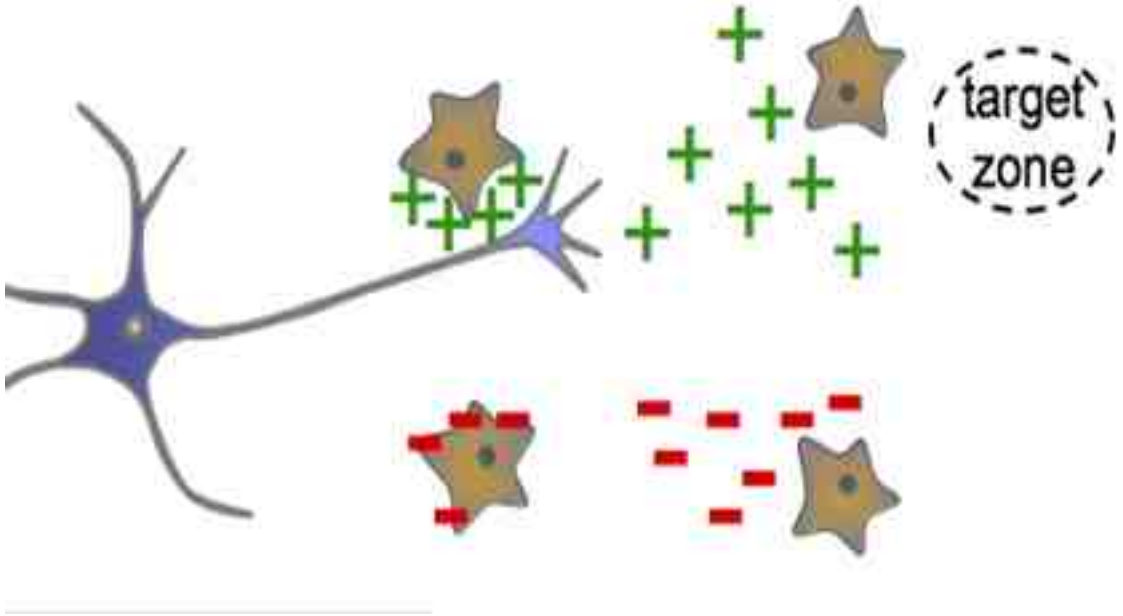
Pathfinding: driven by chemoattraction and chemorepulsion



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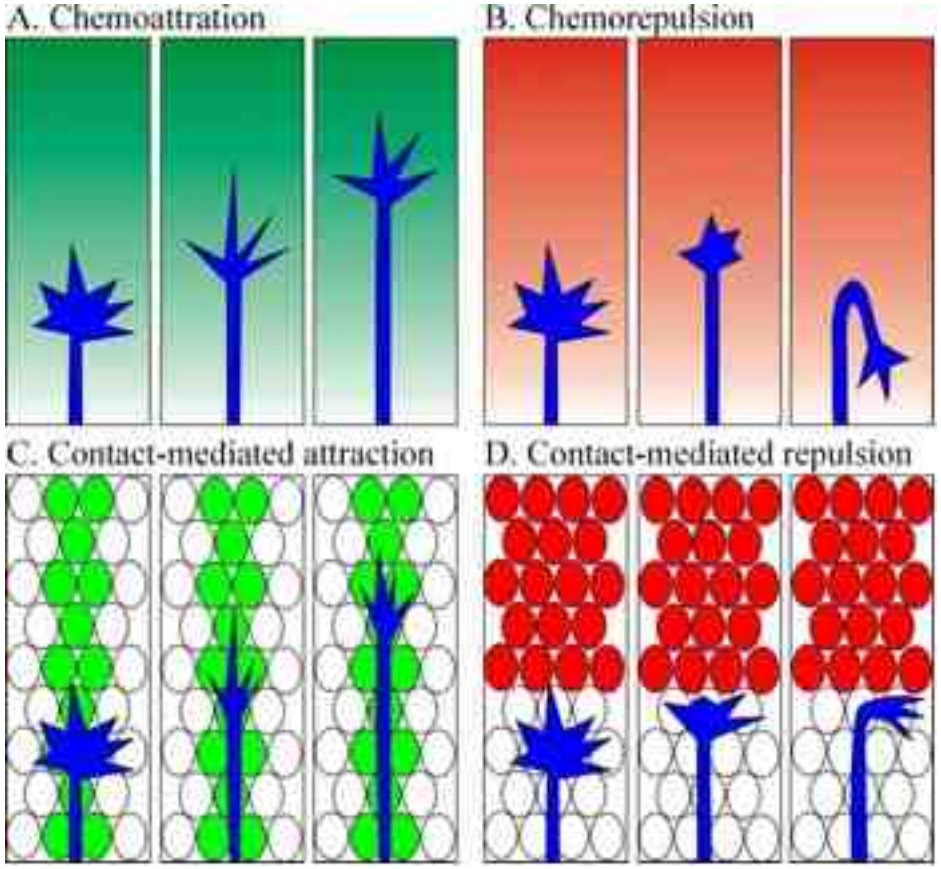
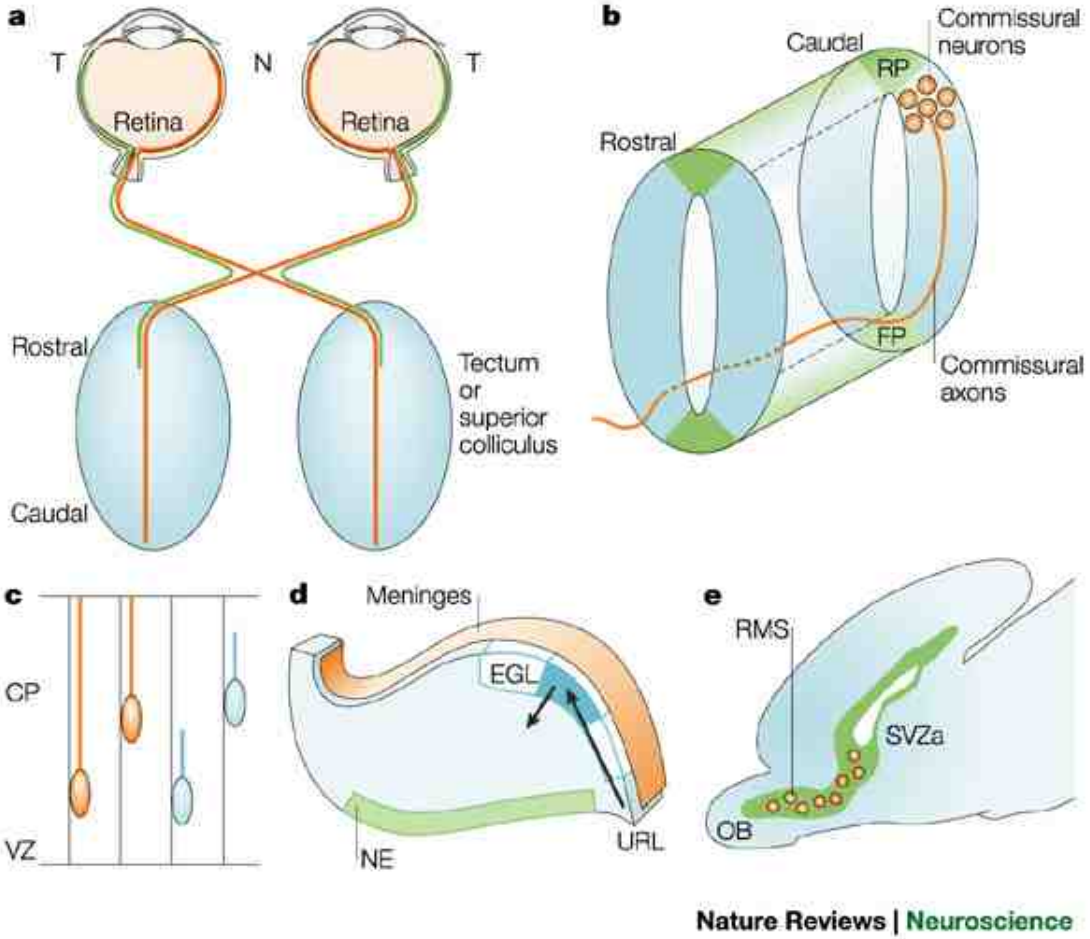


There are several classical models of axon projection and neuronal migration in vertebrates, including the retinotectal projection, the commissural axons of the spinal cord, the radially migrating neuronal precursors in the neocortex, and the tangentially migrating neurons in the rostral migratory stream.

Actin assembly is a key process that controls the growth and steering of axon growth cones (although recent evidence also supports a role for microtubules). The Rho family of small GTPases, which includes Rho, Rac and Cdc42, have important roles in regulating actin cytoskeletal dynamics and have been implicated in growth cone guidance.



Nature Reviews Neuroscience 4, 941-956 (December 2003)

Neuronal migration and axon pathfinding are guided by extracellular cues, including netrins, semaphorins, ephrins and Slits.

Netrins were the first family of directional guidance cues to be found in both invertebrate and vertebrate nervous systems. Netrin-1 and netrin-2 were identified as floor-plate-derived promoters of commissural axon outgrowth. A single netrin can be attractive to some axons and repulsive to others.
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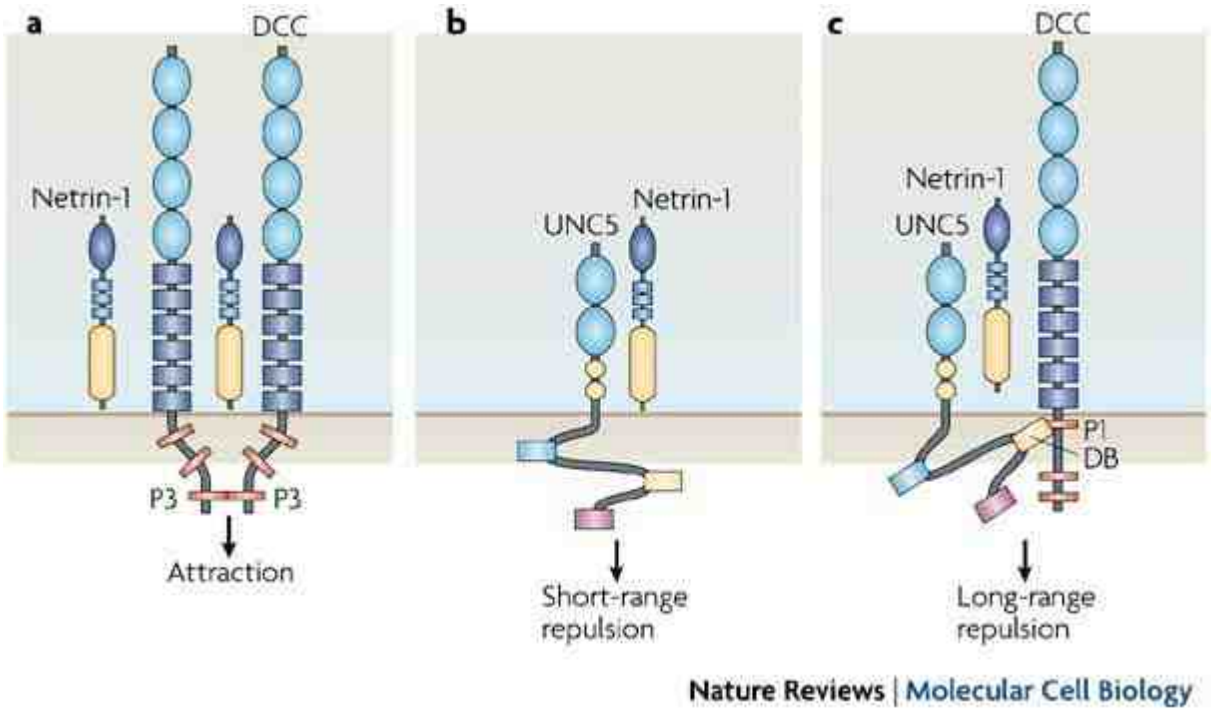
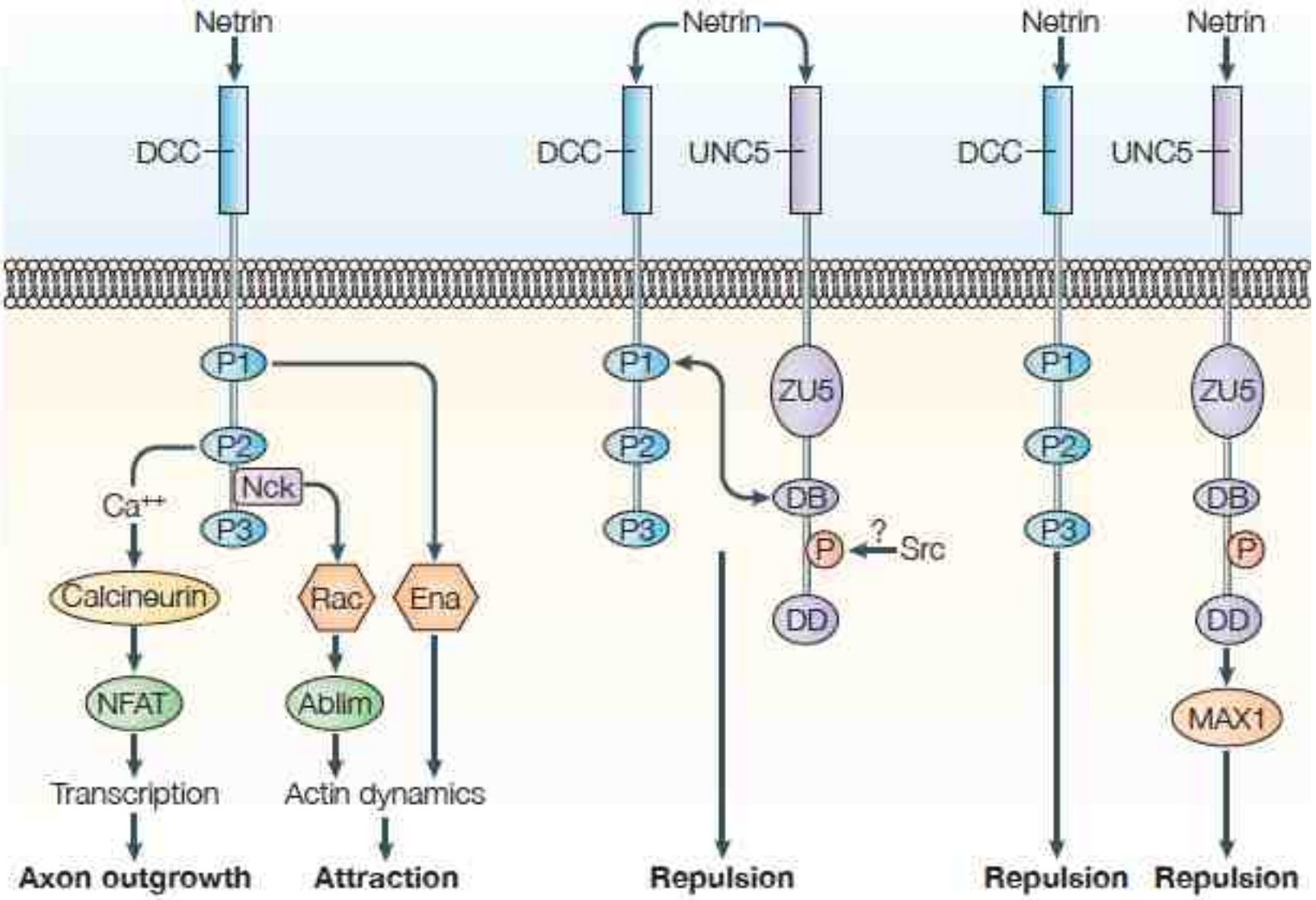
A unique property of netrin-1 in the developing CNS is its functional dichotomy as a guidance cue that can attract some axons while steering others away.



These opposing functions result from separate ligand-dependent mechanisms. Attraction is exclusively mediated by netrin-1 binding to DCC, causing clustering of multiple DCC molecules through their intracellular P3 domains (panel a). By contrast, repulsion might occur through two distinct mechanisms: either through the binding of netrin-1 to UNC5 in instances in which short-range repulsion is desirable (panel b), or through the simultaneous binding of netrin-1 to UNC5 and DCC when long-range repulsion is required. This dual-receptor interaction triggers the conversion of DCC-dependent attraction into repulsion through the formation of a receptor complex that is caused by a ligand-gated association of the cytoplasmic P1 domain of DCC with the DCC-binding (DB) domain of UNC5 (panel c)

Nature Reviews Molecular Cell Biology **8**, 296-306 (April 2007)


Netrin
DCC
P1
P2
P3
Nck
Ca++
Calcineurin
NFAT
Transcription
Axon outgrowth
Rac
Ena
Ablim
Actin dynamics
Attraction
Netrin
DCC
UNC5
P1
P2
P3
ZU5
DB
P
?
Src
DD
Repulsion
Netrin
DCC
P1
P2
P3
Repulsion
Netrin
UNC5
ZU5
DB
P
DD
MAX1
Repulsion

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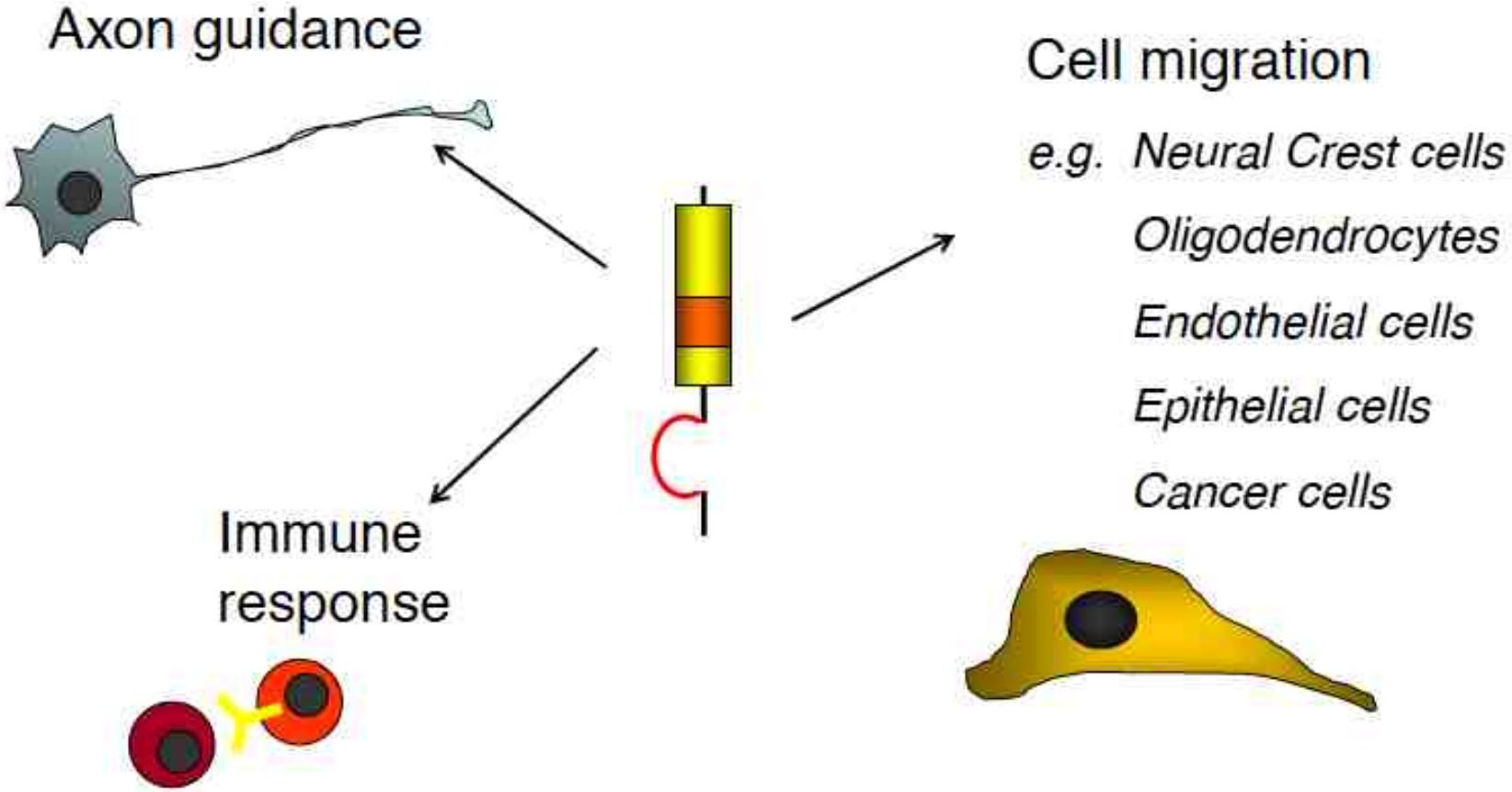
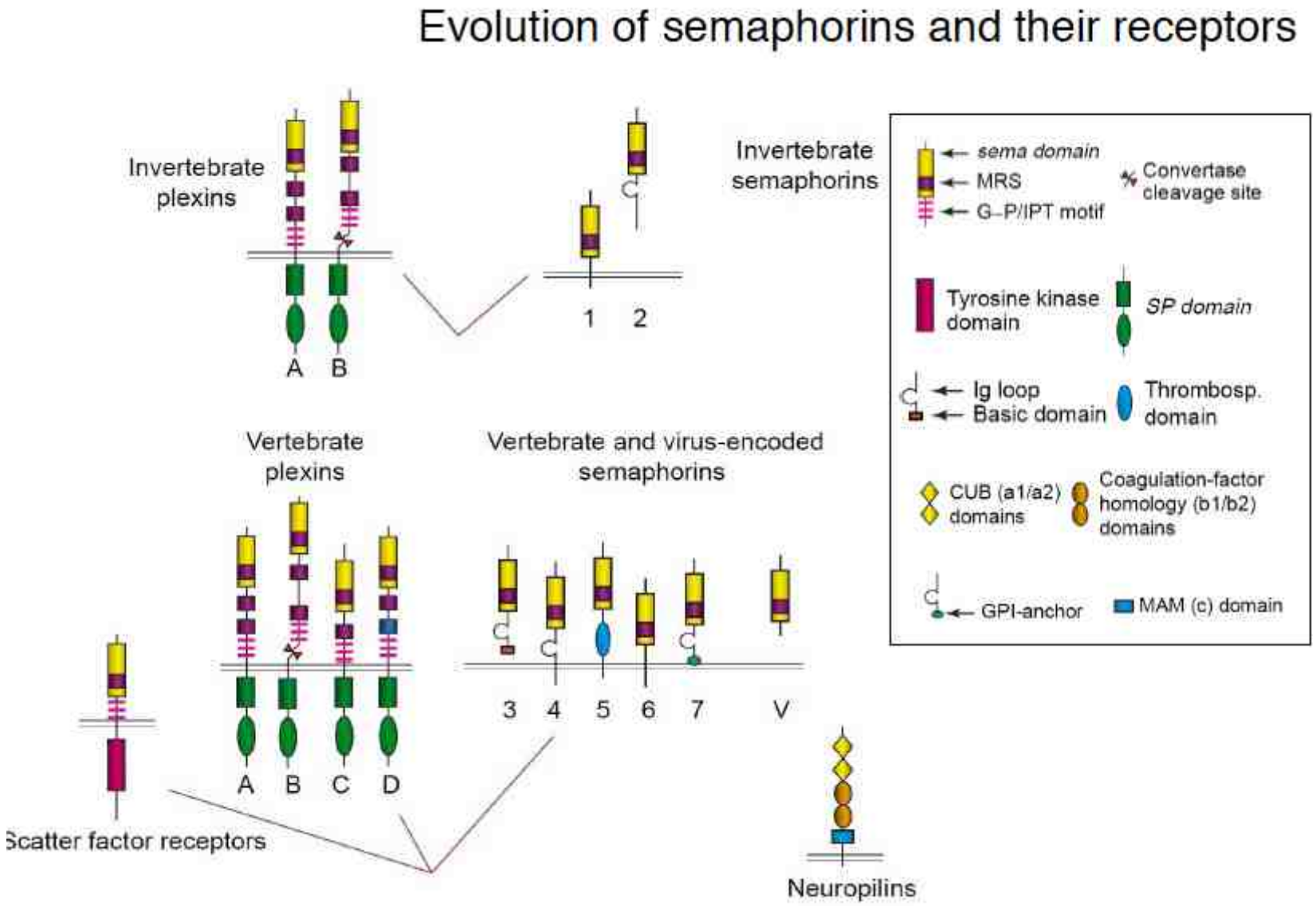
Semaphorins Regulate a Variety of Functions

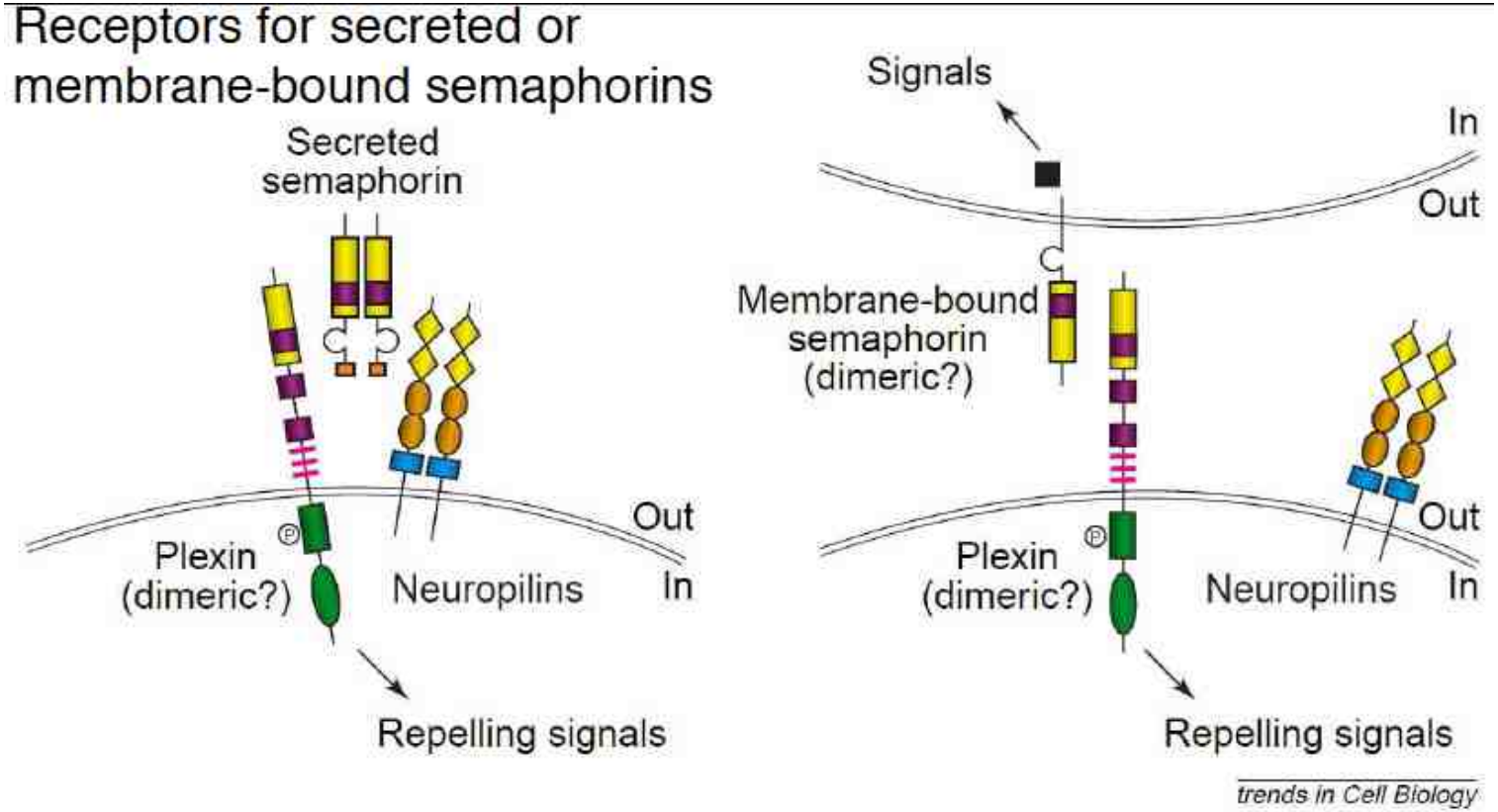
"stop or go"



“Sema Domain”


Evolution of semaphorins and their receptors
Invertebrate plexins
A B
1 2
Invertebrate semaphorins
sema domain
MRS
G–P/IPT motif
Convertase cleavage site
Tyrosine kinase domain
SP domain
Ig loop
Basic domain
Thrombosp. domain
CUB (a1/a2) domains
Coagulation-factor homology (b1/b2) domains
GPI-anchor
MAM (c) domain
Vertebrate plexins
A B C D
Vertebrate and virus-encoded semaphorins
3 4 5 6 7 V
Scatter factor receptors
Neuropilins

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

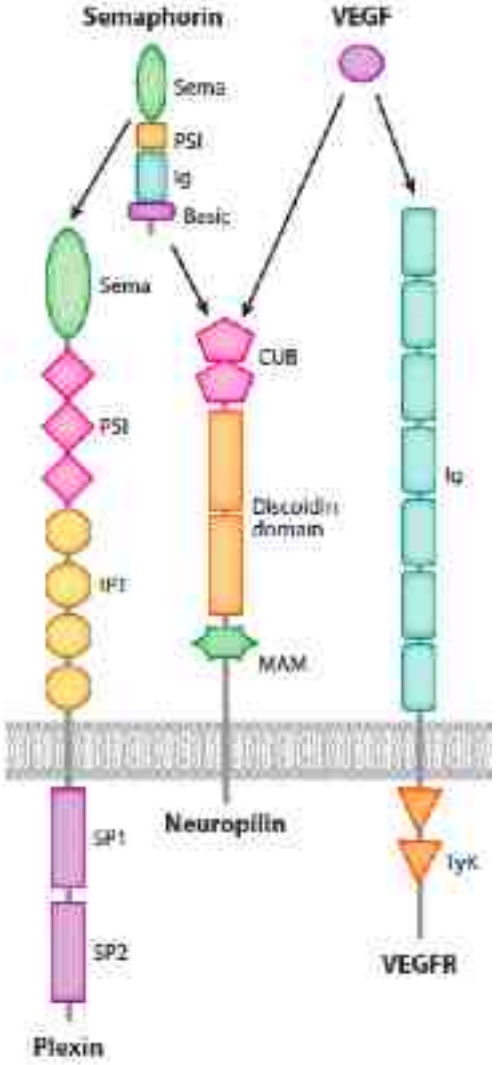


Secreted (subclass 3) semaphorins are dimeric and bind to receptor complexes that include neuropilins (homo or heterodimeric) and plexins (dimeric?).

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.

The semaphorins: versatile regulators of tumour progression and tumour angiogenesis

Gera Neufeld and Ofra Kessler



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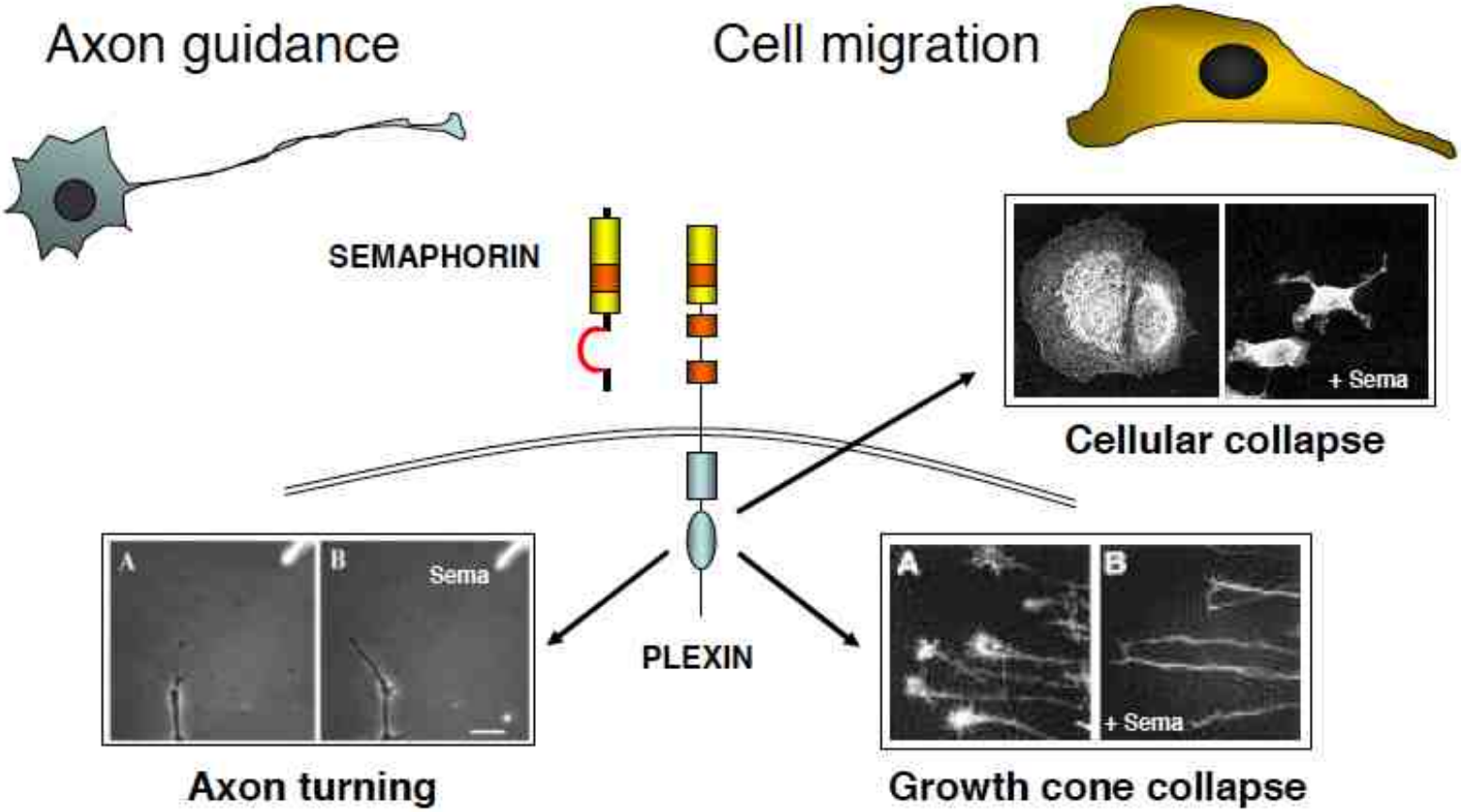
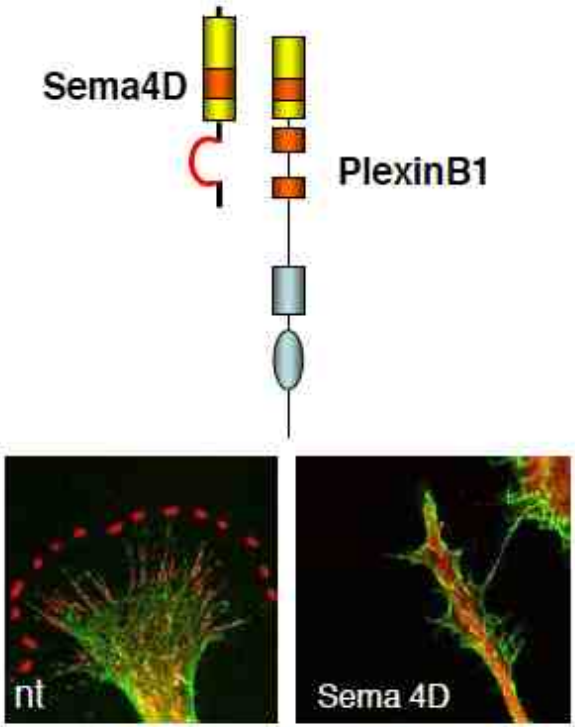
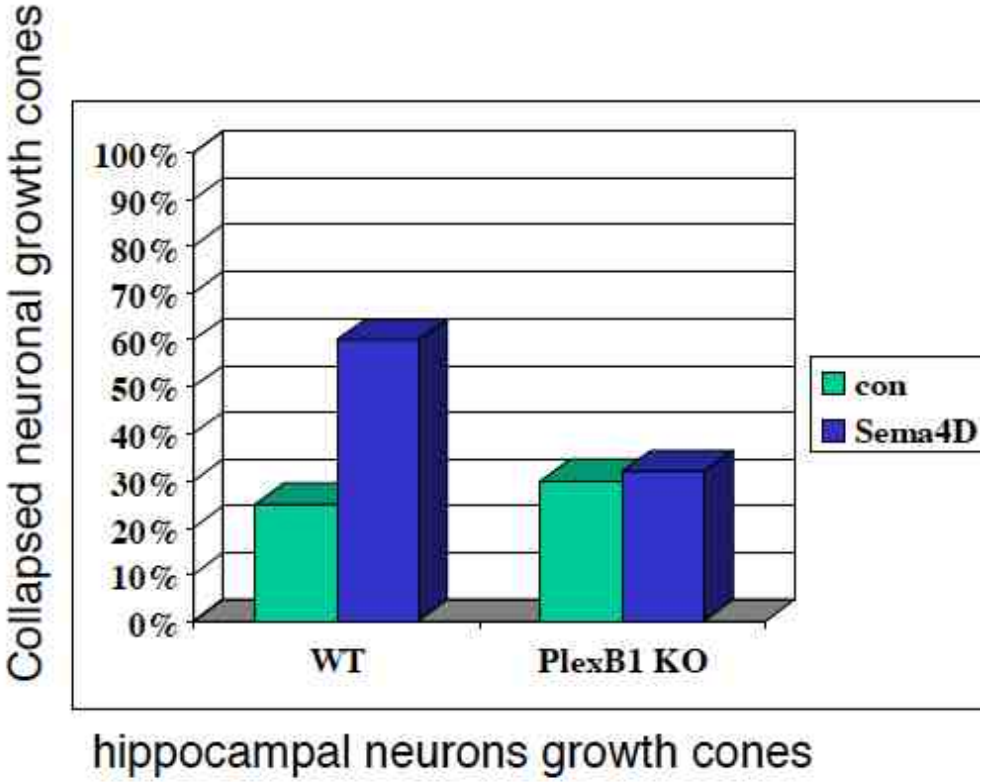


Sema4D-induced collapse of neuronal growth cones is abrogated in PlexinB1-deficient neurons

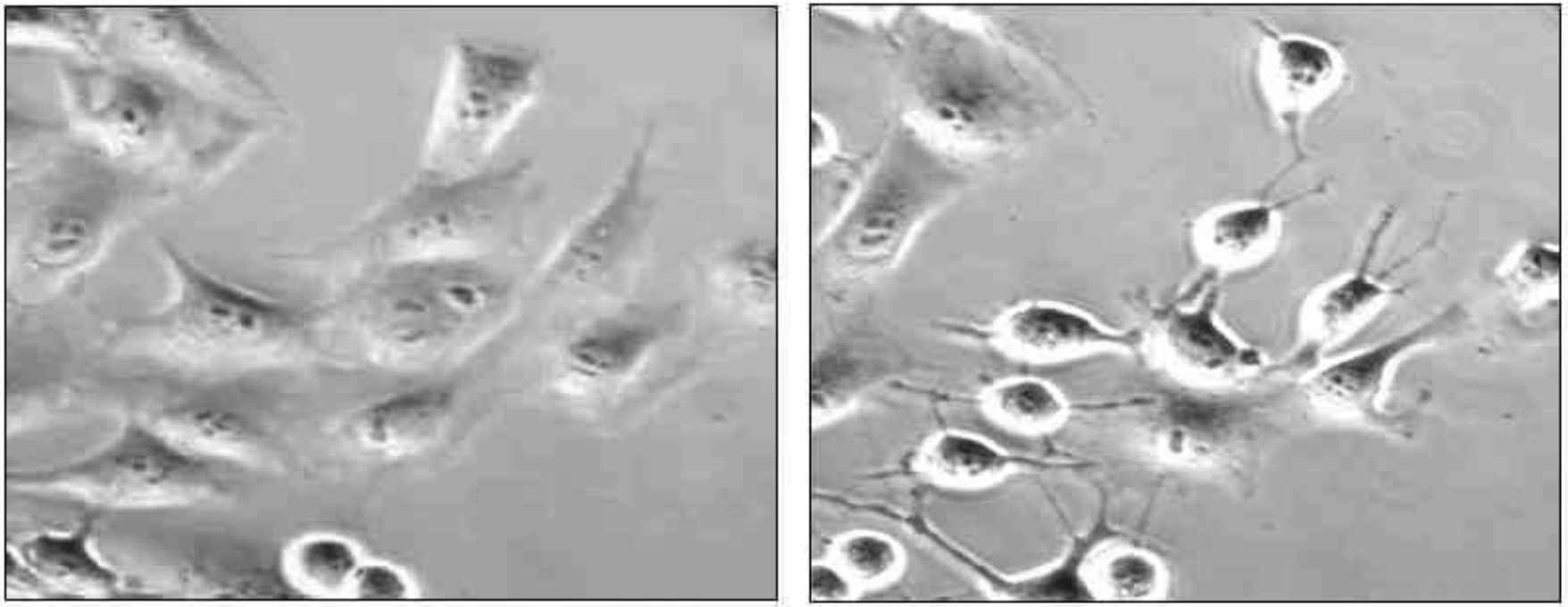




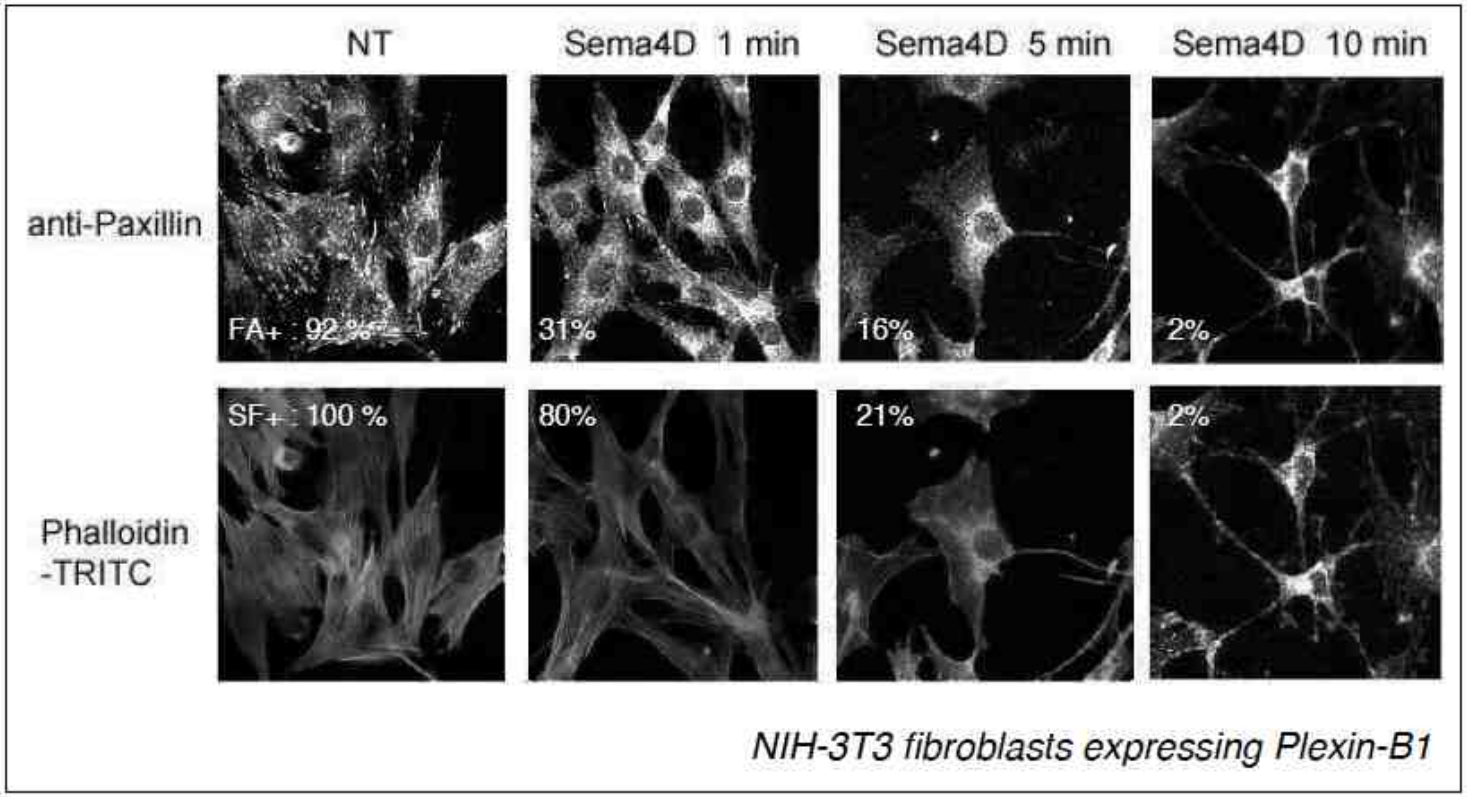
Cellular collapse upon Plexin-B1 activation

control

+ Sema4D

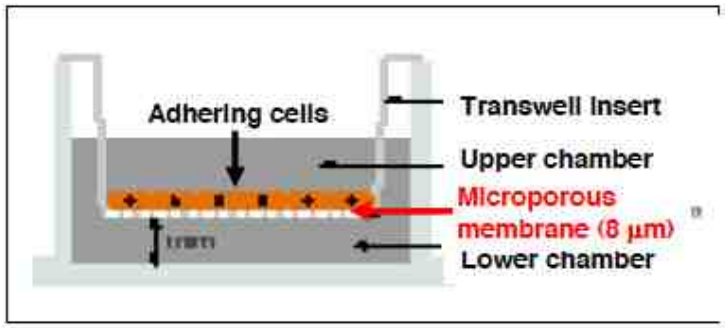


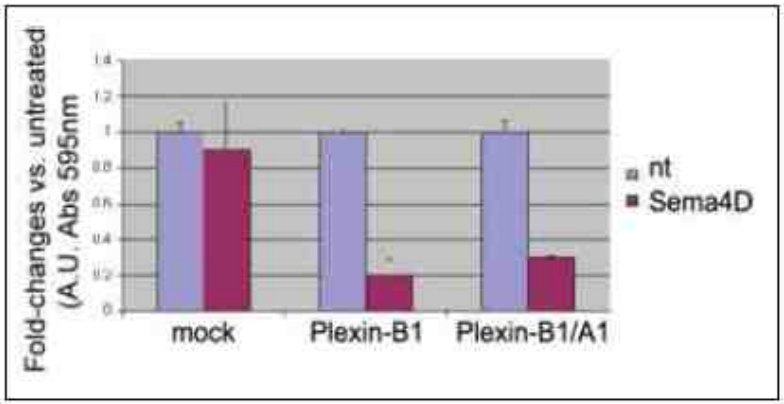
Plexin activation elicits the disassembly of focal adhesions



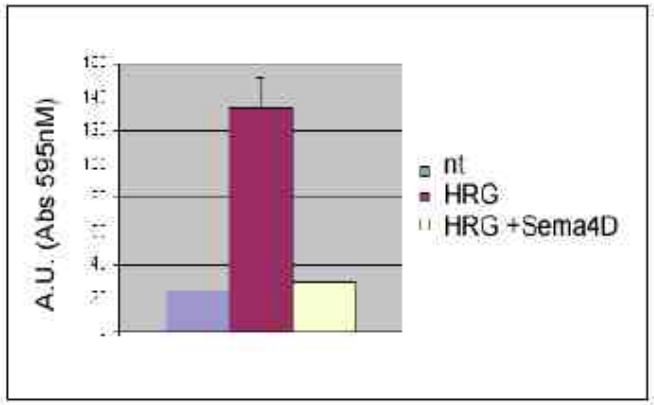
Barberis et al., 2004

Plexin signalling inhibits cell migration





NIH-3T3 fibroblasts



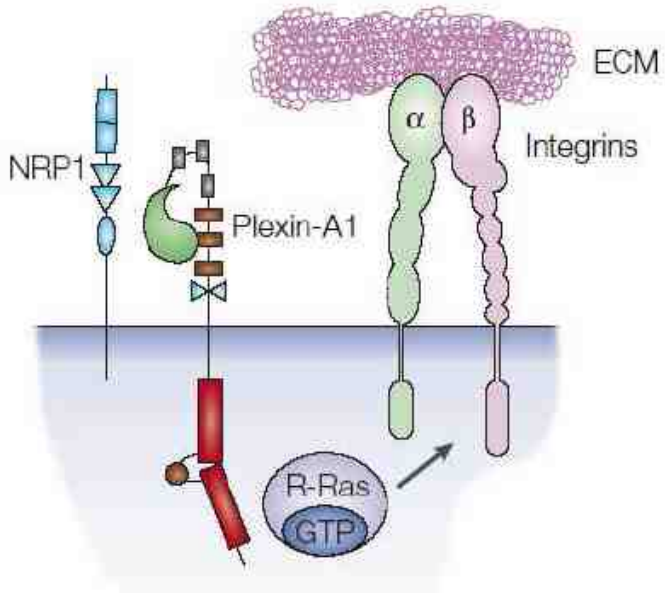
SKBR3 epithelial cells

Plexins have GAP activity towards R-Ras

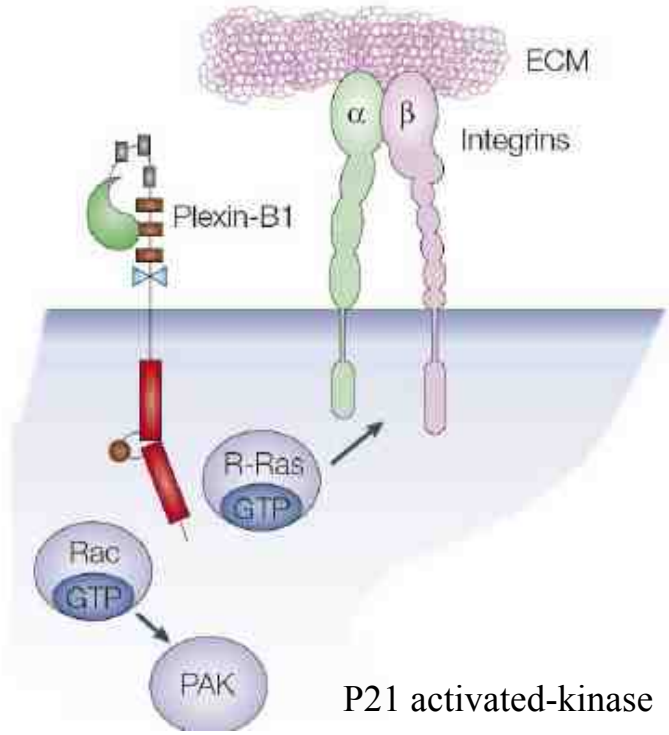
- the intracellular domain of plexins has two highly conserved regions (C1 and C2) that are similar to a GAP (GTPase-activating protein) domain divided in two by a linker region
- the conserved regions contain two arginine residues that are necessary for catalytic activity in GAPs. They are essential 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

Without ligand, R-Ras is active, resulting in integrin-mediated attachment to extra-cellular matrix

Plexin-A1



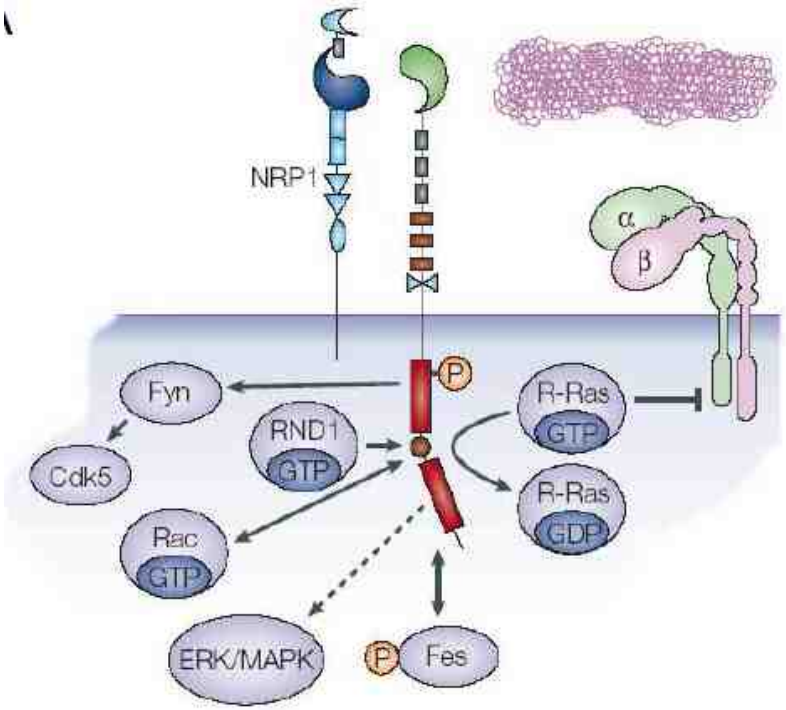
Plexin-B1



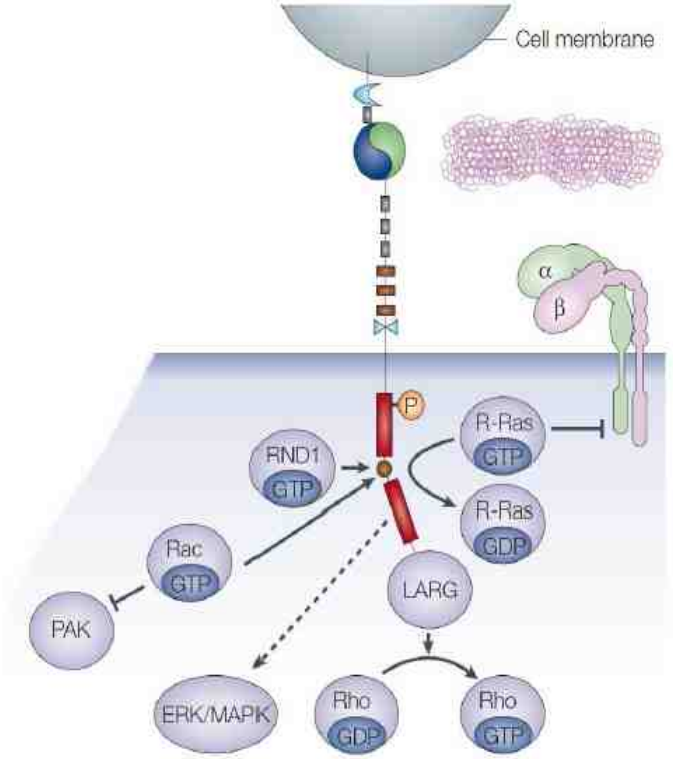
P21 activated-kinase

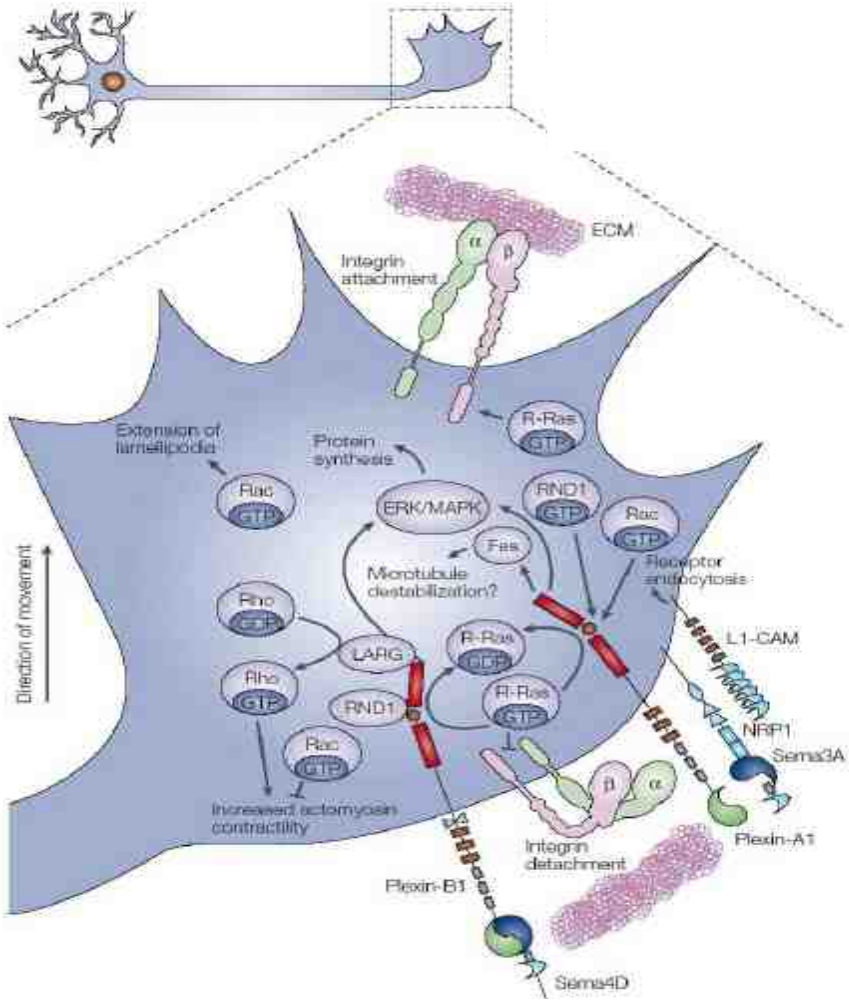
+ ligand = collapsed adhesion

Plexin-A1 + Sema3A



Plexin-B1 + Sema4D





Semaphorins repel axonal growth cones

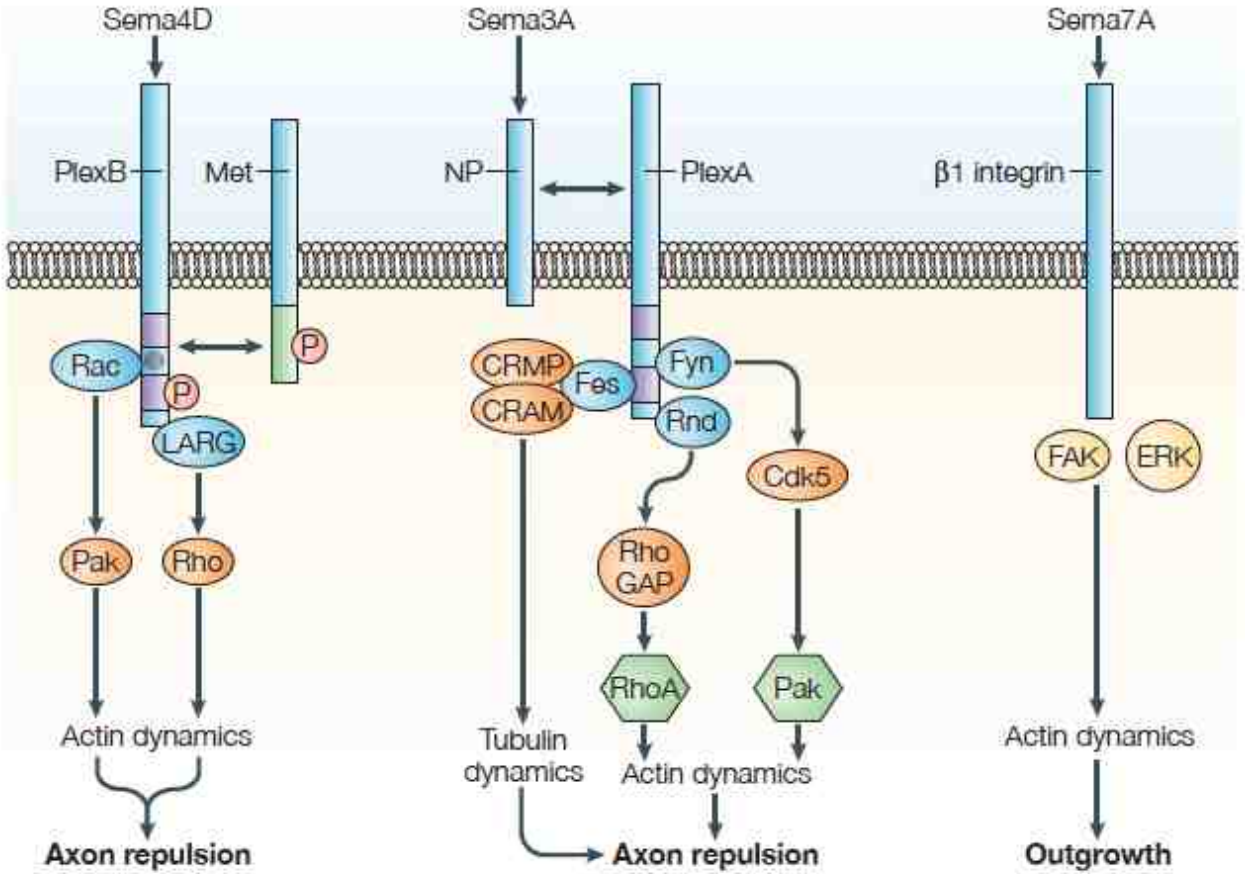


Figure 4 | **Intracellular signalling pathways of semaphorin receptors.** Semaphorins seem to use a variety of receptors for their signalling. CRAM, a collapsin (Sema3A) response mediator protein (CRMP) associated protein; Cdk5, cyclin-dependent kinase 5; ERK, extracellular signal regulated kinase; FAK, focal adhesion kinase; GAP, GTPase-activating protein; LARG, leukaemia-associated Rho-GEF; NP, neuropilin; Pak, p21-activated kinase; Plex, plexin.

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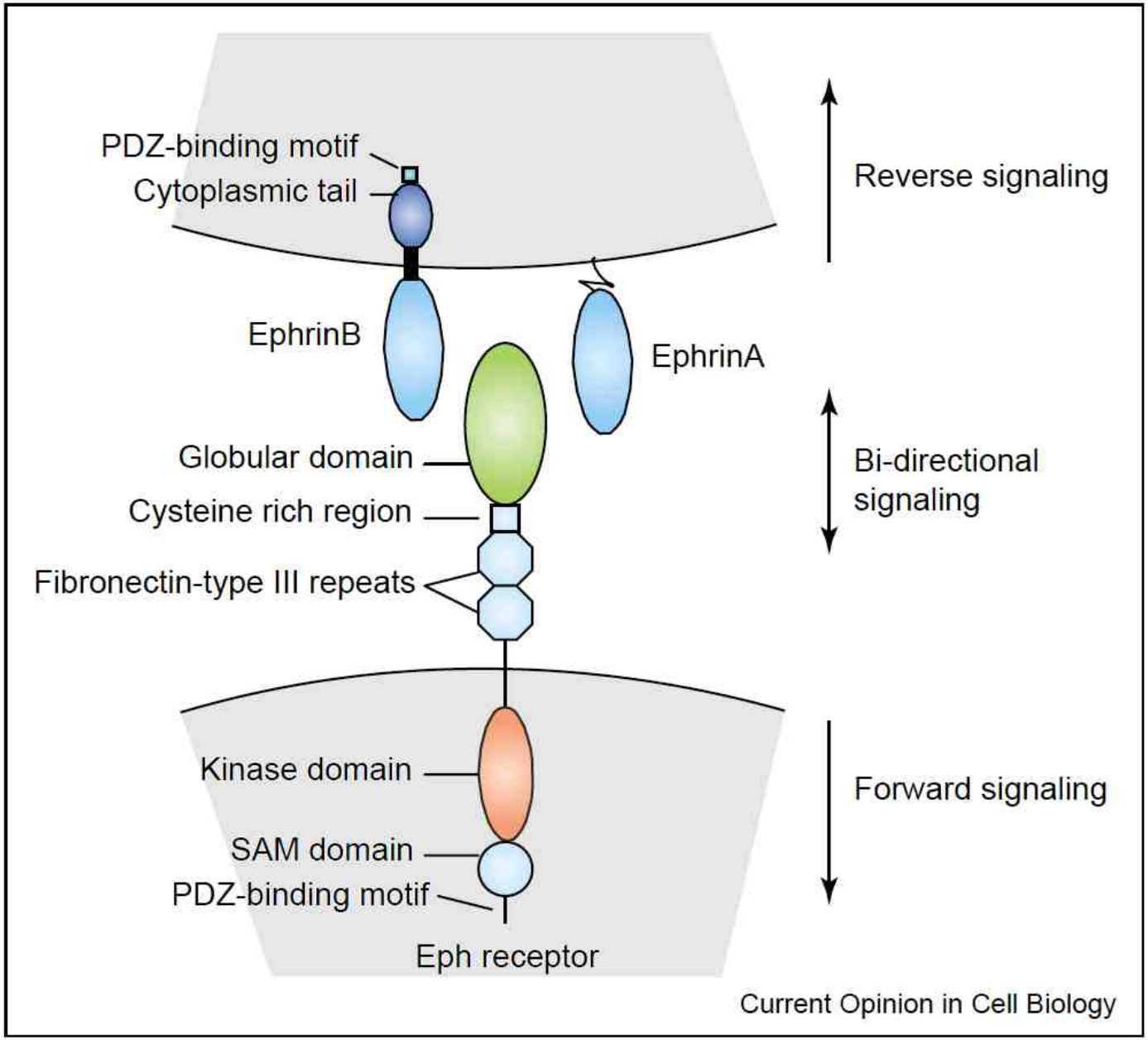
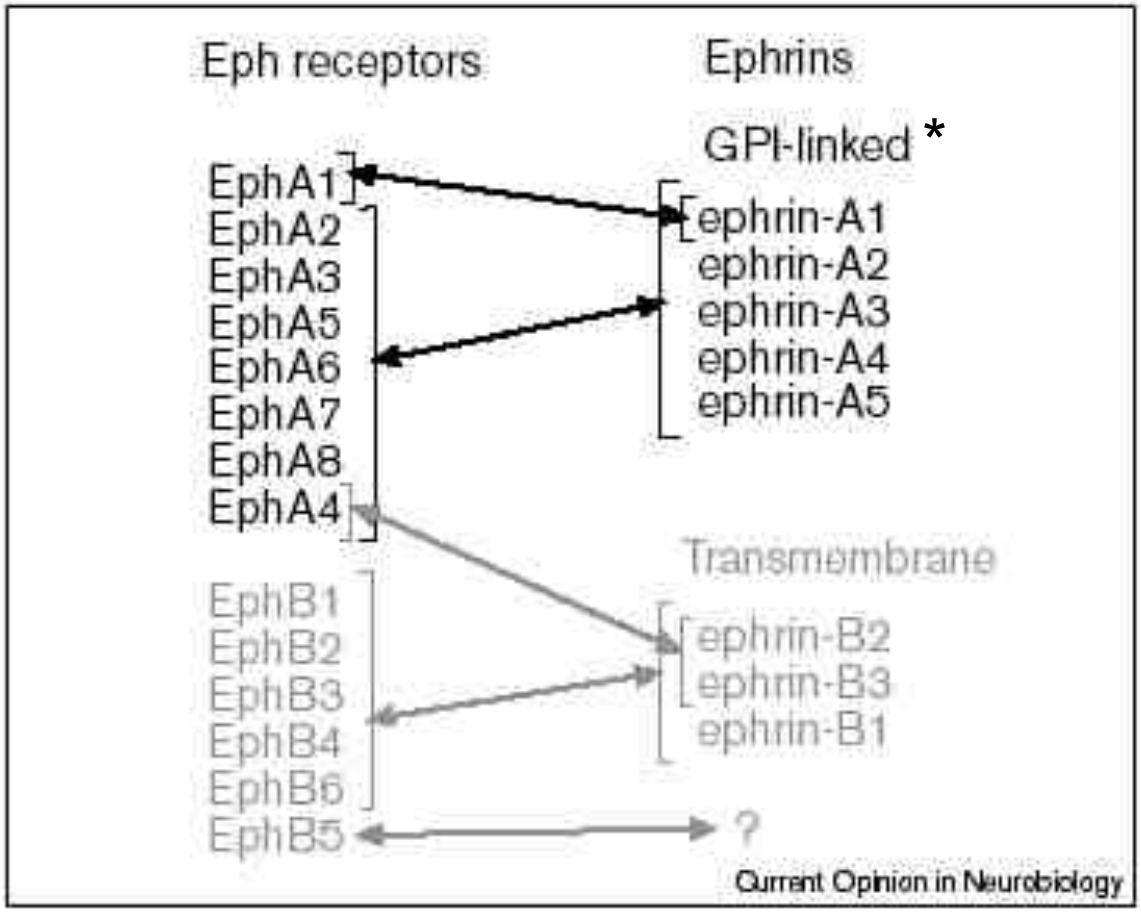

PDZ-binding motif
Cytoplasmic tail
EphrinB
EphrinA
Globular domain
Cysteine rich region
Fibronectin-type III repeats
Kinase domain
SAM domain
PDZ-binding motif
Eph receptor
Reverse signaling
Bi-directional signaling
Forward signaling
Current Opinion in Cell Biology

Eph receptors and ephrin ligands

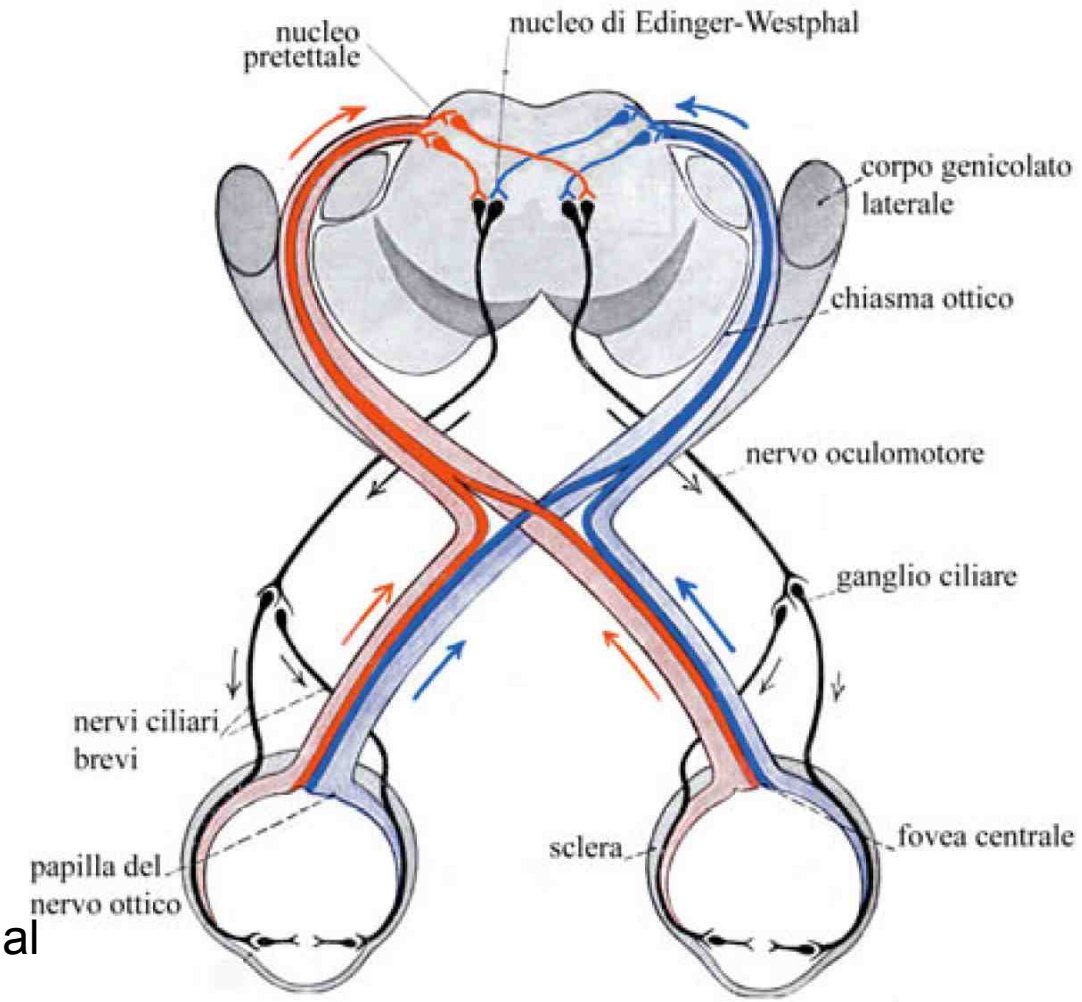


Binding specificities of Eph receptors and ephrins. Eph receptors and ephrins fall largely into two binding specificity classes, with the exception of EphA4, which interacts with ephrin-A and some ephrin-B proteins. Differences exist, however, in the relative affinity of a receptor for different ephrins that may be functionally important. Additional ephrins probably exist, because EphB5 does not bind to any known ephrin.

*glycosylphosphatidyl-inositol membrane anchored

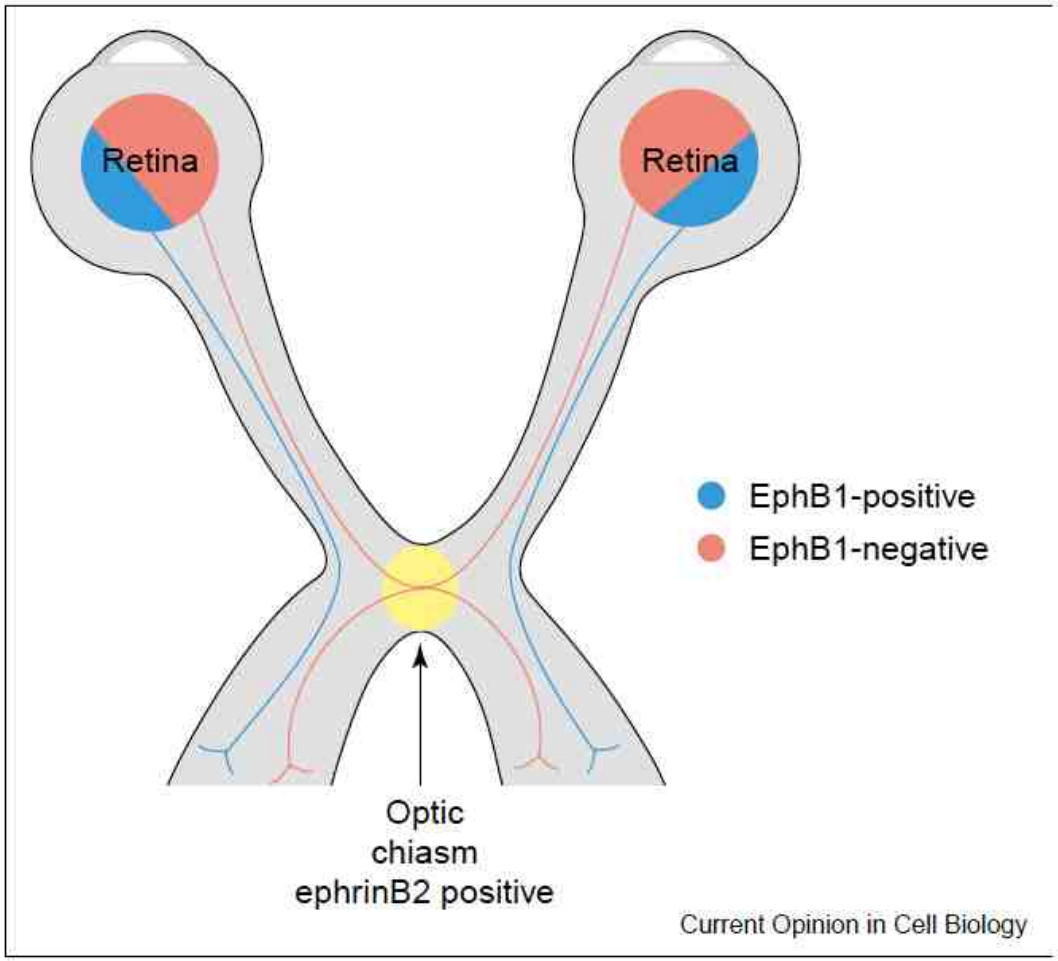
• nel chiasma ottico c'è espressione di ephrin B2
• le fibre che decussano sono EphB1 negative,
• le fibre che non decussano sono EphB1 positive

• le fibre provenienti dalla retina laterale (temporale) proseguono al genicolato dello stesso lato



• le fibre provenienti dalla retina mediale (nasale) si incrociano e si dirigono al genicolato controlaterale

MIDLINE GUIDANCE IN THE VISUAL SYSTEM.



19_bcma_2011

In animals with binocular vision, most retinal axons (red) cross to the contralateral side of the brain, while a smaller subset of retinal axons (blue) project to the ipsilateral side. Retinal axons expressing EphB1 are repelled from the optic chiasm by ephrinB2 and directed to an ipsilateral pathway. Contralaterally projecting axons do not express EphB receptors and therefore are not repelled by ephrinB2.

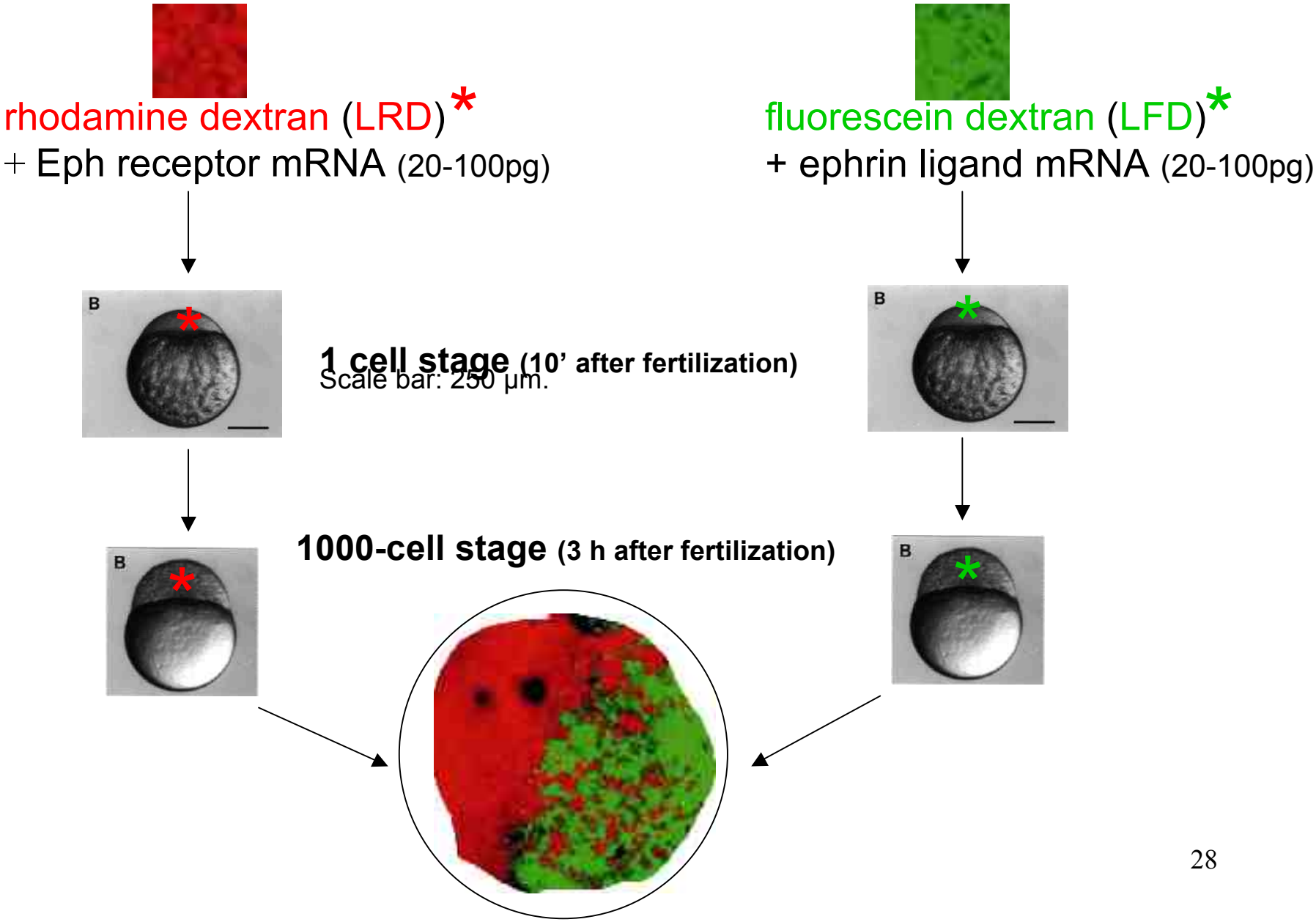
Eph receptors and ephrins restrict cell intermingling and communication

Georg Mellitzer, Qiling Xu & David G. Wilkinson

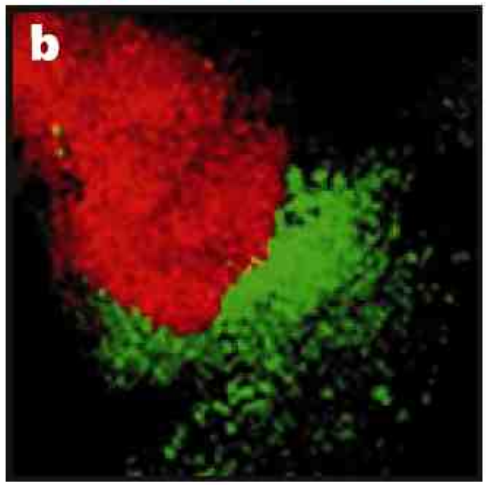
NATURE | VOL 400 | 1 JULY 1999 | www.nature.com

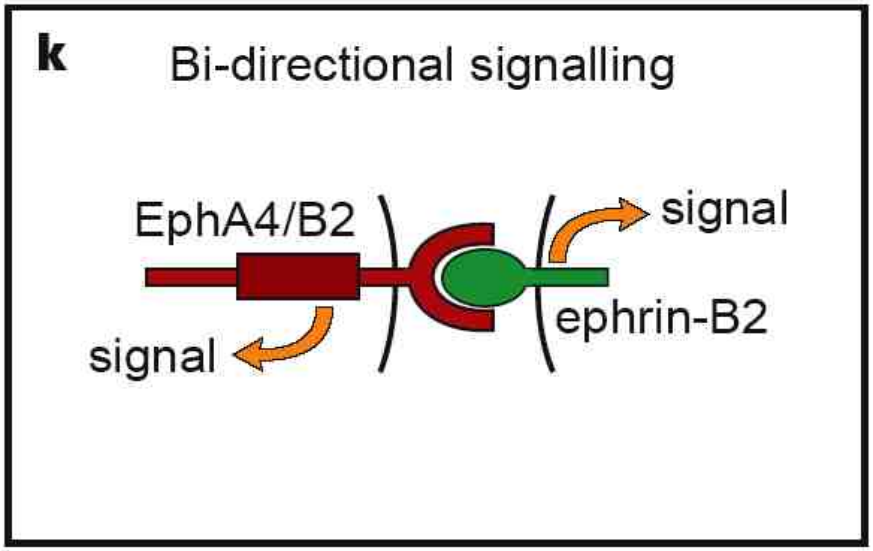
- assay in which an Eph receptor and an ephrin are expressed in adjacent cell populations and the amount of cell intermingling determined
- Zebrafish embryos at the one-cell stage are injected with fluorescent lineage tracer and then animal caps are dissected at the 1,000-cell stage
- animal caps that were labelled with rhodamine dextran (LRD) or fluorescein dextran (LFD) were juxtaposed and co-injected with RNA encoding Eph receptor or ephrin-B, respectively
- after overnight culture, serial confocal sections of the fluorescent tracers were visualized

The zebrafish (*Brachydanio rerio*) is a small aquarium fish used as a model system for vertebrate developmental biology.

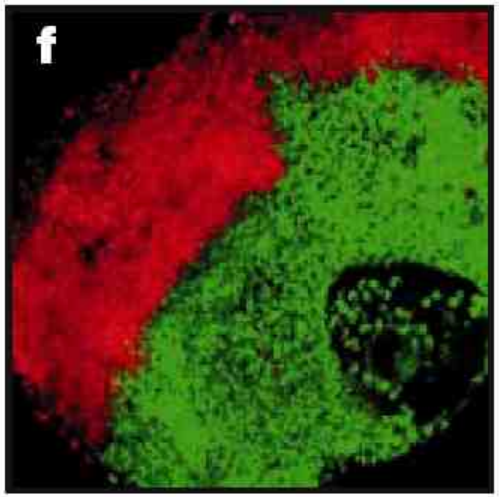


EphA4 /ephrin-B2

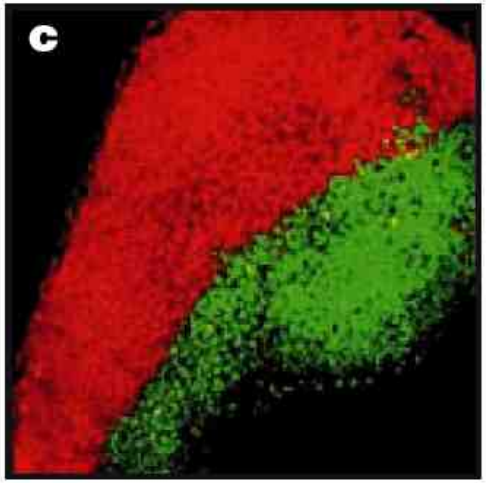


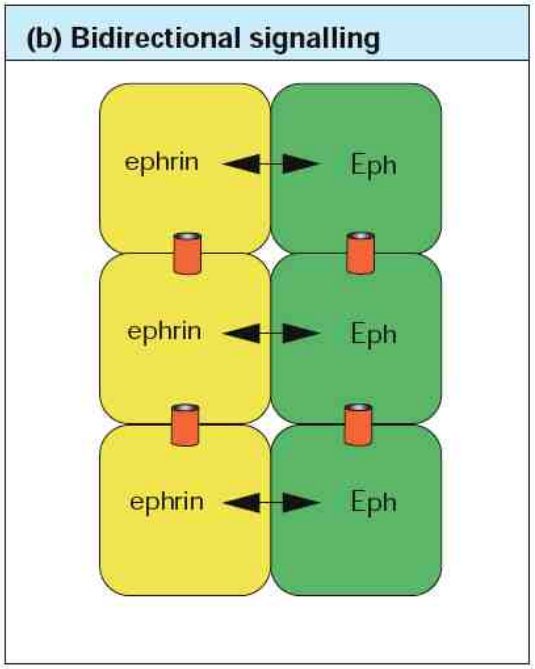


EphB2 /ephrin-B1

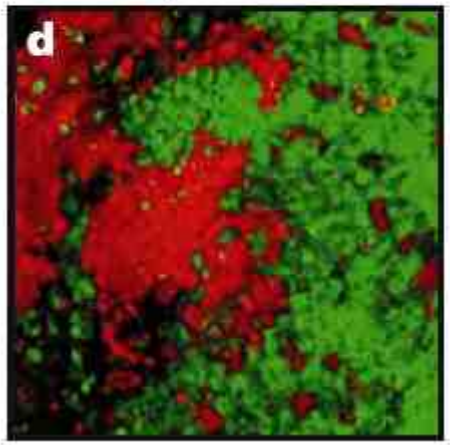


EphB2 /ephrin-B2

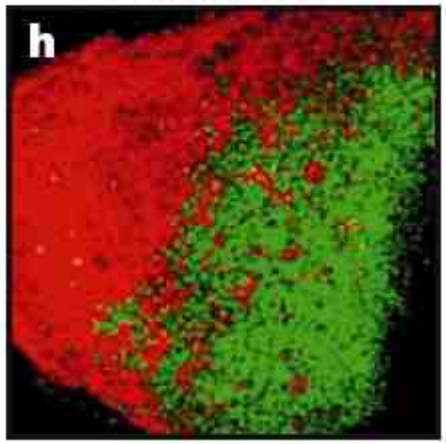


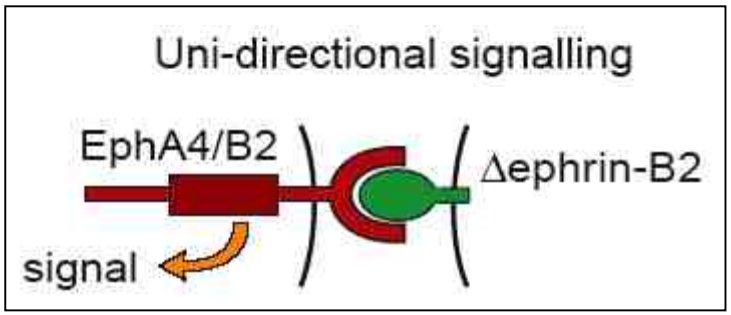


EphB2+EphA4 /
ΔephrinB2

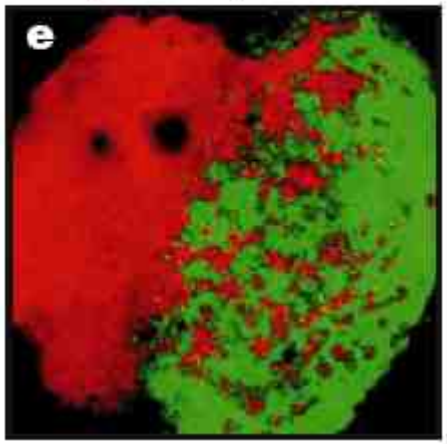


EphA4 /ephrinB1+
ΔephrinB2

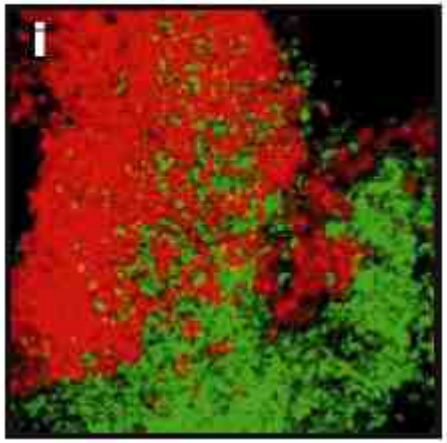


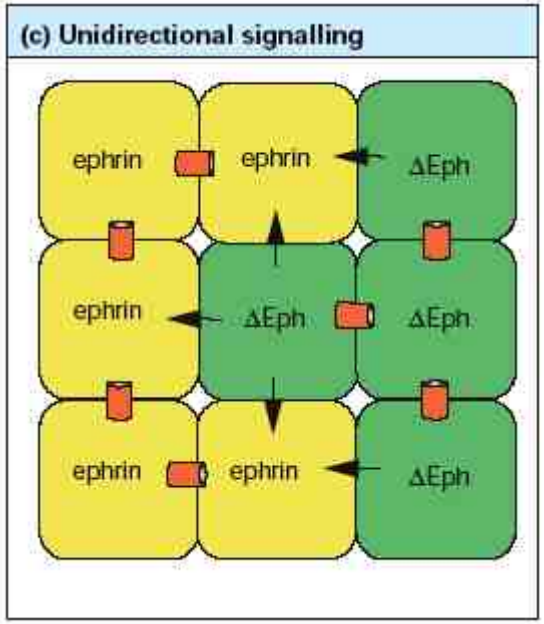


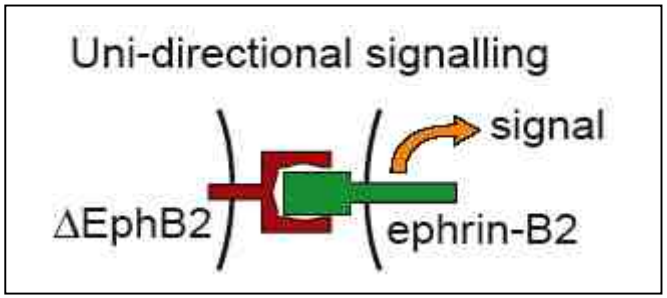
ΔEphB2 /ephrinB2



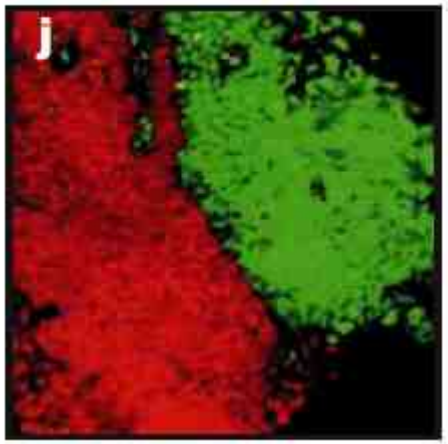
EphA4+ ΔEphB2
/ephrinB1

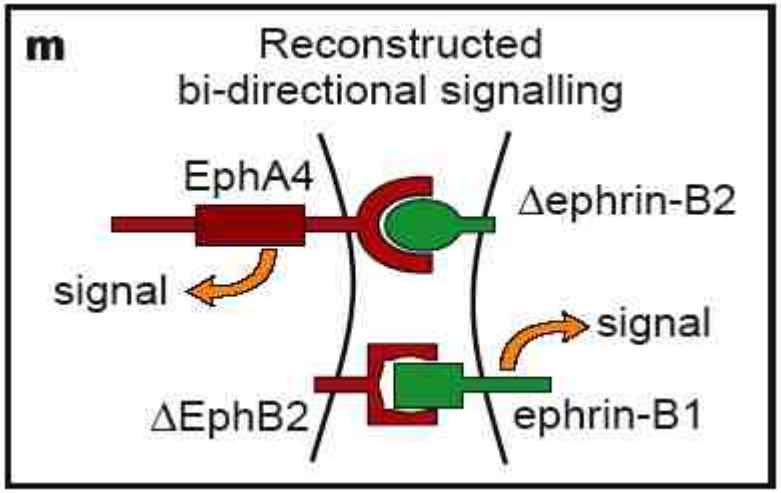


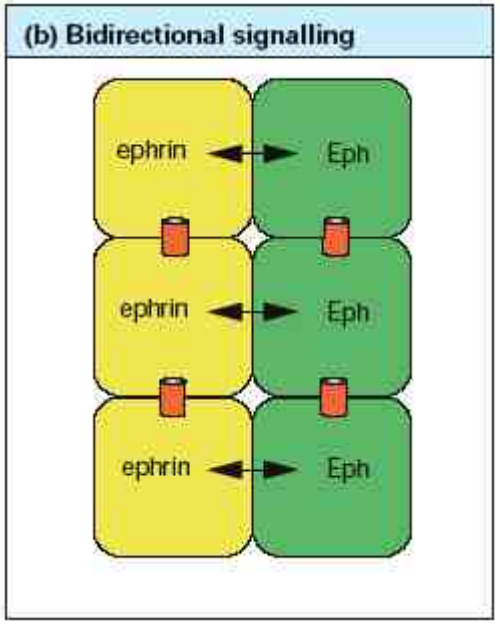




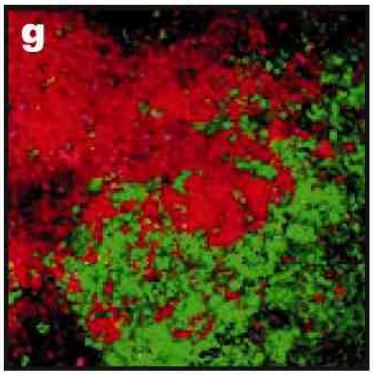
EphA4+ ΔEphB2 /
ephrin-B1+ Δephrin-B2



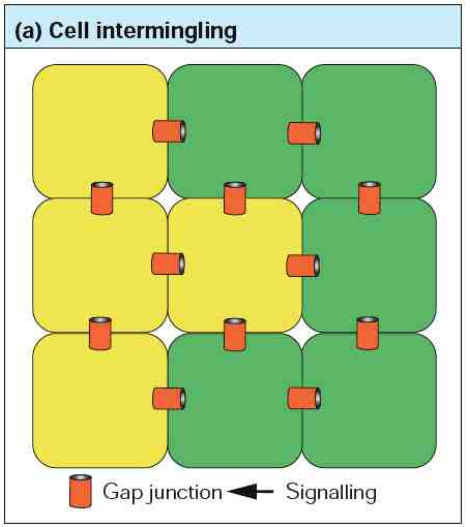




EphA4 /ephrin-B1



Control: EphA4 non
interagisce con Ephrin B1



• many axon guidance molecules, including ephrins, netrins, semaphorins and slits, elicit attractive as well as repulsive responses when bound to their receptors

• some of these factors are diffusible and growth cones respond to concentration gradients, whereas others, including the ephrins, are membrane-bound and repulsion happens after cell–cell contact

• interactions between repellent guidance cues and their receptors are high affinity, contrasting with the rapid process of contact-mediated repulsion

• this results in a paradox: although the formation of a complex between ligand and receptor is an adhesive event, it results in detachment and retraction of cells and their cellular processes

• therefore, there must be a mechanism in place that overcomes adhesion immediately after cell–cell contact.

→ one mechanism that may remove ligand–receptor complexes from the cell surface is PROTEOLYTIC CLEAVAGE

• EphA receptors bind ephrinA (glycosylphosphatidylinositol membrane anchored)
EphB receptors bind ephrinB (transmembrane domain)

• axon repulsion by GPI-anchored ephrinAs requires proteolytic cleavage of the ephrinA ectodomain by the A-Disintegrin-And-Metalloprotease (ADAM)-10

• the growth cones of neurons that encountered cells expressing the ligand ephrinA2 collapsed and withdrew

• when cleavage of ephrinA2 was prevented by mutations in the ephrinA2 ectodomain growth cones still collapsed, but withdrawal was greatly delayed

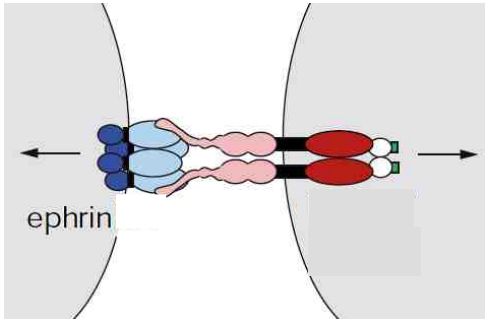
• it was suggested that this mechanism provides a means for efficient axon detachment and termination of signalling

→ ENDOCYTOSIS may provide an alternative mechanism for the removal of ligand–receptor complexes from the surface

Attraction or Repulsion? Ligand or Receptor?

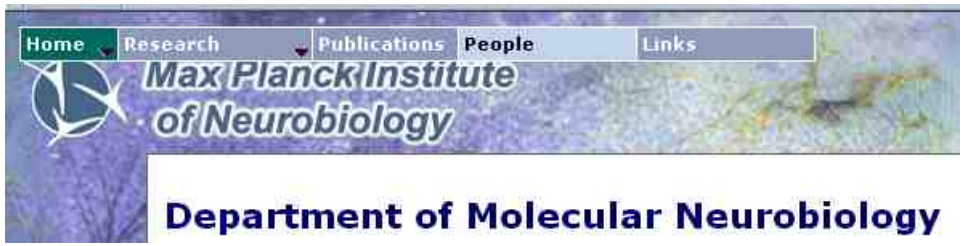
Repulsion by ephrin A ligands requires CLEAVAGE:

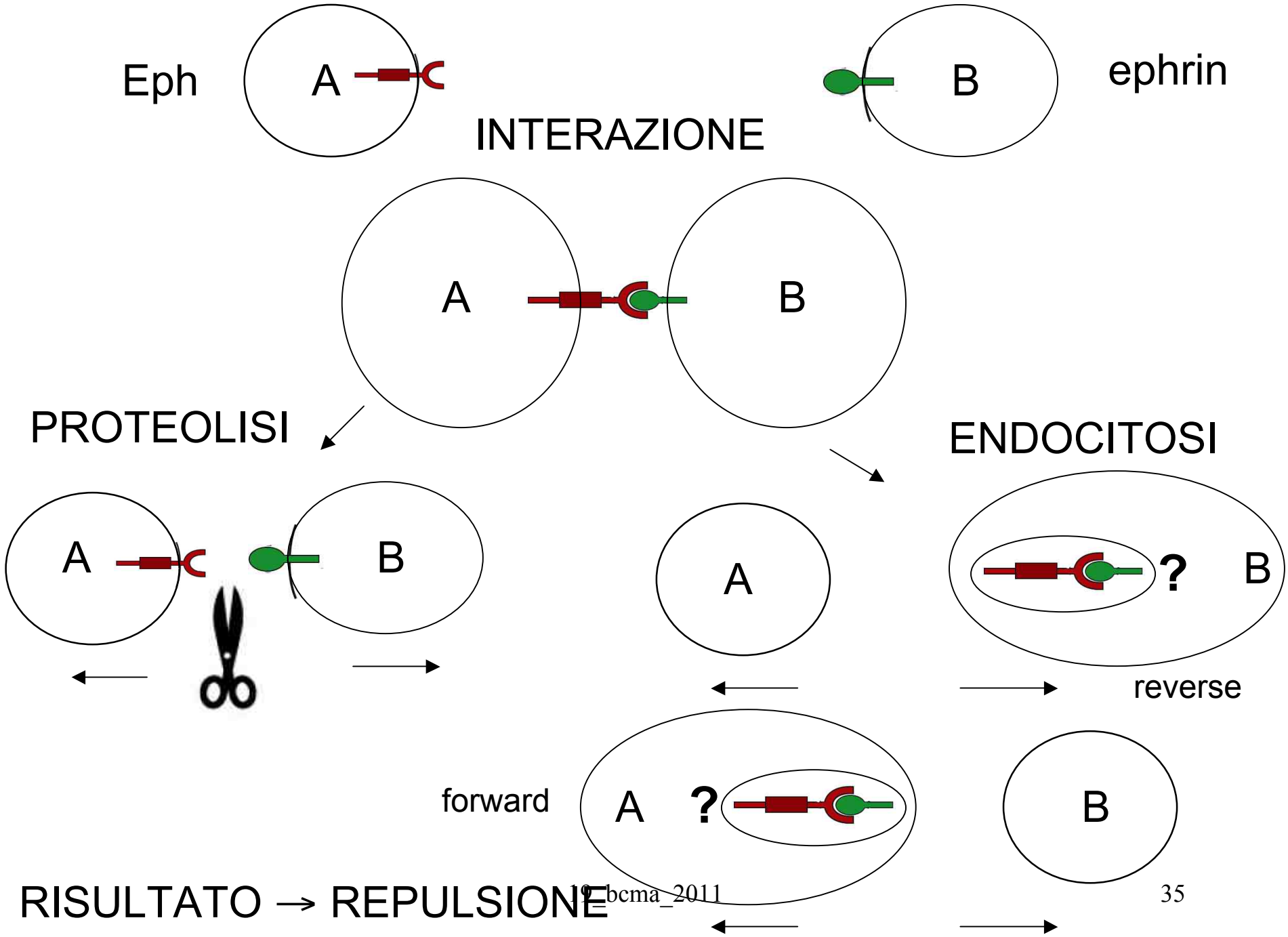
- growth cone contact
- ectodomain shedding
- collapse and withdrawal



Repulsion by ephrin B ligands requires TRANS-ENDOCYTOSIS of ephrinB/EphB complexes

- growth cone contact
- trans-endocytosis
- collapse and withdrawal




Eph
A
B
ephrin
INTERAZIONE
A
B
PROTEOLISI
ENDOCITOSI
A
B
A
?
B
reverse
forward
A
?
B
RISULTATO → REPULSIONE

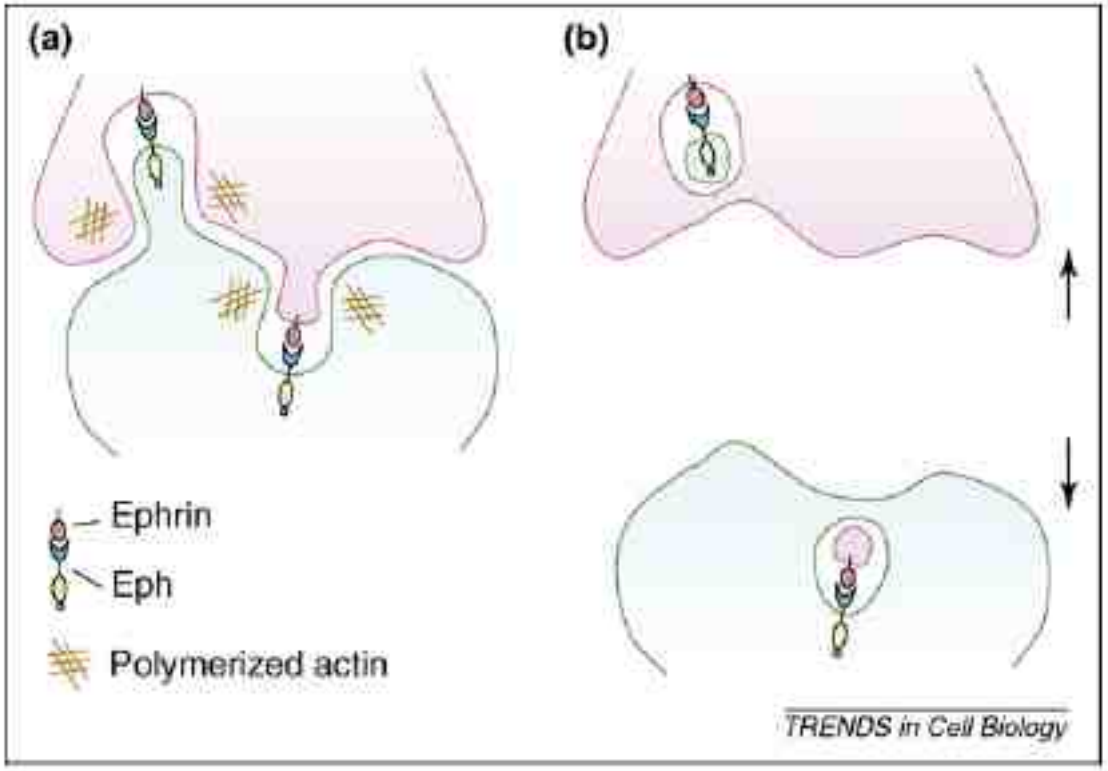


Figure 3. Bidirectional *trans*-endocytosis of ephrinB–EphB complexes. **(a)** The encounter of cells expressing ephrinB with cells expressing EphB receptors can result in bidirectional endocytosis of the ligand–receptor complexes. **(b)** This mechanism, which involves *trans*-internalization of full-length proteins, can turn cell attraction into cell repulsion. EphrinB endocytosis into the EphB-expressing cell requires actin polymerization.

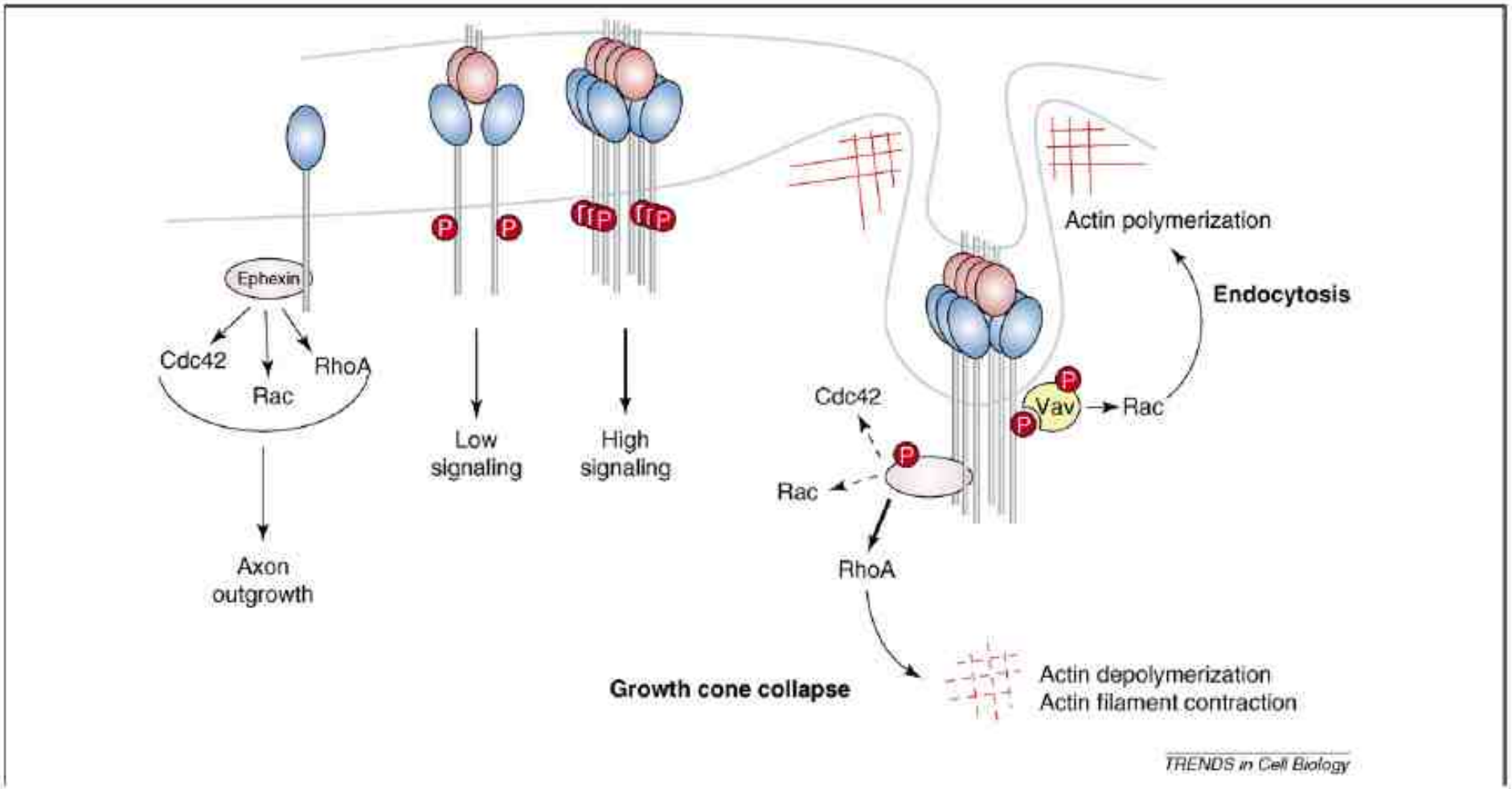


Figure 5. A model for how Rho-GEFs signal downstream Ephs to mediate growth cone collapse and endocytosis. Unclustered Ephs (blue) recruit ephexin to the plasma membrane, where ephexin activates the Rho GTPases Cdc42, Rac and RhoA. This scenario favors axon outgrowth. Ephrin-induced Eph higher-order clustering and activation lead to tyrosine phosphorylation of ephexin (possibly through Src) and a switch to Rho activation. This is necessary for actin disassembly and/or contraction and favors growth cone collapse. Vav proteins are recruited to activated Ephs, become activated and promote Rac-dependent actin polymerization and endocytosis.

Common Factors Regulating Patterning of the Nervous and Vascular Systems*

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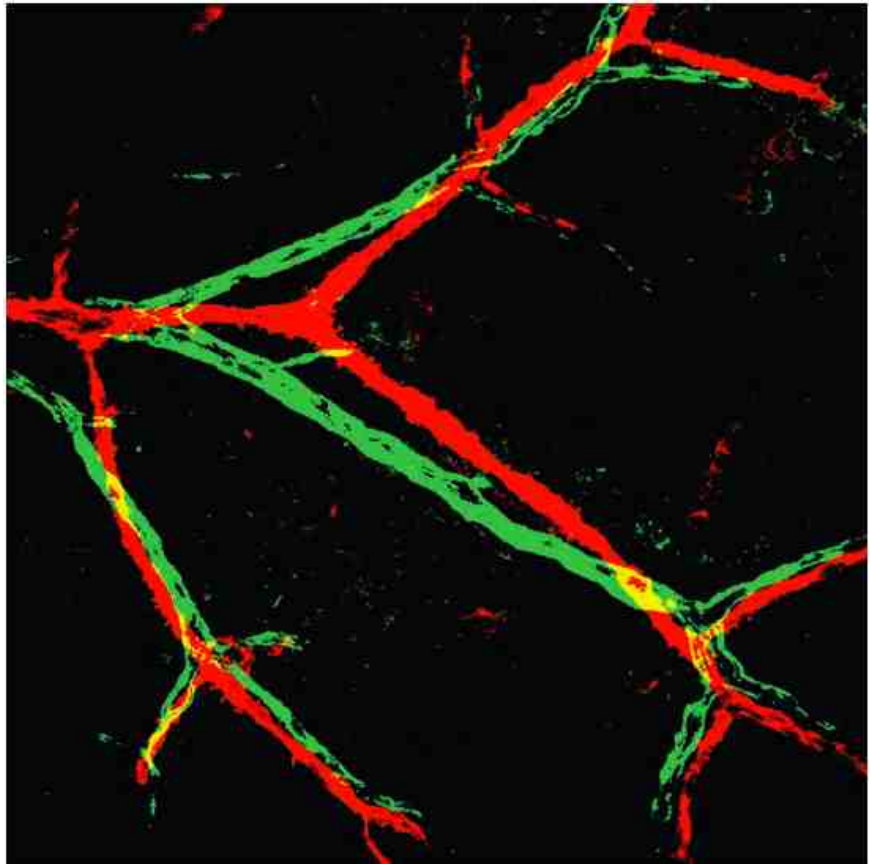
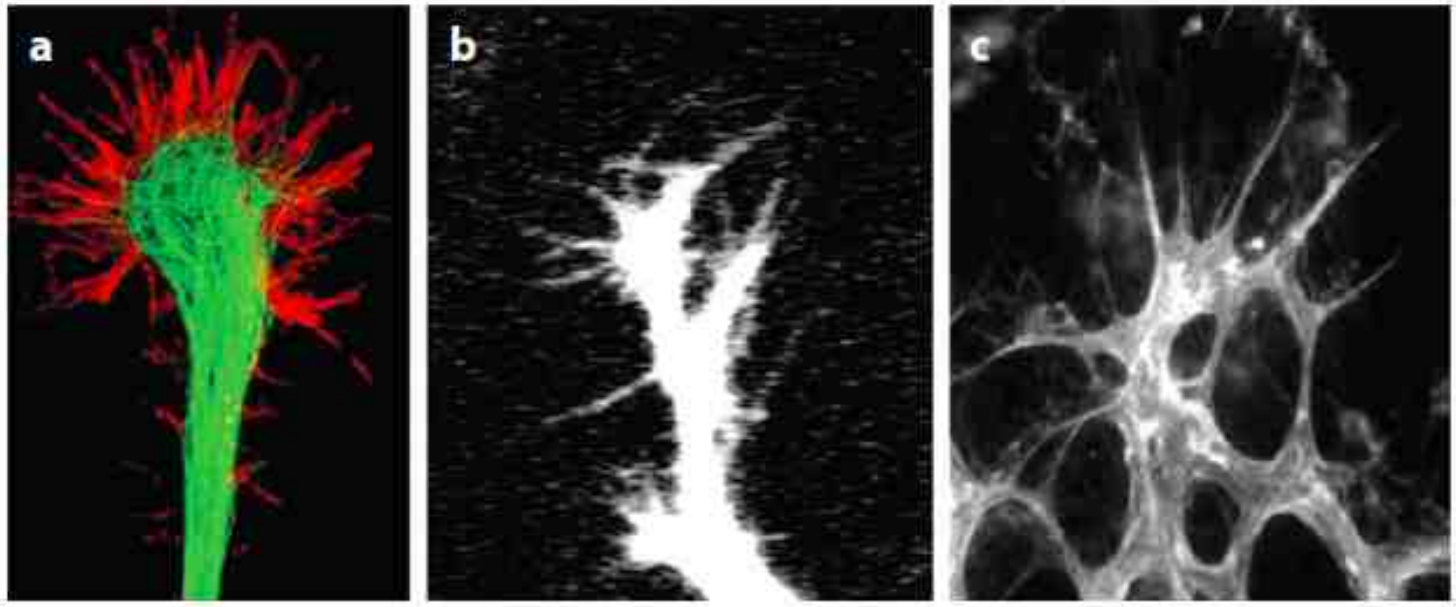
Annu. Rev. Cell Dev. Biol. 2010. 26:639–65



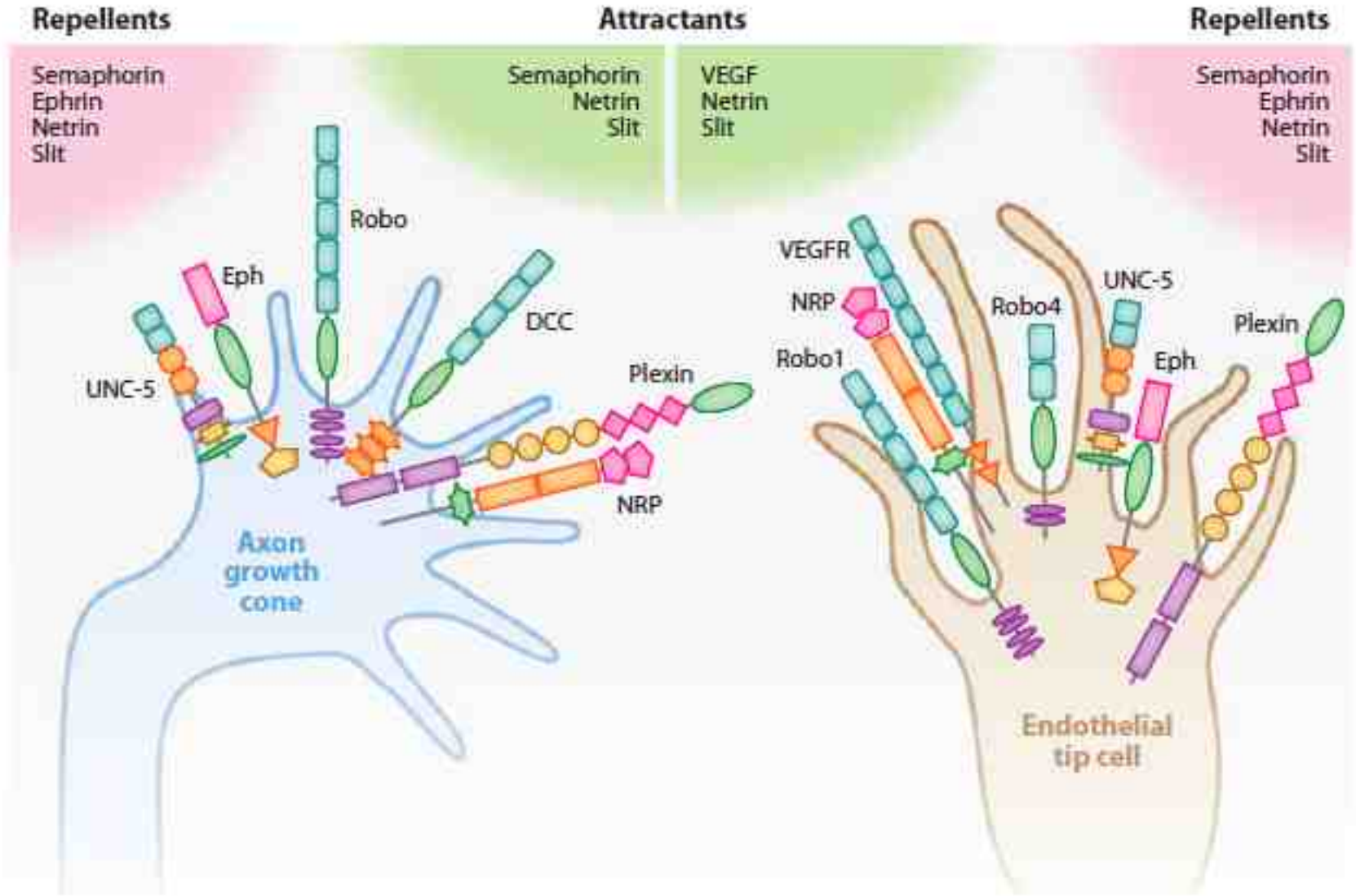
Coalignment of nerves and vessels in mouse skin. Whole-mount double immunofluorescence confocal image of limb skin in E15.5 *ephrinB2taulacZ/+* heterozygous embryos. Arteries (*red*, ephrinB2taulacZ) are aligned with peripheral sensory nerves (*green*, neurofilament) and follow their branching pattern in embryonic limb skin.

Cellular resemblances between axonal growth cones and endothelial tip cells.

Axonal growth cone Endothelial tip cells



(*a*) Axonal growth cone in culture with tubulin-rich filopodia visualized at the distal-most tip of the axon (*red*).
(*b*) Endothelial tip cells at the distal migrating end of a growing intersegmental vessel in the zebrafish trunk
(*c*) Endothelial tip cells at the periphery of the extending superficial retinal vascular plexus. Like neuronal growth cones, endothelial tip cells show evidence of extensive protrusive activity with numerous long filopodia.



Similar ligand-receptor pairs help guide axonal growth cones and endothelial tip cells. Eph-Ephrin signaling is mostly repulsive for both growth cone and endothelial tip cell path finding. Semaphorins act mostly as repellent cues in growth cones and also in endothelial tip cells. Netrins can be both attractants and repellents depending on the cellular context and on the receptor to which they bind. DCC, Deleted in Colorectal Cancer; NRP, Neuropilin; Robo, Roundabout; UNC-5, Uncoordinated-5; VEGF, Vascular endothelial growth factor; VEGFR, Vascular endothelial growth factor receptor.