5- Semaphorin-Plexin-Neuropilin

Table 1. Directional Guidance cues involved in CNS neuronal migration in vivo and in vitro

Ligands	Receptors	Defects in CNS neuronal migration in vivo	Neuronal migration in vitro
Slits	Robo	—	1. Slit repels postnatal SVZa cells[37] 2. Slit repels prenatal SVZ cells of GE[43]
Netrins	DCC	1. Abnormal pontine nuclei in DCC and netrin-1 mutants[46]	1. Netrin-1 attracts pontine nuclei[11]
	Unc-5h	2. Abnormal cerebellar development in unc-5h3[64]*	2. Netrin-1 repels postnatal cerebellar granule cells and prenatal SVZ cells[48,49] 3. Anti-DCC antibody blocks directed migration of postnatal SVZa cells[47]
Semaphorins	Neuropilin Plexin	1. Abnormal GABAergic interneurons in the striatum in neuropilin-2 mutants[50]	—
Ephrins	Eph	—	1. Disruption of Eph-B/Ephrin-B system affects the migration of postnatal SVZa cells[51]

*Unc-5h3/RCM mutant mice showed abnormal development of cerebellum. However, it is still unclear that the defect is primarily caused by migration abnormality or other reasons.

SEMAPHORINS COMMAND CELLS TO MOVE

Robert P. Kruger, Jennifer Aurandt‡ and Kun-Liang Guan§*

Abstract | Semaphorins are secreted or transmembrane proteins that regulate cell motility and attachment in axon guidance, vascular growth, immune cell regulation and tumour progression. The main receptors for semaphorins are plexins, which have established roles in regulating Rho-family GTPases. Recent work shows that plexins can also influence R-Ras, which, in turn, can regulate integrins. Such regulation is probably a common feature of semaphorin signalling and contributes substantially to our understanding of semaphorin biology.

NATURE REVIEWS | MOLECULAR CELL BIOLOGY VOLUME 6 | OCTOBER 2005 | 789

SEMAPHORINS-PLEXINS-NEUROPILINS

ligands receptors co-receptors

• semaphorins and their receptors are known signals for:

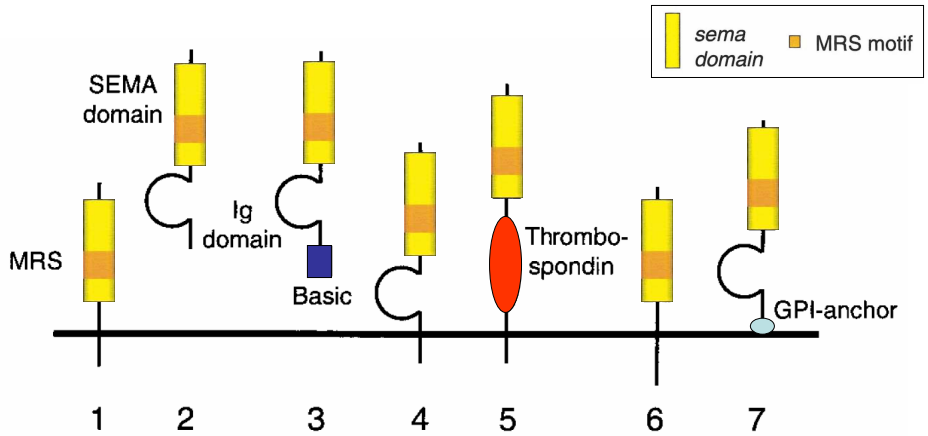
-axon guidance
-cell migration
-morphogenesis
-immune function
-tumor progression

• cell guidance control by semaphorins requires plexins

• the functional receptor for secreted semaphorins is a complex including neuropilins and plexins

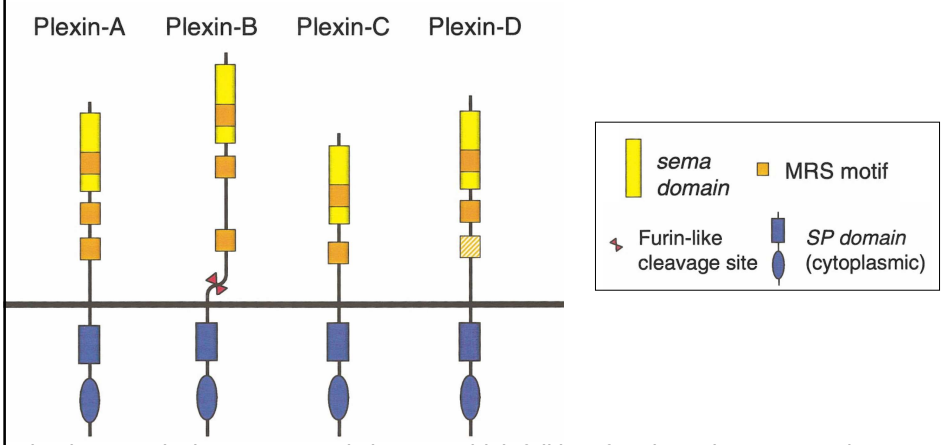
• semaphorins have multiple functions in morphogenesis and tissue remodeling by mediating cell-repelling cues through plexin receptors

SEMAPHORIN SUBFAMILIES



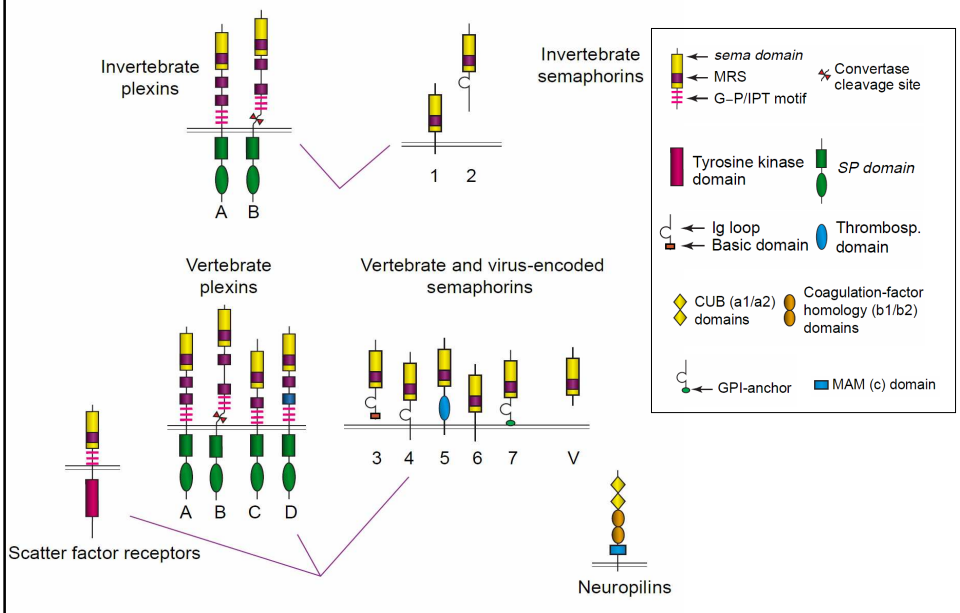
Class 1 and 2 contain transmembrane and secreted semaphorins from invertebrates, respectively. Secreted semaphorins of vertebrates fall into class 3. The other vertebrate semaphorins are membrane bound, either transmembrane (class 4, 5, and 6) or GPI anchored (class 7). The Sema domain is the hallmark of this protein family, and it includes a Met-related sequence (MRS motif). Other conserved domains are immunoglobulin domains (in classes 2, 3, 4, and 7), domains rich in basic amino acids (class 3), and thrombospondin repeats (class 5).

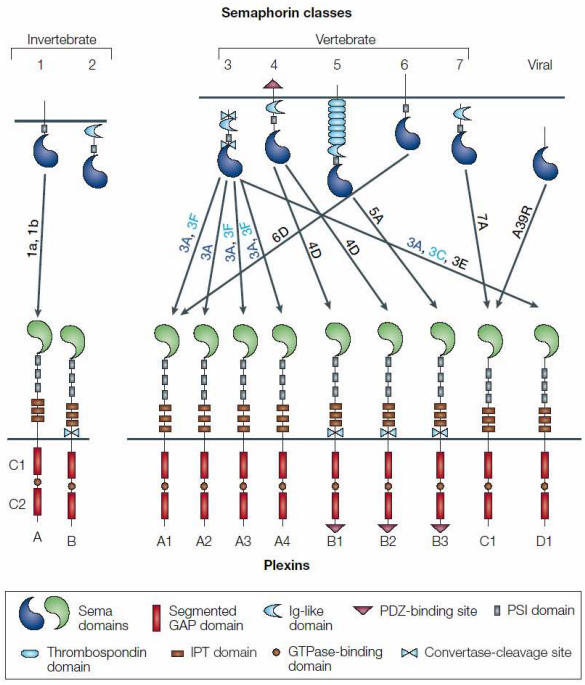
THE SEMAPHORIN RECEPTORS PLEXINS



Nine human plexins are currently known, which fall into four homology groups (A, B, C, and D subfamilies), based on sequence similarity, structural features, and tissue distribution. All plexins include a conserved cytoplasmic domain (sex-plexin domain, SP, blue) and a Sema domain (yellow). Repeated MRS motifs (orange) are found in the extracellular domain. Plexin-B subfamily members include potential cleavage sites for furin-like convertases (also found in plexin-B of *Drosophila,* here marked in red). The prototype member of the plexin-C subfamily (Vespr) is shorter, including only two MRS motifs. Plexin-D1 includes an atypical sequence in its third MRS (stippled).

Evolution of semaphorins and their receptors

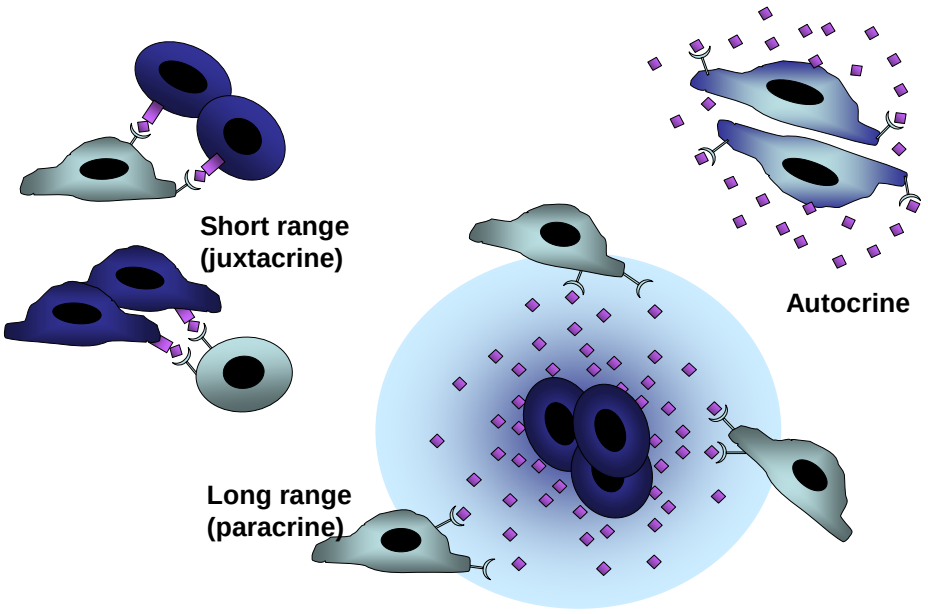




Semaphorins and plexins

Both semaphorins and plexins are characterized by Sema domains. Additional domains that are present in semaphorins include PSI (plexin, semaphorin and integrin) domains, immunoglobulin (Ig)-like domains, thrombospondin domains and PDZ domain-binding sites, both putative and described. Additional domains present in plexins include PSI domains, IPT (Ig-like, plexins and transcription factors) domains, a GTPase-binding domain and a segment GAP (GTPase-activating protein) domain. Some plexins also have PDZ-domain binding sites, and convertase-cleavage sites. Arrows indicate binding interactions detected between semaphorins and plexins. Labels on the arrows indicate which specific semaphorin has been shown to interact with which plexin. Blue labels indicate the necessity for neuropilin-1 (dark blue) or neuropilin-2 (light blue) as co-receptors.

Semaphorin signals can act in different modes



Semaphorin–plexin interactions

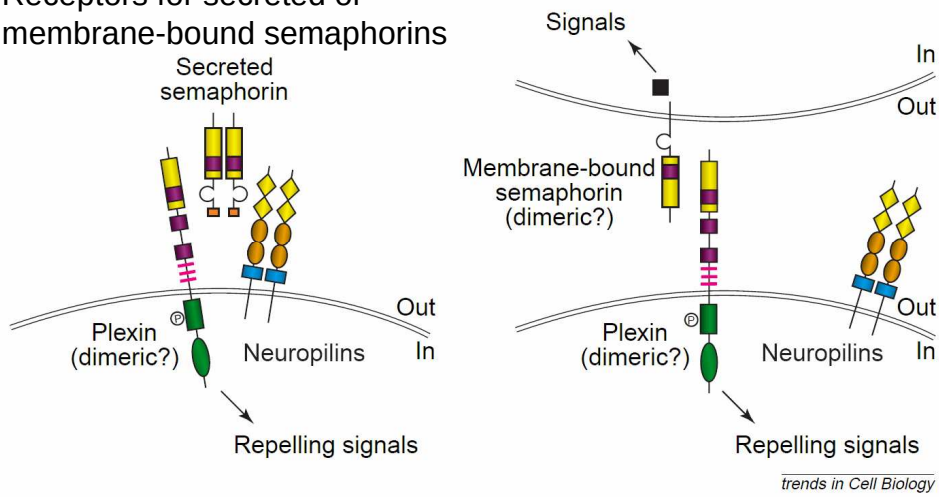
• semaphorins can be both membrane-bound and secreted, plexins can functionally interact with semaphorins on adjacent cells and with semaphorins in the extracellular environment

• semaphorin binding relieves plexin autoinhibition, as Plexin-A1 lacking its Sema domain is constitutively active. An intramolecular interaction between the Sema domain and the rest of the plexin extracellular domain probably inhibits receptor activity in the unbound state

•most plexin–semaphorin interactions are mediated through the Sema domains of both proteins, except for class 3 semaphorins, which, with one exception, require **neuropilins** as essential semaphorin binding co-receptors to signal through class A plexins

• **neuropilins** are transmembrane proteins of ~900 amino acids with short intracellular domains that lack intrinsic enzymatic activity. They function as the ligand-binding partner in co-receptor complexes for both plexins and vascular endothelial growth factor receptors (VEGFRs).

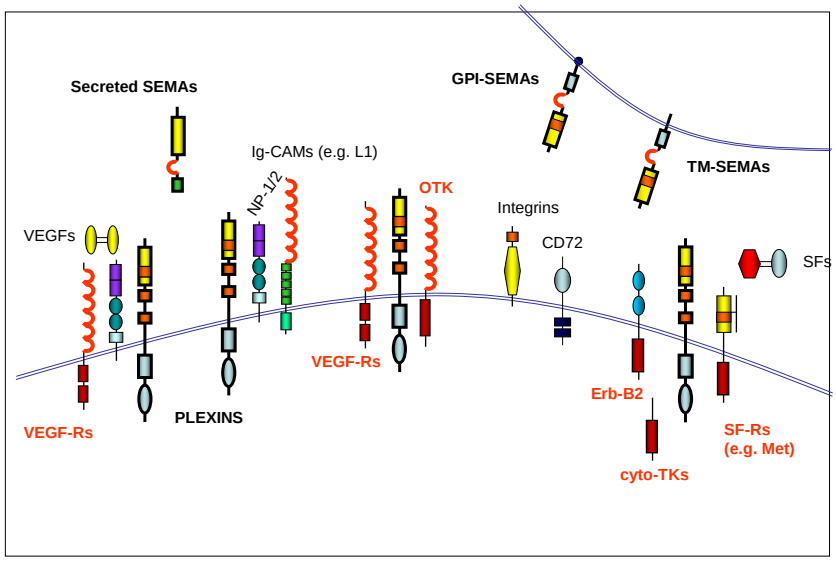
Receptors for secreted or membrane-bound semaphorins



Secreted (subclass 3) semaphorins are dimeric and bind to receptor complexes that include neuropilins (homo or heterodimeric) and plexins (dimeric?).

Membrane-bound semaphorins bind directly to plexins and transduce intracellular signals through the sex-plexin (SP) domain. Transmembrane semaphorins might generate bidirectional signals by associating with cytoplasmic transducers.

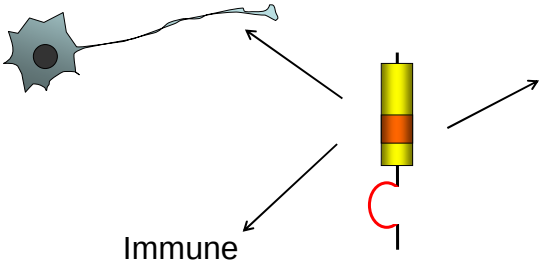
Semaphorin signalling is mediated by Receptor Complexes



Semaphorins Regulate a Variety of Functions

"stop or go"

Axon guidance



Cell migration

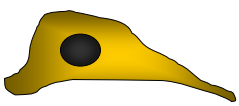
e.g. Neural Crest cells

Oligodendrocytes

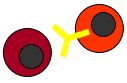
Endothelial cells

Epithelial cells

Cancer cells



Immune response

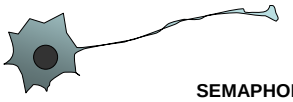


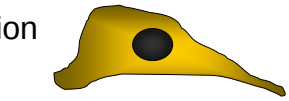
Semaphorins Regulate a Variety of Functions

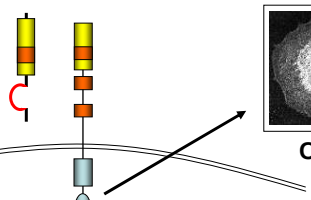
"stop or go"

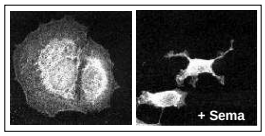
Axon guidance

Cell migration

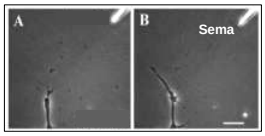




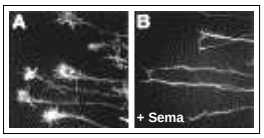




Cellular collapse

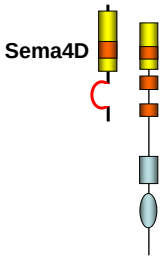


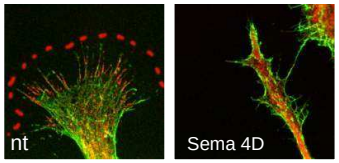
Axon turning



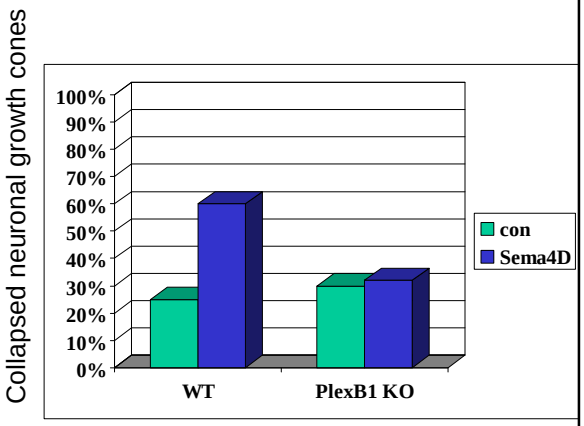
Growth cone collapse

Sema4D-induced collapse of neuronal growth cones is abrogated in PlexinB1-deficient neurons



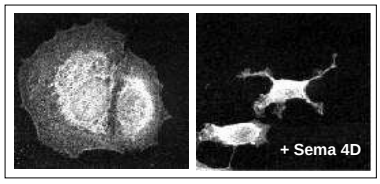


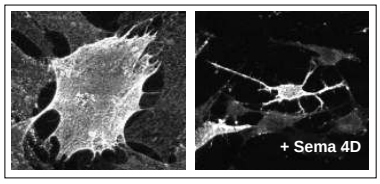
Phalloidin-FITC
α-Vinculin

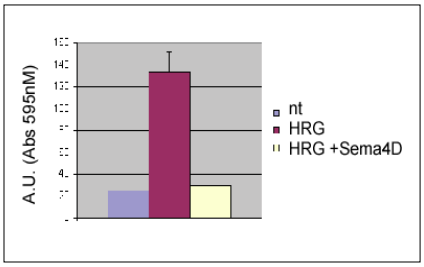


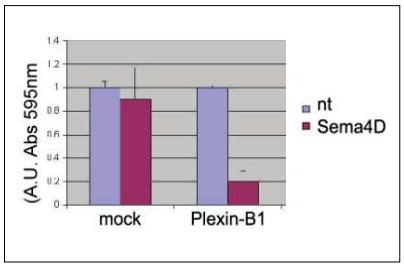
hippocampal neurons growth cones

Plexin signalling and the collapsing response can inhibit cell migration





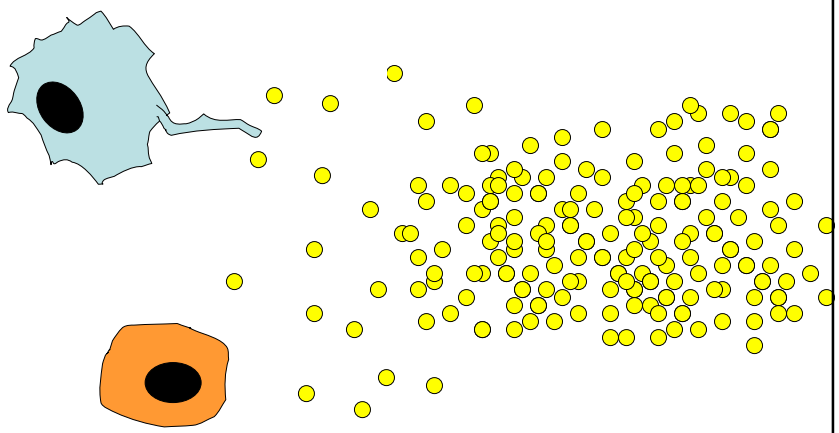




SKBR3 mammary carcinoma cells

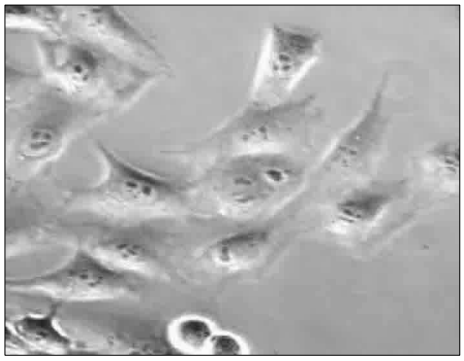
NIH-3T3 fibroblasts

Axons and cells retract in response to semaphorins *(the "collapsing" response)*

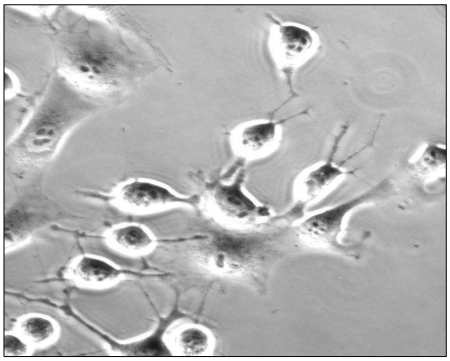


Cellular collapse upon Plexin-B1 activation

control

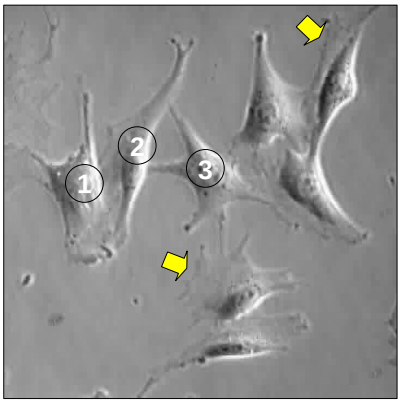


+ Sema4D



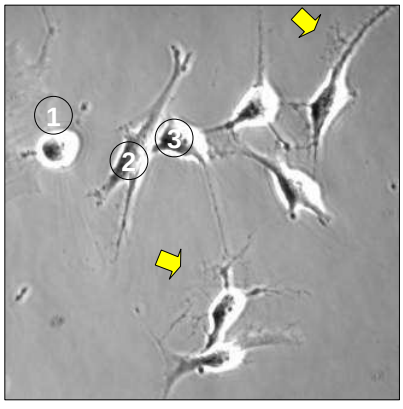
Cellular collapse upon Plexin activation

control



time: 0’

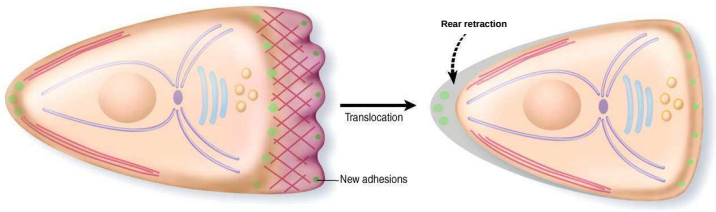
+ Sema4D



time: 15’

What are the molecular mechanisms mediating semaphorin signals?

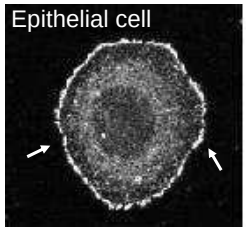
Molecular mechanisms controlling cell migration

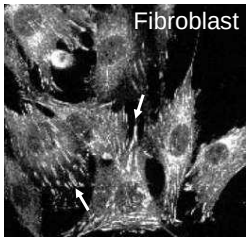


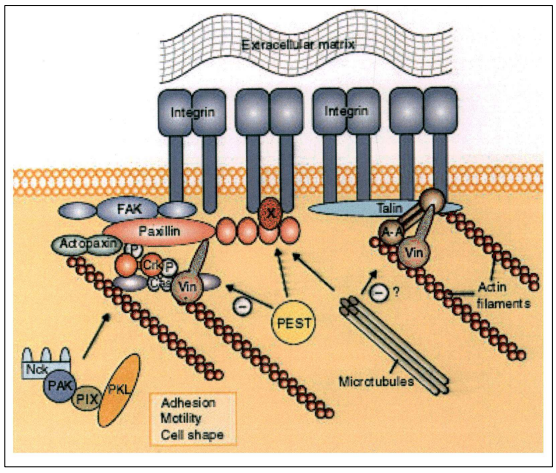
1. Integrin-based adhesion

2. Cytoskeletal remodeling (Rho GTPases)

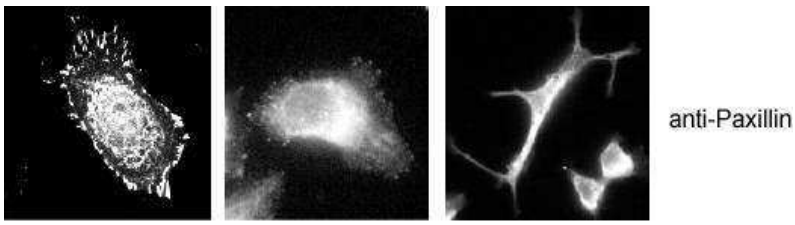
Cell-substrate adhesive complexes





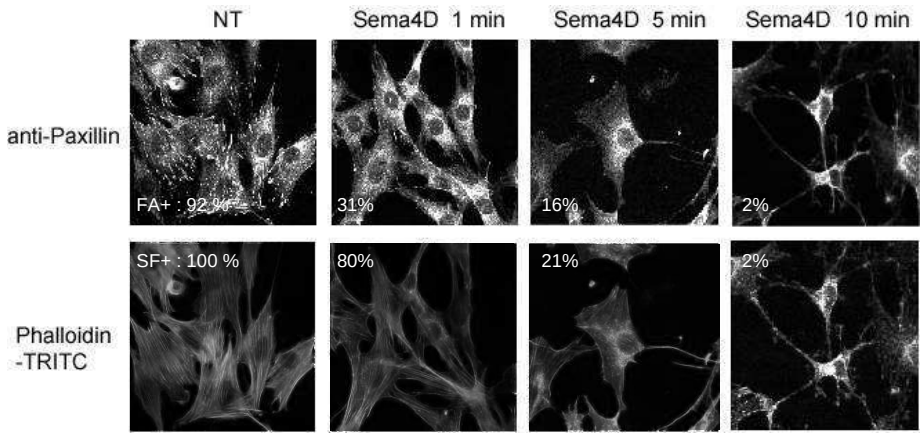


Sema4D triggers the disassambly of adhesive structures in epithelial cells expressing endogenous Plexin-B1



SKBR3 cells

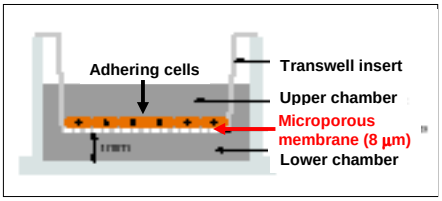
Plexin activation elicits the disassembly of focal adhesions

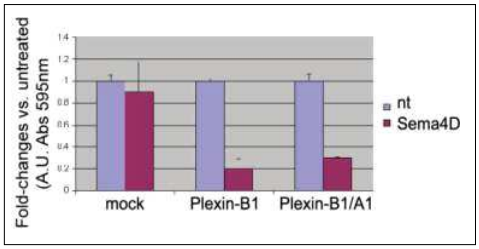


NIH-3T3 fibroblasts expressing Plexin-B1

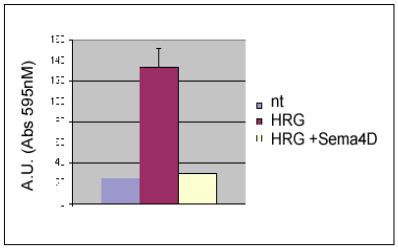
Barberis et al., 2004

Plexin signalling inhibits cell migration





NIH-3T3 fibroblasts



SKBR3 epithelial cells

Semaphorin signalling

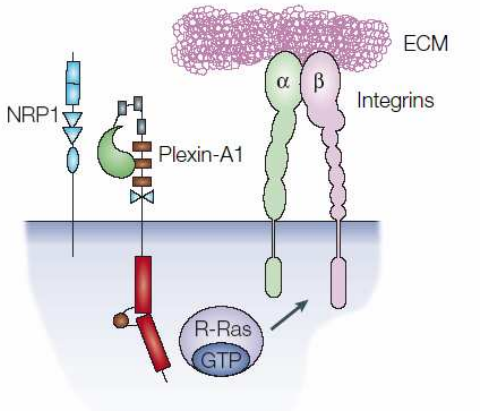
- on activation by semaphorins, plexins become phosphorylated on tyrosine residues in their cytoplasmic domain
- the mechanisms of tyrosine phosphorylation and the role of kinases in plexin signalling are now being unveiled for both class A and B plexins

Plexins have GAP activity towards R-Ras

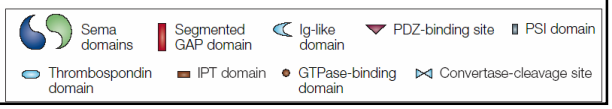
- the intracellular domain of plexins has two highly conserved regions (C1 and C2) that are similar to a GAP (GTPase-activating protein) domain divided in two by a linker region
- the conserved regions contain two arginine residues that are necessary for catalytic activity in GAPs. They are essential in Plexin-A1 signalling and mutating them in Plexin-B1 abolishes the Sema4D induced collapse of COS-7 cells
- consequently, it has been suggested that plexins could be Ras GAPs, but until recently these ideas were only speculative, as efforts to establish GAP activity proved difficult
- it has recently been established that both Plexin-B1 and Plexin-A1 are GAPs for the Ras-family GTPase R-Ras
- this finding establishes a new mechanism of GAP regulation and promotes plexins to the pantheon of **transmembrane receptors with intrinsic enzymatic activity**

Plexin A1 signalling

no ligand

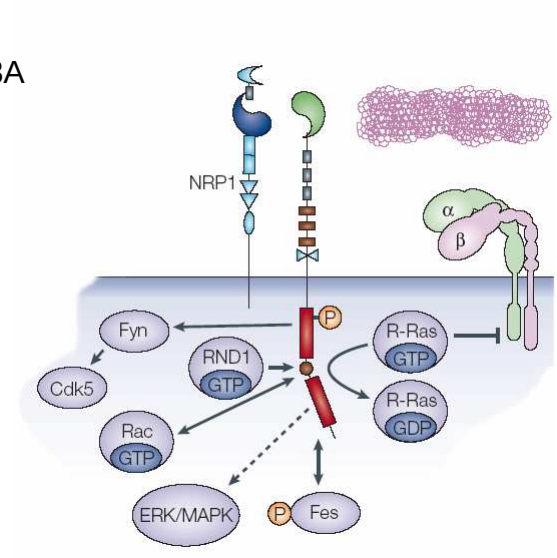


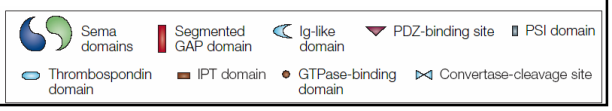
- without ligand, R-Ras is active, resulting in integrin-mediated attachment to the extracellular matrix (ECM).



Plexin A1 signalling

+ Sema3A

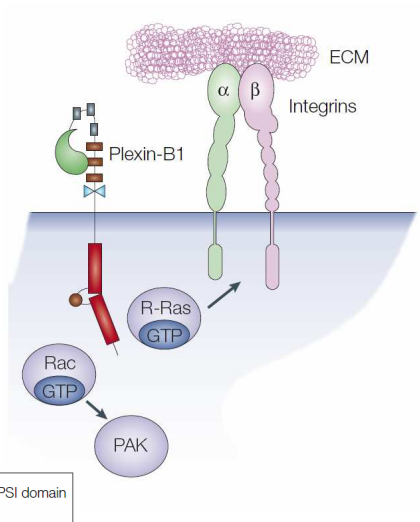


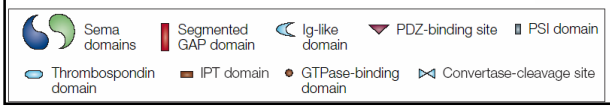


Plexin B1 signalling

no ligand

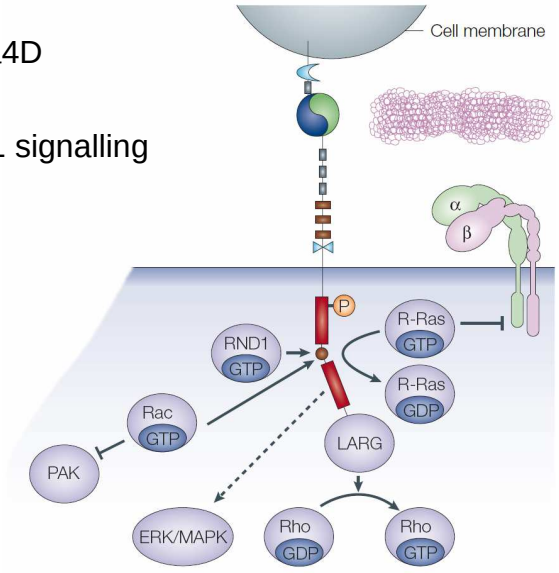
• without bound semaphorin, active Rac promotes the activation of p21-activated kinase (PAK).

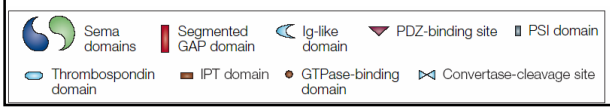




+ Sema4D

Plexin B1 signalling





Semaphorins repel axonal growth cones