Tyrosine phosphorylation in semaphorin signalling: shifting into overdrive

Mélanie Franco & Luca Tamagnone⁺

Institute for Cancer Research and Treatment (IRCC), University of Torino, Candiolo, Italy

The semaphorins constitute a large family of molecular signals with regulatory functions in neuronal development, angiogenesis, cancer progression and immune responses. Accumulating data indicate that semaphorins might trigger multiple signalling pathways, and mediate different and sometimes opposing effects, depending on the cellular context and the particular plexin-associated subunits of the receptor complex, which can include receptor-type or cytoplasmic tyrosine kinases such as MET, ERBB2, VEGFR2, FYN, FES, PYK2 and SRC. It has also been shown that a specific plexin can alternatively associate with different tyrosine kinase receptors, eliciting divergent signalling pathways and functional outcomes. Tyrosine phosphorylation is a pivotal post-translational protein modification that regulates intracellular signalling. Therefore, phosphorylation of tyrosines in the intracellular domain of plexins could determine or modify their interactions with additional signal transducers. Here, we discuss the potential relevance of tyrosine phosphorylation in semaphorin-induced signalling, with an emphasis on its probable role in dictating the choice between multiple pathways and functional outcomes. The identification of implicated tyrosine kinases will pave the way to target individual semaphorin-mediated functions.

Keywords: kinase; plexin; receptor; semaphorin; tyrosine

EMBO reports (2008) 9, 865–871. doi:10.1038/embo.2008.139

See Glossary for abbreviations used in this article.

Introduction

The semaphorins constitute a wide family of membrane-bound and secreted proteins that provide guidance cues for axon pathfinding and cell migration (Tamagnone & Comoglio, 2000; Zhou *et al*, 2008). Moreover, semaphorins are implicated in the regulation of many biological processes, such as neural development and organ morphogenesis in the embryo, and immune response, angiogenesis and invasive tumour growth in the adult. Semaphorin signalling affects cytoskeletal remodelling and integrin-dependent adhesion,

consequently impinging on cell migration; however, it has also been implicated in the regulation of cell proliferation and apoptosis, and, recently, in cell differentiation (for reviews, see Casazza *et al*, 2007; Kruger *et al*, 2005; Tamagnone & Giordano, 2006).

The main functional receptors for semaphorins are members of the plexin family (Takahashi *et al*, 1999; Tamagnone *et al*, 1999); notably, a subset of secreted semaphorins require obligate co-receptors associated with the plexins—which are known as neuropilins—although their potential role in intracellular signal transduction remains controversial. In mammals, nine plexins have been identified and divided into four subfamilies: plexin A1 to plexin A4, plexin B1 to plexin B3, plexin C1 and plexin D1. Plexins are large single-pass transmembrane molecules, the extracellular moiety of which contains conserved protein motifs, such as the ‘sema domain’ that presents a ‘ β -propeller’ structure (for a comprehensive review, see Gherardi *et al*, 2004), the cysteine-rich MET-related-sequences or PSI motifs, and the IPT domains (Artigiani *et al*, 1999). These structural domains are thought to mediate protein–protein interactions, although their specific functional relevance has not yet been elucidated.

The cytoplasmic region of plexins is highly conserved among the family members, and yet it is unique, owing to its lack of apparent homology with other proteins (Maestrini *et al*, 1996). This domain has been shown to associate with several intracellular signal transducers, eliciting multiple signalling pathways in response to semaphorin stimulation (Kruger *et al*, 2005; Fig 1). For instance, the intracellular domains of plexins contain GTPase-activating protein (GAP)-like motifs that are able to interact with—and downregulate—the monomeric G-protein R-Ras (Oinuma *et al*, 2004). Another region in the intracellular domain of plexins has been shown to interact with Rho family GTPases such as Rnd1 and Rac (Rohm *et al*, 2000; Tong *et al*, 2007). Current data indicate that the association of these monomeric G-proteins with plexins could induce the conformational changes required to elicit their GAP activity or to allow for the recruitment of additional signal transducers. Plexins have also been found to interact with GTPase-exchanger factors (GEFs), such as the Rac-GEF FARP2 (Toyofuku *et al*, 2005) and PDZ-Rho-GEF (Driessens *et al*, 2002; Perrot *et al*, 2002; Swiercz *et al*, 2002). By contrast, the cytoplasmic domain of plexin A1 and plexin B1 can interact with p190 Rho-GAP (Barberis *et al*, 2005); therefore, regulation of RhoA might vary in response to semaphorin signals in different cells (Swiercz *et al*, 2008). This is in agreement with the common observation that plexins have

Division of Molecular Oncology, Institute for Cancer Research and Treatment (IRCC), University of Torino, S.P. 142, 10060 Candiolo, Torino, Italy

*Corresponding author. Tel: +39 (0)11 993 3204; Fax: +39 (0)11 993 3225;

E-mail: luca.tamagnone@ircc.it

Submitted 25 January 2008; accepted 24 June 2008; published online 25 July 2008

Glossary

CDK	cyclin-dependent kinase
CRMP	collapsin response-mediator protein
DAP12	DNAX-activating protein of 12 kDa
EGF	epidermal growth factor
ERBB2	erythroblastic leukaemia viral oncogene homologue 2
FAK	focal adhesion kinase
FES	feline sarcoma oncogene
MAPK	mitogen-activated protein kinase
PI(3)K	phosphatidylinositol-3-kinase
PLC γ	phospholipase-C γ
PTB	phosphotyrosine-binding
PYK2	proline-rich tyrosine kinase 2
RhoA	Ras homologue gene family member A
Rnd1	Rho family GTPase 1
Sema4A	semaphorin 4A
SH2	SRC homology 2
TREM2	triggering receptor expressed on myeloid cells 2
VEGFR2	vascular endothelial growth factor receptor 2

been found to trigger multiple intracellular pathways, sometimes leading to opposing functional effects.

In fact, semaphorin signals are often mediated by multimeric receptor complexes that contain additional transmembrane subunits associated with plexins. In particular, receptor tyrosine kinases (RTKs) might associate with plexins on the cell surface, and have been shown to have a pivotal role in semaphorin signalling pathways (Conrotto *et al*, 2004; Giordano *et al*, 2002; Swiercz *et al*, 2004, 2008; Toyofuku *et al*, 2004a; Winberg *et al*, 2001). Moreover, in dendritic cells and osteoclast precursors, plexin A1 can be found in a complex with the transmembrane protein known as TREM2, which is, in turn, associated with the immunoreceptor tyrosine-based activation motif (ITAM) transducer, DAP12 (Takegahara *et al*, 2006), responsible for activating the intracellular tyrosine kinase SYK. Notably, certain semaphorins might also signal through receptors that are distinct from plexins. For instance, in the immune system, Sema4A and Sema4D might use the alternative low-affinity receptors TIM2 and CD72, respectively (Kikutani & Kumanogoh, 2003; Kumanogoh & Kikutani, 2001). CD72 controls tyrosine phosphorylation cascades in B cells

Fig 1 | Tyrosine kinases at the crossroads of semaphorin signalling pathways. Semaphorin receptors can elicit multiple intracellular signalling cascades to control axon guidance, cell migration and invasive growth (reviewed by Kruger *et al*, 2005; Zhou *et al*, 2008). Notably, the cytoplasmic tail of plexins might become tyrosine phosphorylated by either RTKs or cytoplasmic tyrosine kinases, indicating further regulatory mechanisms that have not been characterized (see text and Table 1 for details and specific references). (A) Semaphorin receptor complexes often include plexin-associated RTKs. For example, plexin B1 and plexin A1 have been found in association with tyrosine kinases such as MET, RON, ERBB2, VEGFR2 or OTK (off-track kinase) in a cell-specific manner. On semaphorin binding, these RTKs become activated, resulting in a differential regulation of cell migration, invasive growth and morphogenesis. (B) The intracellular domain of plexins might also associate with cytoplasmic tyrosine kinases implicated in signal transduction. For example, on Sema4D stimulation, endothelial cell chemotaxis might require integrin-dependent activation of the kinases PYK2 and SRC, triggering the PI(3)K/AKT pathway. Moreover, in different neuronal populations, the tyrosine kinases FAK, FYN and FER/FPS regulate neurite outgrowth in response to Sema3A and Sema3B. Therefore, semaphorin signals control cytoskeletal dynamics, integrin function, axon guidance, cell migration and invasive growth, leading sometimes to opposing functional effects, due to the activation of distinct pathways in a cell-specific manner. CDK5, cyclin-dependent kinase 5; CRMP, collapsin response-mediator protein; ERBB2, erythroblastic leukaemia viral oncogene homologue 2; FAK, focal adhesion kinase; FER, feline sarcoma oncogene; GAP, GTPase-activating protein; PI(3)K, phosphatidylinositol-3-kinase; PYK2, proline-rich tyrosine kinase 2; RND, Rho family GTPase 1; RTK, receptor tyrosine kinase; Sema4D, semaphorin 4D; SRC, sarcoma viral oncogene homologue; TK, tyrosine kinase; VEGFR2, vascular endothelial growth factor receptor 2.

Table 1 | Semaphorin signalling pathways that implicate associated tyrosine kinases

Semaphorin	Receptor complex	Cell type	Activity	References
Sema3A	Plexin A1*/NRP1/FES-FPS or FER	DRG neurons, COS	Growth-cone collapse	Mitsui <i>et al</i> , 2002; Shapovalova <i>et al</i> , 2007
	Plexin A2*/NRP1/SRC family kinases	DRG neurons, HEK293 cells	Growth-cone collapse	Sasaki <i>et al</i> , 2002
	Plexin/SRC family kinases	Cortical neurons	Neurite formation, dendritic branching	Morita <i>et al</i> , 2006
	NRP1/L1/FAK kinases	Cortical neurons	Growth-cone collapse	Bechara <i>et al</i> , 2008
Sema3B	NRP2/NrCAM (?) /FAK–SRC family kinases	Commissural neurons	Axon outgrowth attraction (or repulsion/collapse)	Falk <i>et al</i> , 2005
Sema4D	Plexin B1*/MET or RON	Epithelial cells and carcinoma cells, COS, NIH3T3	Increased cell migration, invasive growth	Giordano <i>et al</i> , 2002; Conrotto <i>et al</i> , 2004
	Plexin B1*/SRC/PYK2	HUVEC	Increased migration	Basile <i>et al</i> , 2005
	Plexin B1/MET	HUVEC	Increased migration, angiogenesis	Conrotto <i>et al</i> , 2005
	Plexin B1*/MET	MDA-MB 468	RhoA inhibition, inhibited migration	Swiercz <i>et al</i> , 2008
	Plexin B1*/ERBB2	MCF-7	RhoA activation, increased migration	Swiercz <i>et al</i> , 2008
	Plexin B1*/ERBB2	HEK293 cells, PC12, hippocampal neurons	RhoA activation, growth-cone collapse	Swiercz <i>et al</i> , 2004
Sema5A	Plexin B3/MET	NIH3T3, HUVEC	Increased cell migration (or cell collapse)	Artigiani <i>et al</i> , 2004
Sema6B	Reverse signalling through Sema6B cyto-tail/SRC	COS	Unknown	Eckhardt <i>et al</i> , 1997
Sema6D	Plexin A1/OTK	Endocardiac cells of the ventricle region	Inhibition of migration, cell repulsion	Toyofuku <i>et al</i> , 2004a
	Plexin A1/VEGFR2	Endocardiac cells of the CT segment	Increased cell migration, invasive growth	Toyofuku <i>et al</i> , 2004a
	Plexin A1–TREM2–DAP12/SYK	Dendritic cells	Cell differentiation	Takegahara <i>et al</i> , 2006
	Reverse signalling through Sema6D cyto-tail/ABL (plexin A1 ectodomain)	Myocardiac cells	Increased cell migration, invasive growth	Toyofuku <i>et al</i> , 2004b

Asterisks indicate plexins reported to become tyrosine phosphorylated.

CT, conotruncal; DAP12, DNAX-activating protein of 12 kDa; DRG, dorsal root ganglion; ERBB2, erythroblastic leukaemia viral oncogene homologue 2; FAK, focal adhesion kinase; Fes, feline sarcoma oncogene; HUVEC, human umbilical vein endothelial cell; NrCAM, neuron–glia-related cell–adhesion molecule; NRP, neuropilin; OTK, off-track kinase; PYK2, proline-rich tyrosine kinase 2; RhoA, Ras homologue gene family member A; Sema, semaphorin; Trem2, triggering receptor expressed on myeloid cells 2; Src, sarcoma viral oncogene homologue; VEGFR2, vascular endothelial growth factor receptor 2.

through the regulation of the associated tyrosine phosphatase SHP1. Finally, Sema7A might interact *in trans* with β 1-integrin and trigger the activation of FAK and MAPK signalling cascades (Pasterkamp *et al*, 2003; Suzuki *et al*, 2007).

Tyrosine kinases at the crossroads of semaphorin pathways

Tyrosine phosphorylation is a pivotal post-translational protein modification that regulates intracellular signalling in response to several extracellular signals, which are either receptor ligands or extracellular matrix components. It is mediated by tyrosine kinases, which can be subdivided into receptor type (transmembrane) and non-receptor type (cytoplasmic and commonly membrane associated). Numerous cytoplasmic and receptor-type tyrosine kinases are implicated in controlling integrin-mediated adhesion, cytoskeletal dynamics, cell

migration and axon guidance, which are the main processes regulated by semaphorins. Notably, tyrosine kinase inhibitors have been shown to inhibit Sema3A-mediated and Sema3B-mediated functions in neurons (Falk *et al*, 2005; Morita *et al*, 2006; Sasaki *et al*, 2002). Moreover, the crucial involvement of tyrosine kinases in the signalling pathways mediated by Sema4D and Sema6D in non-neuronal cells has been shown using small-molecule inhibitors, the expression of dominant-negative constructs and RNA interference-based gene-silencing approaches (Conrotto *et al*, 2004, 2005; Giordano *et al*, 2002; Swiercz *et al*, 2004, 2008; Toyofuku *et al*, 2004a,b; Table 1).

As mentioned above, semaphorins might be able to trigger the activation of RTKs associated with plexins in receptor complexes on the cell surface (Fig 1A). For example, Sema4D stimulation can activate and induce tyrosine phosphorylation of MET,

Sidebar A | In need of answers

- (i) How is the association of tyrosine kinases with plexins regulated in different cells?
- (ii) What are the relevant tyrosine phosphorylation sites in the cytoplasmic domain of plexins?
- (iii) What are the functional role(s) of plexin tyrosine phosphorylation—for example, to regulate protein conformation, as docking sites for adaptors/transducers, or in receptor trafficking?

RON and ERBB2 RTKs in different cell types (Conrotto *et al*, 2004; Giordano *et al*, 2002; Swiercz *et al*, 2008). Notably, it has been shown that the pattern of ERBB2 tyrosine phosphorylation induced by Sema4D is not the same as that seen upon EGF stimulation, which could point to the specific recruitment of different downstream effectors in response to these signals (Swiercz *et al*, 2004). Moreover, this cross-talk might be responsible for switching between different signalling pathways. For instance, Swiercz and co-workers found that the pro-migratory and anti-migratory effects observed in response to Sema4D in different epithelial cells seem to correlate with the ability of distinctive receptor complexes to regulate RhoA activity (Swiercz *et al*, 2008). In this regard, the activation of the plexin B1–ERBB2 receptor complex elicits RhoA activation and directional cell migration through the involvement of a plexin-associated PDZ-Rho-GEF, whereas the plexin B1–MET complex seems to mediate Rho inhibition and migration block. However, considering that MET activation in response to Sema4D might also lead to increased migration and invasive growth in other cell types (Giordano *et al*, 2002), the role of MET signalling in response to semaphorins remains controversial, and might be strongly dependent on the cellular context. As another example, Sema6D-mediated signals in different developing cardiac cells can induce opposing migratory effects, due to the involvement of different receptor complexes. In particular, in endocardiac cells of the conotruncal segment, the plexin A1–VEGFR2 complex mediates cell migration and invasive growth in response to Sema6D. By contrast, the migration of cardiac cells of the ventricle region, which express the plexin A1–OTK (off-track kinase) receptor complex, is inhibited in response to Sema6D (Toyofuku *et al*, 2004a). Notably, although OTK is an unusual RTK that is devoid of catalytic activity, VEGFR2 is known to activate intracellular signalling pathways, including PLC γ and PI(3)K/AKT (Olsson *et al*, 2006).

The mechanisms that mediate the activation of plexin-associated tyrosine kinases in response to semaphorin stimulation have not been clearly elucidated (Sidebar A). As the association of RTKs with plexins seems to pre-date ligand binding, it has been proposed—although still not shown—that semaphorins can cluster in large receptor complexes on the cell surface, which is a process that is known to activate RTKs.

Cytoplasmic tyrosine kinases have also been implicated in semaphorin signalling, and have been found to be associated with semaphorin receptors (Fig 1B). The mechanism mediating the recruitment and functional activation of these plexin-associated non-receptor tyrosine kinases is less obvious, and might be direct or indirect. The intracellular domain of plexins might contain motifs that recognize conserved protein domains such as SH2 and SH3, which are frequently found in cytoplasmic tyrosine kinases;

the conformational change after recruitment to the plexin might be sufficient to disturb the auto-inhibitory intramolecular interaction, leading to its functional activation (Moarefi *et al*, 1997). For example, plexin A1 and plexin A2 include proline-rich putative SH3-domain-binding sequences (Sasaki *et al*, 2002; M.F., unpublished observations). Notably, independent studies have shown that the intracellular tyrosine kinases FYN and FES/FPS can associate with plexins of the A-subfamily in a ligand-independent manner (Mitsui *et al*, 2002; Sasaki *et al*, 2002), and that PYK2 and SRC are recruited to the plexin B1 receptor complex, and are functionally activated in endothelial cells on Sema4D stimulation (Basile *et al*, 2005). Moreover, additional transmembrane molecules within semaphorin-receptor complexes might control the association and activation of intracellular tyrosine kinases; for example, the Sema3A co-receptor neuropilin 1 has been implicated in the recruitment of FES and FAK tyrosine kinases (Bechara *et al*, 2008; Mitsui *et al*, 2002).

The functional role of FYN in response to Sema3A in neurons seems to implicate the activation of the CDK5 phosphorylation cascade—known to regulate cytoskeletal rearrangement and membrane endocytosis (Sasaki *et al*, 2002; Yamashita *et al*, 2007). Moreover, FES and the related kinase FER were reported to phosphorylate CRMP-associated molecules, which are substrates of CDK5 (Mitsui *et al*, 2002; Yamashita *et al*, 2007). It was shown recently that Sema3A stimulation of neurons expressing neuropilin-1/L1 receptor complexes also leads to the recruitment and activation of FAK, which is a pathway that is implicated in the turnover of integrin-based adhesions and the inhibition of axonal outgrowth (Bechara *et al*, 2008). Intriguingly, the same group had shown previously that FAK and SRC kinases are activated in response to Sema3B in anterior commissural neurons, the axons of which are attracted by the semaphorin (Falk *et al*, 2005). By contrast, FAK and SRC activation are not observed in posterior commissural axons, which are repelled by Sema3B. It seems that the addition of a specific SRC family kinase inhibitor not only abolishes the attractive response to Sema3B, but also converts it into axon repulsion (Falk *et al*, 2005). Therefore, FAK/SRC signalling induced by Sema3B is a crucial component of the attractive response in commissural neurons, and the kinase activity seems to tightly control the switch between opposing outcomes. Sema7A-dependent axonal outgrowth was further explained by integrin engagement, as well as activation of the FAK and MAPK signalling cascades (Pasterkamp *et al*, 2003). In endothelial cells, Sema4D stimulation leads to the recruitment and activation of the integrin-associated kinase PYK2, thereby eliciting the PI(3)K/AKT pathway, which is implicated in cell motility and angiogenesis (Basile *et al*, 2005, 2007). Therefore, the association of semaphorin signals with the activation of tyrosine kinases that regulate integrin functions, such as SRC-family kinases FAK and PYK2, as well as MET and ERBB2 (Guo *et al*, 2006; Trusolino *et al*, 2001), might represent another mechanism of controlling integrin-mediated adhesion, in addition to the inactivation of monomeric GTPases.

Cytoplasmic tyrosine kinases have also been implicated in the so-called ‘reverse’ signalling pathways mediated by the intracellular domain of transmembrane semaphorins. In particular, Toyofuku and co-workers used the ABL kinase inhibitor STI-571 to show that this tyrosine kinase is required for Sema6D/plexin A1-induced migration of endocardiac cells during heart development (Toyofuku *et al*, 2004b). The intracellular domain of Sema6D associates with the ABL substrate Enabled, the tyrosine phosphorylation of which is required

Fig 2 | Conserved tyrosine residues in the cytoplasmic domain of plexin family members. Alignment of protein sequences of human plexin intracellular domains. In the consensus line (shown under the sequences), amino-acid identity is marked with asterisks and similarity is indicated by dots or columns, as assigned by the CLUSTALW algorithm. Background colours highlight residues or domains of particular interest. In total, 13 tyrosine residues (Y) are conserved in all plexins (or in all but one family member) and are shown in red; moreover, three of these residues are included in highly conserved amino-acid stretches (blue background). The positions of highly conserved tyrosines are indicated on top of the sequences, with reference to the amino-acid coordinates in plexin A1 (accession number NP_115618.2). Notably, the residues diverging from this consensus in plexin B3 and plexin A4 (underlined) are conserved between the human and mouse genes. Tyrosine residues that are conserved in at least one entire plexin subfamily are shown in blue. The three conserved arginines found to be functionally required in the GTPase-activating protein-like motifs are shown in green (Vikis *et al*, 2002). A grey background highlights the presumptive RhoGTPase-binding domain (Tong *et al*, 2008).

for semaphorin-dependent myocardial cell motility. Notably, other transmembrane semaphorins of subclass 6 (such as Sema6B) contain proline-rich SH3-domain-binding motifs that are potentially implicated in the interaction with cytoplasmic SRC-like tyrosine kinases and in their functional activation (Comoglio *et al*, 2004; Eckhardt *et al*, 1997).

Potential role of plexin tyrosine phosphorylation

Initial experiments had shown that plexins might become phosphorylated in tyrosine residues when overexpressed (Tamagnone *et al*, 1999). There is now consistent evidence that plexins might be substrates for associated tyrosine kinases, both receptor type and cytoplasmic (Table 1). Moreover, in the presence of dominant-negative mutants of ERBB2, MET and FYN that lack kinase activity, the tyrosine phosphorylation of associated plexins is strongly reduced (Giordano *et al*, 2002; Sasaki *et al*, 2002; Swiercz *et al*, 2004).

Although the tyrosine phosphorylation of plexins is thought to exert a regulatory role in semaphorin signalling, this has not yet been specifically investigated. Tyrosine phosphorylation of plexins could provide docking sites for SH2 or PTB domains (Yaffe, 2002), which are contained in several adaptors and transducer molecules that are implicated in plexin signalling, such as SRC-like tyrosine kinases (SRC, ABL, FAK and PYK2) and the regulatory subunit of PI(3)K, p85. Moreover, the tyrosine phosphorylation of plexins could modulate the conformational state of the cytoplasmic domain and allow for the recruitment of specific signal transducers. Recently, oncogenic mutations found in the cytoplasmic domain of plexin B1—and linked to loss of association with Rac1 and Rnd1—have been found to have a strong impact on plexin B1 structure and function (Tong *et al*, 2008; Wong *et al*, 2007). Therefore, plexin tyrosine phosphorylation by specific RTKs might elicit relevant conformational changes in the cytoplasmic tail, differentially regulating the accessibility of specific protein modules. Along the same lines, as plexin B1 has been previously reported to differentially regulate Rho activity through the recruitment of either a RhoGEF or a RhoGAP, one could speculate that plexin B1 tyrosine phosphorylation mediated by ERBB2 might elicit RhoA activation by favouring the accessibility of its PDZ-domain-binding sequence. However, the interaction of plexin B1 with MET could also result in Rho inhibition by promoting the association of p190 RhoGAP with the cytoplasmic tail of plexin B1.

The specific tyrosine residues phosphorylated in the cytoplasmic domain of plexins in response to semaphorin stimulation have not yet been identified, although this region contains numerous conserved sites that could be targets of tyrosine kinases. Sequence alignment reveals the presence of 13 conserved tyrosine residues (Fig 2). Notably, some of these tyrosines have been found, by using high-throughput screening experiments, to be phosphorylated in living cells (www.phosphosite.org). Using the ScanSite algorithm (scansite.mit.edu), we found that certain conserved tyrosines might be substrates for known kinases (for example, SRC for Tyr1833 in plexin A1 and LCK for Tyr2053 in plexin B1). Moreover, some of these residues, when phosphorylated, might become docking sites for SH2- or PTB-domain-containing proteins. For example, phosphorylated Tyr1833 in plexin A1 and the corresponding tyrosine in plexin B1 might bind the SH2 domains found in kinases of the SRC family. Therefore, tyrosine phosphorylation of plexins triggered by semaphorin signals could mediate the recruitment of distinctive transducers through specific phosphotyrosine docking sites and/or important conformational changes in the

plexin cytoplasmic tail, thereby allowing for a switch between multiple intracellular signalling routes. Clearly, these speculative hypotheses await experimental validation.

In conclusion, tyrosine kinases associated with semaphorins or semaphorin receptors—and subsequent tyrosine phosphorylation cascades—have an important role in semaphorin functions. They seem to control the switch between multiple signalling routes that mediate diverse functional outcomes. Although the tyrosine phosphorylation of plexins has been observed on semaphorin stimulation, site-directed mutagenesis experiments are required to characterize its function. Eventually, the identification of distinctive tyrosine kinases implicated in semaphorin signalling pathways might allow us to test pharmacological inhibitors that target individual semaphorin functions, and could potentially be used to modulate processes such as nerve regeneration, immune response and cancer progression.

ACKNOWLEDGEMENTS

The authors acknowledge all of the members of the Tamagnone laboratory for helpful discussions. Research activity carried out by M.F. and L.T. is supported by the Italian Association for Cancer Research (AIRC).

REFERENCES

- Artigiani S, Comoglio PM, Tamagnone L (1999) Plexins, semaphorins, and scatter factor receptors: a common root for cell guidance signals? *IUBMB Life* **48**: 477–482
- Artigiani S, Conrotto P, Fazzari P, Gilestro GF, Barberis D, Giordano S, Comoglio PM, Tamagnone L (2004) Plexin-B3 is a functional receptor for semaphorin 5A. *EMBO Rep* **5**: 710–714
- Barberis D, Casazza A, Sordella R, Corso S, Artigiani S, Settleman J, Comoglio PM, Tamagnone L (2005) p190 Rho-GTPase activating protein associates with plexins and it is required for semaphorin signalling. *J Cell Sci* **118**: 4689–4700
- Basile JR, Afkhami T, Gutkind JS (2005) Semaphorin 4D/plexin-B1 induces endothelial cell migration through the activation of PYK2, Src, and the phosphatidylinositol 3-kinase–Akt pathway. *Mol Cell Biol* **25**: 6889–6898
- Basile JR, Gavard J, Gutkind JS (2007) Plexin-B1 utilizes RhoA and Rho kinase to promote the integrin-dependent activation of Akt and ERK and endothelial cell motility. *J Biol Chem* **282**: 34888–34895
- Bechara A *et al* (2008) FAK-MAPK-dependent adhesion disassembly downstream of L1 contributes to semaphorin3A-induced collapse. *EMBO J* **27**: 1549–1562
- Casazza A, Fazzari P, Tamagnone L (2007) Semaphorin signals in cell adhesion and cell migration: functional role and molecular mechanisms. *Adv Exp Med Biol* **600**: 90–108
- Comoglio PM, Tamagnone L, Giordano S (2004) Invasive growth: a two-way street for semaphorin signalling. *Nat Cell Biol* **6**: 1155–1157
- Conrotto P, Corso S, Gamberini S, Comoglio PM, Giordano S (2004) Interplay between scatter factor receptors and B plexins controls invasive growth. *Oncogene* **23**: 5131–5137
- Conrotto P, Valdembrì D, Corso S, Serini G, Tamagnone L, Comoglio PM, Bussolino F, Giordano S (2005) Sema4D induces angiogenesis through Met recruitment by Plexin B1. *Blood* **105**: 4321–4329
- Driessens MH, Olivo C, Nagata K, Inagaki M, Collard JG (2002) B plexins activate Rho through PDZ-RhoGEF. *FEBS Lett* **529**: 168–172
- Eckhardt F, Behar O, Calautti E, Yonezawa K, Nishimoto I, Fishman MC (1997) A novel transmembrane semaphorin can bind c-src. *Mol Cell Neurosci* **9**: 409–419
- Falk J *et al* (2005) Dual functional activity of semaphorin 3B is required for positioning the anterior commissure. *Neuron* **48**: 63–75
- Gherardi E, Love CA, Esnouf RM, Jones EY (2004) The sema domain. *Curr Opin Struct Biol* **14**: 669–678
- Giordano S, Corso S, Conrotto P, Artigiani S, Gilestro G, Barberis D, Tamagnone L, Comoglio PM (2002) The semaphorin 4D receptor controls invasive growth by coupling with Met. *Nat Cell Biol* **4**: 720–724
- Guo W, Pylayeva Y, Pepe A, Yoshioka T, Muller WJ, Inghirami G, Giancotti FG (2006) Beta 4 integrin amplifies ErbB2 signaling to promote mammary tumorigenesis. *Cell* **126**: 489–502

- Kikutani H, Kumanogoh A (2003) Semaphorins in interactions between T cells and antigen-presenting cells. *Nat Rev Immunol* **3**: 159–167
- Kruger RP, Aurandt J, Guan KL (2005) Semaphorins command cells to move. *Nat Rev Mol Cell Biol* **6**: 789–800
- Kumanogoh A, Kikutani H (2001) The CD100–CD72 interaction: a novel mechanism of immune regulation. *Trends Immunol* **22**: 670–676
- Maestrini E *et al* (1996) A family of transmembrane proteins with homology to the MET–hepatocyte growth factor receptor. *Proc Natl Acad Sci USA* **93**: 674–678
- Mitsui N, Inatome R, Takahashi S, Goshima Y, Yamamura H, Yanagi S (2002) Involvement of Fes/Fps tyrosine kinase in semaphorin3A signaling. *EMBO J* **21**: 3274–3285
- Moarefi I, LaFevre-Bernt M, Sicheri F, Huse M, Lee CH, Kuriyan J, Miller WT (1997) Activation of the Src-family tyrosine kinase Hck by SH3 domain displacement. *Nature* **385**: 650–653
- Morita A *et al* (2006) Regulation of dendritic branching and spine maturation by semaphorin3A–Fyn signaling. *J Neurosci* **26**: 2971–2980
- Oinuma I, Ishikawa Y, Katoh H, Negishi M (2004) The Semaphorin 4D receptor Plexin-B1 is a GTPase activating protein for R-Ras. *Science* **305**: 862–865
- Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L (2006) VEGF receptor signalling: in control of vascular function. *Nat Rev Mol Cell Biol* **7**: 359–371
- Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL (2003) Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. *Nature* **424**: 398–405
- Perrot V, Vazquez-Prado J, Gutkind JS (2002) Plexin B regulates Rho through the guanine nucleotide exchange factors leukemia-associated Rho GEF (LARG) and PDZ-RhoGEF. *J Biol Chem* **277**: 43115–43120
- Rohm B, Rahim B, Kleiber B, Hovatta I, Puschel AW (2000) The semaphorin 3A receptor may directly regulate the activity of small GTPases. *FEBS Lett* **486**: 68–72
- Sasaki Y *et al* (2002) Fyn and Cdk5 mediate semaphorin-3A signaling, which is involved in regulation of dendrite orientation in cerebral cortex. *Neuron* **35**: 907–920
- Shapovalova Z, Tabunshchik K, Greer PA (2007) The Fer tyrosine kinase regulates an axon retraction response to Semaphorin 3A in dorsal root ganglion neurons. *BMC Dev Biol* **7**: 133
- Suzuki K *et al* (2007) Semaphorin 7A initiates T-cell-mediated inflammatory responses through alpha1beta1 integrin. *Nature* **446**: 680–684
- Swiercz JM, Kuner R, Behrens J, Offermanns S (2002) Plexin-B1 directly interacts with PDZ-RhoGEF/LARG to regulate RhoA and growth cone morphology. *Neuron* **35**: 51–63
- Swiercz JM, Kuner R, Offermanns S (2004) Plexin-B1/RhoGEF-mediated RhoA activation involves the receptor tyrosine kinase ErbB-2. *J Cell Biol* **165**: 869–880
- Swiercz JM, Worzfeld T, Offermanns S (2008) ErbB-2 and Met reciprocally regulate cellular signaling via plexin-B1. *J Biol Chem* **283**: 1893–1901
- Takahashi T, Fournier A, Nakamura F, Wang LH, Murakami Y, Kalb RG, Fujisawa H, Strittmatter SM (1999) Plexin–neuropilin-1 complexes form functional semaphorin-3A receptors. *Cell* **99**: 59–69
- Takegahara N *et al* (2006) Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. *Nat Cell Biol* **8**: 615–622
- Tamagnone L, Comoglio PM (2000) Signalling by semaphorin receptors: cell guidance and beyond. *Trends Cell Biol* **10**: 377–383
- Tamagnone L, Giordano S (2006) Semaphorin pathways orchestrate osteogenesis. *Nat Cell Biol* **8**: 545–547
- Tamagnone L *et al* (1999) Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. *Cell* **99**: 71–80
- Tong Y, Chugha P, Hota PK, Alviani RS, Li M, Tempel W, Shen L, Park HW, Buck M (2007) Binding of Rac1, Rnd1, and RhoD to a novel Rho GTPase interaction motif destabilizes dimerization of the plexin-B1 effector domain. *J Biol Chem* **282**: 37215–37224
- Tong Y, Hota PK, Hamaneh MB, Buck M (2008) Insights into oncogenic mutations of plexin-B1 based on the solution structure of the Rho GTPase binding domain. *Structure* **16**: 246–258
- Toyofuku T *et al* (2004a) Dual roles of Sema6D in cardiac morphogenesis through region-specific association of its receptor, Plexin-A1, with off-track and vascular endothelial growth factor receptor type 2. *Genes Dev* **18**: 435–447
- Toyofuku T, Zhang H, Kumanogoh A, Takegahara N, Yabuki M, Harada K, Hori M, Kikutani H (2004b) Guidance of myocardial patterning in cardiac development by Sema6D reverse signalling. *Nat Cell Biol* **6**: 1204–1211
- Toyofuku T, Yoshida J, Sugimoto T, Zhang H, Kumanogoh A, Hori M, Kikutani H (2005) FARP2 triggers signals for Sema3A-mediated axonal repulsion. *Nat Neurosci* **8**: 1712–1719
- Trusolino L, Bertotti A, Comoglio PM (2001) A signaling adapter function for alpha6beta4 integrin in the control of HGF-dependent invasive growth. *Cell* **107**: 643–654
- Vikis HG, Li W, Guan KL (2002) The plexin-B1/Rac interaction inhibits PAK activation and enhances Sema4D ligand binding. *Genes Dev* **16**: 836–845
- Winberg ML, Tamagnone L, Bai J, Comoglio PM, Montell D, Goodman CS (2001) The transmembrane protein Off-track associates with plexins and functions downstream of semaphorin signaling during axon guidance. *Neuron* **32**: 53–62
- Wong OG *et al* (2007) Plexin-B1 mutations in prostate cancer. *Proc Natl Acad Sci USA* **104**: 19040–19045
- Yaffe MB (2002) Phosphotyrosine-binding domains in signal transduction. *Nat Rev Mol Cell Biol* **3**: 177–186
- Yamashita N *et al* (2007) Regulation of spine development by semaphorin3A through cyclin-dependent kinase 5 phosphorylation of collapsin response mediator protein 1. *J Neurosci* **27**: 12546–12554
- Zhou Y, Gunput RA, Pasterkamp RJ (2008) Semaphorin signaling: progress made and promises ahead. *Trends Biochem Sci* **33**: 161–170

Luca Tamagnone & Mélanie Franco