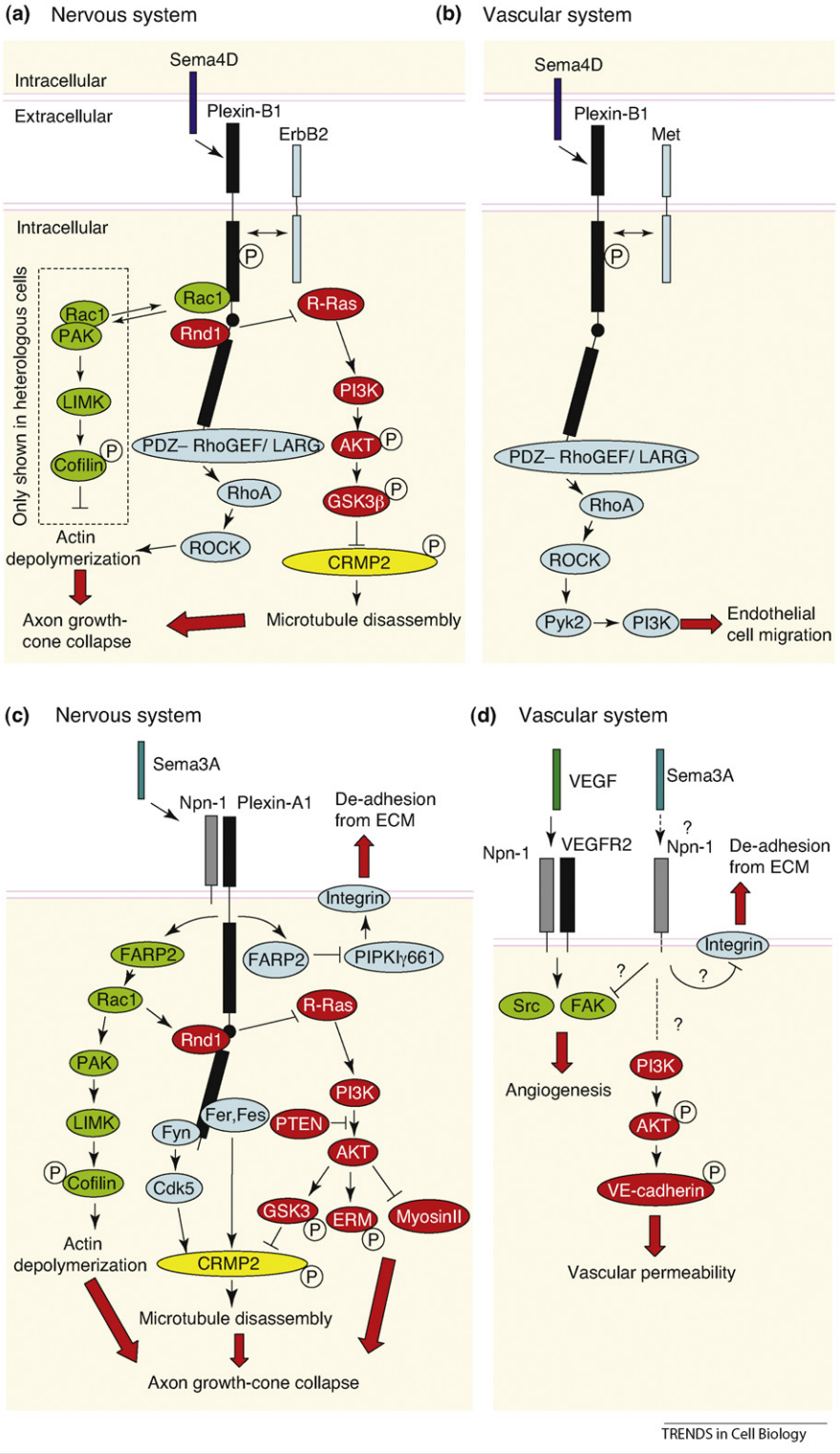

Figure 1. Sema4D and Sema3A signaling in the nervous and vascular systems. Plexin-B1 and Plexin-A1 are shown in black, with the intracellular C1 and C2 domains represented by black rectangles and the linker region in between as a black circle. **(a)** Sema4D signaling in the nervous system. Proteins in the R-Ras pathway are shown in red: in the presence of Sema4D, Rnd1 is recruited to Plexin-B1. Plexin-B1 R-RasGAP activity is activated and downregulates the active form of R-Ras. The decrease of active R-Ras inhibits PI3K–Akt activity, decreasing GSK3β phosphorylation and, thus, activating it. GSK3β then phosphorylates and deactivates CRMP2 and causes microtubule disassembly. Proteins in the RhoA pathway are shown in blue: in the presence of Sema4D, receptor tyrosine kinase ErbB2 binds and subsequently phosphorylates Plexin-B1 (as indicated by the double-headed arrow) and then activates PDZ–RhoGEF and LARG, which associate with Plexin-B1. PDZ–RhoGEF and LARG activate RhoA, causing actin depolymerization through ROCK. Proteins in the Rac1 pathway are shown in green. Upon Sema4D binding, activated Plexin-B1 competes for active Rac1 with PAK. The shift in the equilibrium between Plexin-B1- and PAK-bound Rac1 results in decrease of PAK activity, LIMK activity and Cofilin phosphorylation, thus, causing actin depolymerization. So far, this pathway has only been shown in heterologous cells, as indicated by the dashed box. Both the actin depolymerization and microtubule disassembly lead to axon growth-cone collapse. **(b)** Sema4D signaling in the vascular system. Proteins in the RhoA pathway are shown in blue: in the presence of Sema4D, the receptor tyrosine kinase Met binds and phosphorylates Plexin-B1 (as indicated by the double-headed arrow) and then activates PDZ–RhoGEF and LARG, which activates RhoA and leads to endothelial cell migration through the ROCK, Pyk2 and PI3K pathway. It is not clear how this pathway affects actin dynamics or microtubule dynamics in vascular system. **(c)** Sema3A signaling in the nervous system. Rac1-regulating proteins are shown in green: in the presence of Sema3A, FARP2 is released from Plexin-A1 and actives Rac1. Rac1 then activates PAK and LIMK and, as a result, phosphorylates Cofilin, which finally causes actin depolymerization. R-Ras-regulating proteins are

neurons and endothelial cells, Plexin-B1 binds to the PDZ-domain-containing proteins PDZ–RhoGEF and leukemia-associated RhoGEF (LARG) through its C-terminal PDZ-binding motif (Figure 1a,b). Upon Sema4D binding, a receptor tyrosine kinase (ErbB2 in neurons and Met in endothelial cells [15,16]) binds to and subsequently phosphorylates Plexin-B1, activating PDZ–RhoGEF and LARG, and increasing active RhoA and RhoA-activated kinase (ROCK) [17]. The activation of RhoA–ROCK then induces the collapse of cultured hippocampal neuron growth cones and promotes endothelial cell migration and vessel formation [12] through different downstream signaling pathways.

In addition to the different upstream regulators of RhoA activation in neuron and endothelial cells (i.e. ErbB2 and Met), the signals downstream of RhoA are also distinct in the two systems. Downstream of RhoA, endothelial cells have a unique pathway through Pyk2, a non-receptor tyrosine kinase that can be activated by RhoA–ROCK. Activation of Pyk2 triggers the phosphoinositide 3 kinase (PI3K)–protein kinase B (Akt) pathway and leads to cytoskeletal reorganization and endothelial cell migration [18] (Figure 1b).

Sema4D–Plexin-B1 signaling in neurons also involves two small GTPases not yet shown to be crucial for this signaling in endothelial cells (Figure 1a). One of these is R-Ras. Plexin-B1 has two conserved R-RasGAP homology motifs located in its C1 and C2 domains, which are autoinhibited in the absence of Sema4D [9]. In cultured hippocampal neurons upon Sema4D treatment, Rnd1, a small GTPase that lacks intrinsic GTPase activity and exists constitutively in a GTP-bound form, is recruited to Plexin-B1 and induces the R-RasGAP activity of Plexin-B1 by releasing the inhibitory interaction between the C1 and C2 domains in the intracellular region [8,9]; this leads to conversion of active R-Ras–GTP to inactive R-Ras–GDP. Sema4D-induced downregulation of R-Ras activity dephosphorylates PI3K–Akt and activates glycogen synthase kinase 3β (GSK3β). GSK3β then phosphorylates and inactivates collapsin-response-mediator protein 2 (CRMP2), causing hippocampal neuron growth-cone collapse [10,19]. CRMP2 is a member of the collapsin-response-mediator protein family, which stimulates microtubule assembly in neurons [19]. The second small GTPase involved in Sema4D–Plexin-B1 signaling in neurons is Rac1, a member of the Rho small GTPase family. Sema4D binding to Plexin-B1 promotes the binding of active Rac1 to Plexin-B1, which results in a reduction of the amount of active Rac1 bound to the p21-activated kinase (PAK) and, therefore, reduces PAK activity [15,16]. In cultured fibroblasts, Rac1 activates LIM-kinase (LIMK) via PAK, and LIMK phosphorylates and inactivates Cofilin, an actin-severing protein. Thus, Sema4D binding to Plexin-B1 sequesters active Rac1 and therefore downregulates PAK–LIMK activity, releasing the inhibition of Cofilin and causing growth-cone collapse [15,16] (Figure 1a). It will be interesting to test whether comparable mechanisms involving R-Ras and/or Rac1 exist in Sema4D–Plexin-B1 signaling in the vascular system.

Sema3A signaling in the nervous and vascular systems

Sema3A was the first semaphorin identified in vertebrates and genetic studies have implicated it in a variety of neural wiring processes including axon guidance, fasciculation (axon bundling), branching, pruning, dendrite development and neuronal migration [7]. In neurons, it is well established that Sema3A signals through the neuropilin-1 (Npn-1)–Plexin-A complex to induce cytoskeletal reorganization and growth-cone collapse. Both *Sema3A* null mutant embryos and *Npn-1*$^{Sema-}$ knock-in mutants, in which Sema3A–Npn-1 binding is selectively abolished owing to an engineered mutation in the Sema3A-binding site of Npn-1, have severe abnormalities in many central nervous system and peripheral nervous system axon projections [7].

In neurons, similarly to Sema4D, small GTPases (Rac1 and R-Ras) and kinases are involved in Sema3A signaling. For Rac1, the same pathway of Rac1–PAK–LIMK–Cofilin activation used in Sema4D also participates in Sema3A signaling. However, in contrast to the Sema4D signaling pathway, Rac1 activity is enhanced immediately in the presence of Sema3A. Rac1 activation is achieved by the FERM, RhoGEF and pleckstrin-domain-containing protein FARP2, a Rac1GEF released from Plexin-A and activated upon Sema3A binding [20] (Figure 1c). Another difference between Sema3A and 4D signaling is that an as yet unknown factor downstream of Sema3A can re-activate Cofilin after its inactivation by LIMK [20]. Therefore, even though Sema3A increases Rac1 activity, the end result of Rac1 signaling is still growth-cone collapse.

Regarding the pathway regulated by R-Ras, Sema3A uses the same Rnd1–R-Ras activation mechanism and downstream PI3K–Akt–GSK3β–CRMP2 pathway as Sema4D [21]. In addition, Sema3A-induced inactivation of PI3K can also trigger two other cytoskeletal changes that contribute to growth-cone collapse. First, the Sema3A-induced signal inhibits ERM phosphorylation in growth-cone filopodia and causes growth-cone collapse [22]. Second, the Sema3A-induced signal activates myosin II through the PI3K–Akt pathway. Activated myosin II then associates with actin to generate cellular contractile forces in dorsal root ganglion (DRG) neurons, contributing to growth-cone collapse [23]. In addition, a recent report indicates that phosphatase and tensin homolog (PTEN), an antagonist of PI3K, induces GSK3β activation in DRG

shown in red: in the presence of Sema3A, Rac1 facilitates Rnd1 recruitment to Plexin-A1, which induces the R-RasGAP activity of Plexin-A1 and downregulates active R-Ras. A decrease in active R-Ras downregulates PI3K–AKT activity and leads to axon growth-cone collapse through three different pathways: reduced phosphorylation of GSK3β, reduced phosphorylation of ERM and activation of myosin II. Kinases are shown in blue: in the presence of Sema3A, FARP2 inhibits PIPKIγ661 and suppresses integrin-induced adhesion. Fer and Fes are activated upon Sema3A binding to Plexin-A1 and phosphorylate and inactivate CRMP2, which leads to microtubule disassembly. Fyn is also activated after its binding to Plexin-A1 and inactivates CRMP2 by phosphorylating and activating Cdk5. Both actin depolymerization and microtubule disassembly lead to axon growth-cone collapse. **(d)** Sema3A signaling in the vascular system. Sema3A, through an unknown mechanism (possibly through Npn-1 and/or a co-receptor, shown as a dashed line and '?'), inhibits VEGF-induced activation of Src and FAK and contributes to angiogenesis. Sema3A might also function through Npn-1 to inhibit integrin-mediated adhesion of endothelial cells to the ECM. Sema3A can induce VE-cadherin phosphorylation and causes vascular permeability through unknown mechanisms (indicated by '?'), in which PI3K–Akt is involved.

neurons upon Sema3A treatment, probably through the suppression of PI3K activity [24] (Figure 1c).

In addition to the aforementioned small GTPases, several kinases have also been shown to have important roles in Sema3A signaling. The first is phosphatidylinositol phosphate kinase type I γ (PIPKIγ) 661, which is inhibited by the FARP2 released from Plexin-A1 and suppresses integrin-induced adhesion in the leading edge of growth cone [20]. Second, Fyn, which is activated by associating with Plexin-A1, activates another kinase, Cdk5, which then phosphorylates CRMP2 [25,26]. Finally, Fer and Fes bind Plexin-A1 and directly phosphorylate CRMP2 after Sema3A treatment [27,28] (Figure 1c). For information about additional Sema3A signaling pathways in neurons, see the review by Tran and colleagues [7].

In vitro treatment of endothelial cells with Sema3A inhibits their migration and vessel formation and promotes permeability [29–33]. Whether Sema3A–Npn-1–PlexA signaling mediates these effects in endothelial cells *in vivo* is unclear, even though endothelial cells express Npn-1 and plexin receptors [34]. One complication of studying this signaling event in endothelial cells is that Npn-1 is also a receptor for VEGF, a protein that is crucially important for endothelial cell development and function [35]. In zebrafish, either overexpression or a deficiency of Sema3A orthologs lead to vessel-patterning defects [32], indicating that Sema3A is essential for vascular development, although the penetrance of these defects is low. However, no detectable vascular defect was found in either *Sema3A* knockout mice or *Npn-1*$^{Sema-}$ mice lacking Sema3A–Npn-1 signaling. This indicates that Sema3A–Npn-1 signaling is not essential for general vascular development in mice [36,37], but it would be interesting to test whether Sema3A signaling functions in pathological processes.

In endothelial cells, three possible mechanisms for Sema3A signaling have been proposed based on *in vitro* studies (Figure 1d). First, Sema3A changes integrin affinity for the extracellular matrix (ECM) by an unknown mechanism; this inhibits integrin-mediated adhesion of endothelial cells to the ECM and enables the de-adhesion necessary for vascular remodeling [31]. Second, Sema3A blocks VEGF-induced focal adhesion kinase (FAK) and Src phosphorylation through unknown mechanisms and consequently disrupts VEGF-mediated angiogenesis [29]. Although it has been suggested that Sema3A affects VEGF-induced angiogenesis by competing with VEGF for Npn-1 [30], more evidence has shown that Sema3A and VEGF use different, non-overlapping sites on Npn-1 for binding, thereby making competitive binding unlikely [38,39]. Finally, Sema3A induces tyrosine phosphorylation of VE-cadherin through the PI3K–Akt pathway, thus promoting vascular permeability [29]. How Sema3A is linked to the PI3K–Akt pathway in the vascular system is not yet clear, and it would be interesting to see whether the molecules involved in Sema3A-induced PI3K–Akt signaling in the nervous system also participate in endothelial cells. It is important to point out that if indeed there is Npn-1-mediated Sema3A signaling in endothelial cells, it is not clear whether Plexin-A receptors or VEGF receptor 2 (VEGFR2) are its co-receptors. Recent evidence indicates that another plexin, Plexin-D1, might be the co-receptor of Npn-1 for Sema3A signaling. Studies in zebrafish show that knockdown of Sema3a1, Sema3a2 and Plexin-D1 lead to vessel-patterning defects, presumably through an Npn-1–Plexin-D1 complex [33]. However, in mice, another class 3 semaphorin (Sema3E, not Sema3A) binds and signals through Plexin-D1 directly and independently of Npn-1 to pattern developing blood vessels [40].

The recent discovery of Sema3E and its receptor Plexin-D1 might serve as a better example to demonstrate the dual function of class 3 semaphorins in neurons and endothelial cells. Both *in vitro* and *in vivo* studies have demonstrated that Sema3E exerts a repulsive effect through Plexin-D1 in the vascular and nervous systems [40,41] and the presence of Npn-1 converts this repulsive effect into attraction in neurons [41]. Identification of their downstream signaling pathways in both neurons and endothelial cells will provide further insight into the similarities and differences of class 3 semaphorin signaling in neurons and endothelial cells.

In summary, semaphorins have been studied as key guidance cues regulating neural and vascular development via similar and distinct signaling mechanisms. Although there are many different regulators in the two systems, partly owing to the difference in cell type, semaphorin signaling uses similar principles. For example, in both systems, Sema4D uses the same small GTPases (for example, RhoA) as its main mode of action, but is also able to either use the same (PDZ–RhoGEF and LARG) or different (ErbB2 or Met) regulators to activate the network. Another example is the PI3K–Akt pathway, which is used in both Sema3A and Sema4D signaling cascades and is shared in the nervous and vascular systems. Moreover, further investigation of the signaling pathways of other semaphorin family members that also have guidance functions in both neurons and endothelial cells, including Sema3C, 3E, 3F, Sema5A, 5B and Sema6A [7,42,43], will shed new light on the mechanistic similarities between both systems. Finally, it is worth pointing out that most of the mechanistic work described in this section has been performed *in vitro*, so *in vivo* validation of these signaling molecules and how they function together will be required.

Ephrins

Ephrin ligands and their Eph receptors have important roles in neural and vascular development [44]. There are two families of ephrins, the ephrin-A and ephrin-B family. Ephrin-A ligands are glycosylphosphatidylinositol (GPI)-anchored to the membrane and bind to EphA receptors, whereas ephrin-B ligands are transmembrane proteins that bind to EphB receptors (Box 2).

Ephrin-A–EphA signaling in the nervous and vascular systems

Ephrin-A signaling has been studied more extensively in the neuronal system than in the vascular system. Ephrin-A–EphA signaling leads to axon growth-cone collapse and dendrite retraction (Figure 2a). EphAs have important roles in establishing axon topographic maps, especially from the retina to the superior colliculus, and *EphA7* knockout mice exhibit an aberrant innervation pattern in the superior colliculus [45]. EphA–ephrin-A signaling

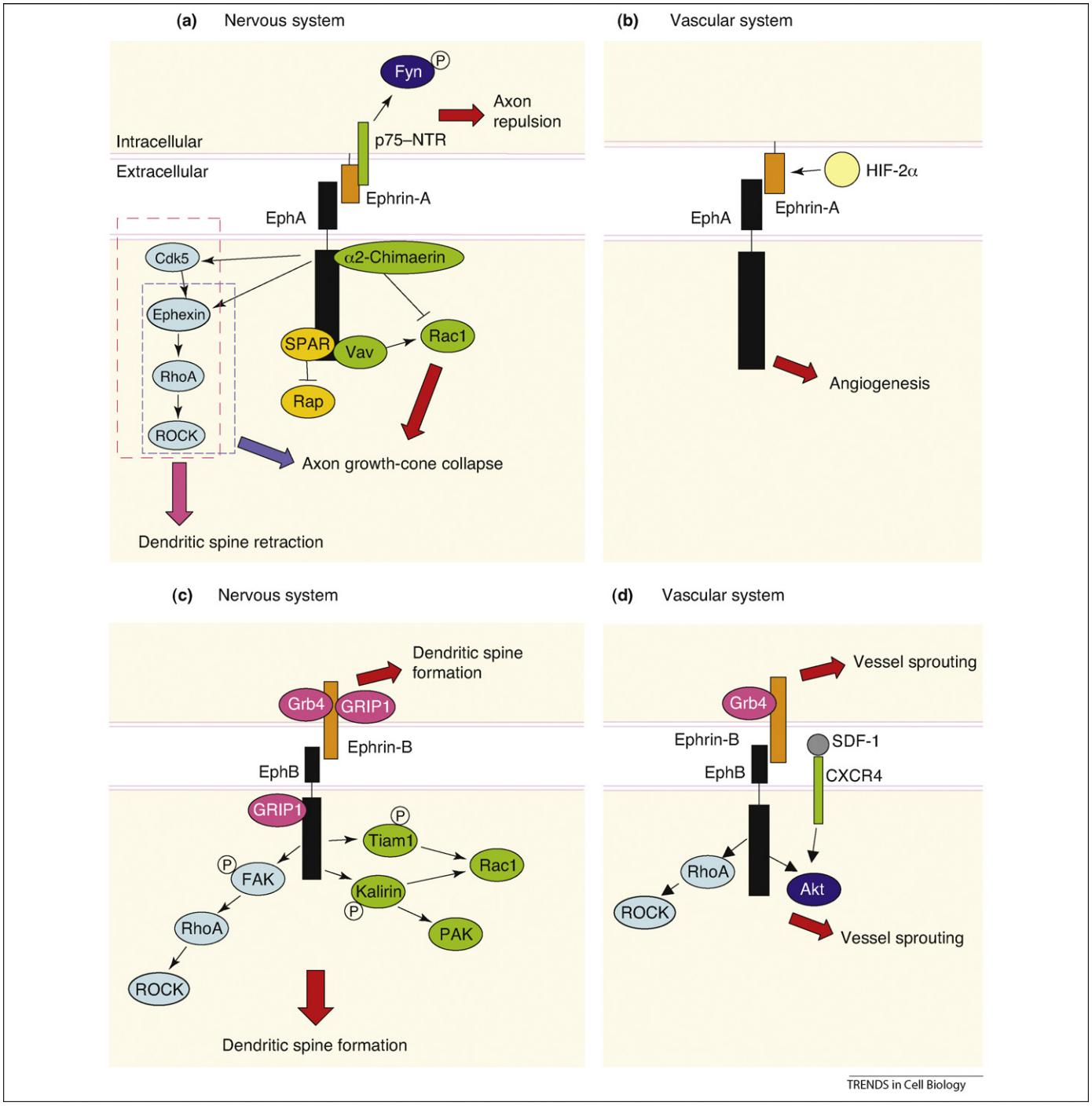

Figure 2. Ephrin–Eph signaling in the nervous and vascular systems. **(a)** Ephrin-A–EphA signaling in the nervous system. The Rap pathway is shown in gold: EphA activation leads to tyrosine phosphorylation of SPAR, a RapGAP that binds to EphA4 through its PDZ domain. SPAR then inactivates Rap. The RhoA pathway is shown in light blue: upon ephrin-A treatment, EphA recruits and phosphorylates Cdk5, which in turn phosphorylates and activates a RhoGEF, ephexin, activating RhoA. RhoA then activates ROCK, leading to dendritic spine retraction (as shown by the dashed pink box). The dashed blue box indicates the part of this pathway that has been shown to be important for growth-cone collapse. The Rac pathway is shown in green: activation of EphA receptors leads to binding and phosphorylation of the RacGAP α2-chimaerin, which then inactivates Rac1 in cortical neurons, leading to growth-cone collapse. In retinal axons, ephrin binding to EphA receptors leads to activation of the Rho family GEF Vav2, which then activates Rac1. Vav2-mediated activation of Rac1 leads to endocytosis of the membrane, eventually leading to growth-cone collapse. In reverse signaling, ephrin-A associates with the p75–NTR receptor. This complex works to phosphorylate Fyn and cause axon repulsion from EphA-expressing cells when EphA binds ephrin-A. **(b)** Ephrin-A–EphA signaling in the vascular system. There is limited information on how this signaling regulates angiogenesis, but ephrin-A expression is known to be regulated by HIF-2α expression in surrounding tissue and signaling through EphA in the vascular system can lead to angiogenesis in tumor models. **(c)** Ephrin-B–EphB signaling in the nervous system. EphB is recruited to the membrane by GRIP1, a multi-PDZ domain scaffolding protein. The Rac1 pathway is shown in green. Rac1 is activated by the GEFs Kalirin and Tiam1. Both Tiam1 and Kalirin bind to and are phosphorylated by the activated receptor and then activate Rac1, leading to increased dendrite morphogenesis. The RhoA pathway is shown in blue. EphA activation leads to phosphorylation of FAK, which then activates RhoA, activating ROCK and leading to increased dendrite morphogenesis. For reverse signaling through the ephrin-B ligand, several adaptor proteins are required. GRIP1 binds to ephrin-B3 and is perhaps involved in clustering it at the synapse. The SH2–SH3-domain-containing adaptor protein Grb4 binds ephrin-Bs and links them with multiple downstream regulators and enhancing dendrite morphogenesis in the ligand-expressing cell. **(d)** Ephrin-B–EphB signaling in the vascular system. EphB activation leads to the activation of RhoA and ROCK. In addition, EphB signaling enhances SFD–CXCR4 signaling as seen by increased phosphorylation of Akt. Both pathways lead to endothelial cell migration and sprouting. For reverse signaling, ephrin-B binds the SH2–SH3-domain-containing adaptor protein Grb4, leading to vessel sprouting, presumably through multiple downstream regulators recruited by Grb4.

has not been extensively studied in the developing vasculature but, rather, in pathological contexts. Ephrin-A1 and EphA2 are expressed in tumor neovasculatures and in human umbilical vein endothelial cells (HUVECs). Treatment of HUVECs expressing a dominant-negative EphA2 with ephrin-A1 inhibits capillary tube-like formation [46]. Ephrin-A1 expression in tumor vasculature is regulated by hypoxia-inducible response factor-2α in the surrounding tissue, and blocking ephrin-A1 with an inhibitor can impair angiogenesis [47] (Figure 2b).

Small GTPases have a crucial role in ephrin-A signaling (Figure 2a). First, in hippocampal axons, EphAs have been shown to interact with the spine-associated RapGAP (SPAR), leading to the inactivation of the small GTPase Rap1 and causing growth-cone collapse [48]. Second, Rac1 regulation of filopodial dynamics and membrane internalization is involved in EphA-mediated growth-cone collapse. Both the activation and inactivation of Rac1 can cause growth-cone collapse, and whether these events both occur in the same cell using the same players is unclear. In cortical neurons, Rac1 is inactivated by the RacGAP α2-chimaerin, which interacts with EphA4 and leads to actin depolymerization followed by growth-cone collapse. Blocking α2-chimaerin prevents growth-cone collapse *in vitro* and, importantly, *α2-chimaerin* knockout mice phenocopy the *EphA4* knockout neuronal phenotype of abnormal corticospinal and spinal interneuron circuitry, ultimately leading to a hopping gait [49–51]. In retinal ganglion cells, Rac1 is activated by the Vav family of GEFs, leading to internalization of the membrane, reorganization of F-actin and growth-cone collapse [52]. Third, RhoA activity is involved in ephrin-A–EphA-triggered growth-cone collapse. The activation of EphA receptors leads to the activation of ephexin, a RhoGEF that activates RhoA, leading to ROCK activation and growth-cone collapse via depolymerization of F-actin and inhibition of F-actin turnover [53]. In hippocampal neurons, EphA4 activation leads to Cdk5 phosphorylation, which then phosphorylates ephexin, leading to RhoA activation and dendritic spine retraction [54]. EphA4 regulates spine morphogenesis via activation of phospholipase Cγ (PLCγ), leading to activation of Cofilin, actin-fiber destabilization and resulting dendritic spine retraction [55]. Whether these same small GTPases have a role in ephrin-A–EphA signaling in endothelial cells is unknown.

Even though ephrin-A ligands are not transmembrane proteins, they do engage in reverse signaling (Figure 2a). A recent study has shown that, in retinal axons, ephrin-A forms a complex with the p75 neurotrophin receptor (NTR), causing Fyn phosphorylation upon activation of EphA receptors and leading to axon repulsion from inappropriate targets [56]. Retinal-specific *p75(NTR)* knockout mice have defects in retinal axon target innervation in the brain. Whether or not this is a mechanism employed by endothelial cells is still an open question.

Ephrin-B–EphB signaling in the nervous and vascular systems

In neurons, ephrin-Bs regulate both axon guidance and dendritic spine morphogenesis (Box 2). Dendritic spines are post-synaptic structures that are primarily composed of actin and are capable of changing their shape in a very short time scale [57] (Box 1). Hippocampal neurons express ephrin-B ligands both pre- and post-synaptically and EphB receptors post-synaptically [58]. Treatment of hippocampal neurons with exogenous ephrin-B or EphB receptor leads to increased spine morphogenesis and *EphB1/B2/B3* triple knockout mice have reduced dendritic spine density [59]. Ephrin-B–EphB signaling has also been shown to have a role in the repulsive axon guidance of several neuronal populations; for a review, see Ref. [60].

In the vascular system, ephrin-B2 is expressed in the arteries, whereas EphB4 is expressed in the veins, and this division enables demarcation of the vessel types. *Ephrin-B2* and *EphB2/B3* null mice die early in development with disorganized vasculature. Animals lacking the cytoplasmic domain of ephrin-B2 phenocopy the *ephrin-B2* nulls, indicating that reverse signaling through the ephrin ligand is also important for vascular development. In addition, just as treatment of neurons with exogenous ephrin-B2 leads to excess dendritic spine formation, treatment with ephrin-B1 causes excess capillary sprouting *in vitro* [61].

Forward signaling through Eph receptors is used in both neurons and endothelial cells, and common players such as the small GTPase RhoA have important roles in both systems. In neurons, after receptor phosphorylation upon ligand binding, EphB2 is targeted and maintained at the post-synaptic membrane via the multi-PDZ domain scaffold protein glutamate receptor interaction protein (GRIP1), which binds many post-synaptic proteins [59]. Ephrin-B1 activation of EphB2 leads to several events that ultimately affect RhoA or Rac1. First, the Rac1GEF Tiam1 (Table 1) is phosphorylated and interacts with EphB2, leading to cytoskeletal rearrangement and increased spine formation *in vitro*. The elimination of Tiam1 from neurons using RNAi silencing leads to a dearth of spines [62]. Second, the RhoGEF Kalirin is activated and translocates to the dendrite, leading to the activation of Rac1 and PAK, thus increasing spine morphogenesis [63]. Third, FAK is phosphorylated upon ligand binding and activates RhoA and, consequently, knockout of either of these genes in cultured neurons blocks ephrin-induced increases in dendrite number [64] (Figure 2c). Similarly, in endothelial cells, RhoA is also activated after EphB activation. Ephrin-B treatment of HUVECs leads to cell retraction, and this effect can be partially blocked by the application of RhoA–ROCK inhibitors [65] (Figure 2d).

Reverse signaling through ephrin-B ligands is also involved in neural and vascular development. For example, common adaptor proteins are recruited to the ephrin-B complex in both neurons and endothelial cells (Figure 2c,d). In neurons, the scaffolding protein GRIP1 interacts with post-synaptic ephrin-B3 and regulates synapse number in the post-synaptic membrane [66]. In addition, Grb4, a SH2- and SH3-domain-containing adaptor protein is localized in hippocampal dendrites and mediates ephrin-mediated dendrite morphogenesis [67] by linking ephrin-B to downstream effectors including Abi-1, Pak1, Cbl-associated protein (CAP) and Dynamin [68]. Similarly, in endothelial cells, Grb4 is also recruited to the ephrin-B–EphB complex after EphB activation and

causes vessel sprouting [69]. For additional information about ephrin-B reverse signaling, see Ref. [70].

Ephrin-B signaling in endothelial cells is also mediated by molecules not found to be involved in the nervous system. EphBs can cross-talk with other ligand-receptor pairs. For example, ephrin-B1 and -B2 activate human endothelial cells that express EphB2 and B4, enhancing the effects of the ligand–receptor pair SDF-1–CXCR4, as seen by enhanced Akt phosphorylation during *in vitro* endothelial cell assembly into capillary-like structures [71] (Figure 2d).

In summary, ephrin-A and -B signaling is important for both neuronal and vascular development and some common themes emerge. First, both in the vascular and nervous systems, ephrins–Ephs are trafficked and carefully regulated at the membrane by scaffolding proteins such as Grb4. In the nervous system, Ephs are also precisely compartmentalized within the cell, with EphBs primarily found in dendrites and EphAs mostly localized in axons. Although endothelial cells in the vasculature lack this complex cytoarchitecture, it would be interesting to see whether Ephs are differentially localized in endothelial cells with differing functions, such as tip or stalk cells, or whether different Eph receptors are localized at the leading or trailing end of the migrating cell. Second, processes in both systems, including dendritic spine formation, endothelial cell migration and angiogenesis, use both forward and reverse signaling to regulate cytoskeletal dynamics via small GTPases (RhoA). However, there are still some challenges in the field. Unlike the semaphorin family, ephrins are very promiscuous in their binding, with most A-family ligands binding most EphA receptors and most B-family ligands binding most EphB receptors. Knocking down ephrins or Ephs *in vivo* can often lead to compensation by other family members, making the identification of their exact signaling and function difficult. Additionally, although some of the signaling molecules downstream of these proteins have been identified and even confirmed *in vivo*, how these molecules are linked together to form a coherent picture to mediate ephrin–Eph effects is not known. As more molecules involved in the vascular signaling of ephrins and Ephs are identified, the similarities or differences between the two systems will become more apparent.

VEGF

VEGF is a key regulator of angiogenesis (Box 2). VEGF mediates vasculogenesis, angiogenesis and vascular permeability by affecting cellular processes such as proliferation and cell migration. Recently, VEGF was also shown to have an autocrine role in the maintenance of adult vasculature because mice lacking VEGF in adult vasculature undergo progressive endothelial degeneration, eventually leading to death [72]. VEGF and its receptors are also expressed in neurons and glia, and preliminary reports have indicated that they have a role in the main-

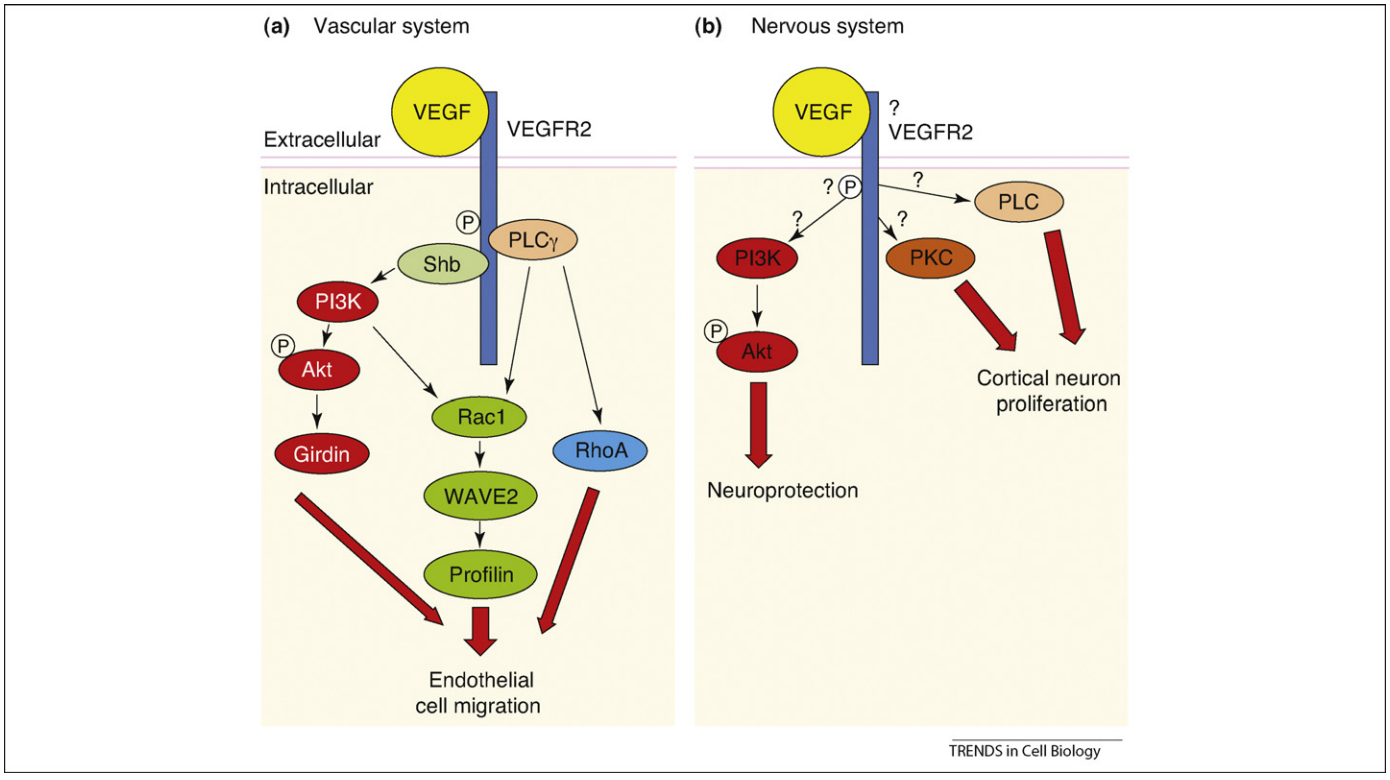

Figure 3. VEGF–VEGFR2 signaling in the nervous and vascular systems. **(a)** VEGF signaling in the vascular system. The PI3K–Akt pathway is shown in red. VEGF binding to VEGFR2 causes autophosphorylation of the receptor. Phosphorylation of the receptor at Tyr1175 causes the recruitment of the adaptor protein Shb, which then activates PI3K. PI3K phosphorylates Akt, which acts through substrates such as the actin-binding protein Girdin to regulate endothelial cell migration. The Rac1 pathway is shown in green. PI3K also activates Rac1, which regulates migration through the effectors WAVE2 and the actin-binding protein Profilin. Phosphorylation of Tyr1175 also causes activation of PLCγ, which activates the small GTPases Rac1 and RhoA to regulate endothelial cell migration. **(b)** VEGF–VEGFR2 signaling in the nervous system. Many of the same proteins used by VEGFR2 signaling in the vascular system are also implicated to act in the nervous system, but the evidence for this is less clear (indicated by '?'). PI3K inhibitors abrogate VEGFs neuroprotective effect, whereas PLC and PKC inhibitors prevented the neurotrophic effect exerted by VEGF on cortical neurons.

tenance of neural precursor identity and proliferation, neuroprotection, adult neurogenesis and neuronal migration [73].

VEGF-A signaling in the vascular and nervous systems

In endothelial cells, VEGF induces VEGR2 phosphorylation via several sites. Tyr1175 is crucial for proper vascular development because knock-in mice with an amino acid substitution at Tyr1175 (Tyr→Phe) lack Tyr1175 autophosphorylation and phenocopy *VEGFR2* knockout mice, which die early in development with impaired vasculogenesis [74]. Phosphorylation of VEGFR2 at Tyr1175 leads to binding of adaptor proteins such as Shb and subsequently activates PI3K. PI3K activates Akt, which mediates endothelial cell migration through substrates such as Girdin, an actin-binding protein [75] (Figure 3a). PI3K also leads to the activation of the small GTPase Rac1. Rac1 stimulates membrane ruffling or the formation of new actin-rich cell protrusions by associating with Wiskott-Aldrich syndrome protein family member WAVE2, which regulates profilin, an actin-binding protein involved in the polymerization of actin filaments. In addition, VEGFR2 can directly activate PLCγ. PLC activates the small GTPases Rac1 and RhoA to induce endothelial cell migration [76]. For further discussion of VEGF signaling, see Ref. [77] and Box 2.

Similarly, the PI3K–Akt pathway is also involved in VEGFR2 signaling in neurons. VEGF treatment of several neuronal types leads to neuroprotection against injury. Spinal cord motor neurons of superoxide dismutase (SOD) mice, a genetic model of amyotrophic lateral sclerosis (ALS), are protected from degeneration by exogenous treatment with VEGF [78] and PI3K inhibitors prevent this protective effect [79]. However, whether this protective effect affects neurons directly or indirectly is still unknown. Axotomized retinal ganglion cells have also been found to be protected from degeneration by treatment with VEGF, and they show increased levels of phosphorylated Akt after VEGF treatment. The neuroprotective effect exerted by VEGF on retinal neurons is blocked by Akt inhibitors [80]. *In vitro* studies have also indicated that VEGF signaling involves PI3K, PLC and PKC to mediate cortical neuron proliferation [81] (Figure 3b), but whether these pathways are also involved in processes such as VEGF-mediated neuronal migration is not known.

The role of the VEGFR2 co-receptor Npn-1 in VEGF signaling is starting to be uncovered. As mentioned previously, Npn-1 binds both VEGF and Sema3A. *In vivo* studies show that an endothelial-specific *Npn-1* knockout mouse has severe vascular defects. However, a *Npn-1*$^{Sema-}$ knock-in mouse that has no Npn-1–Sema3 binding has none of these defects, indicating that Sema3–Npn-1 interaction is not necessary for vascular development [36]. This indicates that the VEGF–Npn-1 interaction has a vital role in vascular development. Several lines of evidence point to the fact that Npn-1 can enhance VEGF–VEGFR2-mediated vascular function. VEGFR2–Npn-1 complex formation is induced by VEGF binding and both components are internalized together via a clathrin-dependent mechanism [82]. VEGF has a greater anti-apoptotic effect on Npn-1–VEGFR2-expressing cells than on cells expressing only VEGFR2 [83]. In addition, injection of function-blocking antibodies against Npn-1 and VEGF into the postnatal retina inhibited angiogenesis more severely than that of an anti-VEGF antibody alone. The same effect is seen with antibody injection in a tumor angiogenesis model [84]. Npn-1 has also been shown to enhance VEGFR2-induced p38MAPK activation [85].

Recent studies indicate that Npn-1 can also signal independently of VEGFR2. When the transmembrane and cytoplasmic domains of Npn-1 were fused to the extracellular portion of the endothelial growth factor (EGF) receptor and transfected into HUVECs, EGF treatment could still induce cell migration in the presence of VEGFR2 function-blocking antibodies [86]. Conversely, function-blocking antibodies against Npn-1 block $VEGF_{121}$-mediated endothelial cell migration despite the fact that $VEGF_{121}$ is unable to promote Npn-1 and VEGFR2 complex formation [39]. To definitively identify the role of VEGF–Npn-1 function in both the nervous and vascular systems, a mouse model that selectively eliminates VEGF binding to Npn-1 in a temporally and spatially controlled manner is needed.

In summary, more is known about VEGF-A signaling in the vascular than the nervous system. However, these studies have mostly been performed *in vitro* using biochemical approaches or by antibody injection in animals. Mouse models are required to fully dissect the *in vivo* roles of individual signaling molecules in each process. Owing to the importance of many of these signaling components to the survival of the organism, mouse models must be both temporally and spatially tractable. Moreover, because some of the signaling pathways used in the vascular system have begun to be implicated in the neuronal system, future studies should focus on their *in vivo* importance. It will also be important to begin to address the VEGF signaling pathways involved in neuronal migration and neurogenesis. Finally, there is no doubt that knowledge about VEGF signaling in the vascular system could guide future studies on its signaling in the nervous system.

Concluding remarks

Both the nervous and vascular systems are dynamic, highly adaptable organ systems in which development is controlled using a multitude of cues, including the proteins covered here and molecules such as Netrins and Slits. Similarities exist between the signaling mechanisms used in the nervous and vascular systems on several levels. Firstly, the working principles used in both systems are similar. For example, the activation of each receptor leads to the re-arrangement of the cytoskeleton through small GTPases, whether for the purpose of growth-cone guidance, dendrite formation or vascular tip cell guidance. Secondly, some common receptors are used by different ligands to regulate both neuronal and vascular development. For example, Npn-1 is a receptor both for semaphorins and VEGF, and VEGFR2 is a receptor for Sema6D in addition to VEGF [87]. Third, there is cross-talk between the downstream signaling molecules from different ligand-receptor pairs. For example, Sema3A influences VEGF function by blocking some of its downstream signaling molecules [29]. An interesting question for the future is

how growth cones or endothelial cells integrate multiple signals to control cell behavior when they encounter multiple environmental cues. Moreover, whether ligands and receptors expressed in nerves interact with their counterparts on vessels, and vice versa, and how their signaling contributes to vessel–nerve interaction is an exciting question for the future. Future studies on this topic can focus on identifying more players, not only in terms of the signals that directly lead to cytoskeletal changes, but also signals regulating receptor expression and trafficking. This will generate a better picture of the relationships between all of the involved players and a more thorough understanding of the *in vivo* roles of each of the proteins involved in this process. Furthermore, identification of molecules involved downstream of ephrins, semaphorins and VEGF can provide novel therapeutic targets for both vascular and neurological diseases. Because some of the same molecules are used in both systems, drugs currently used in one system could potentially be applicable to disorders of the other system.

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