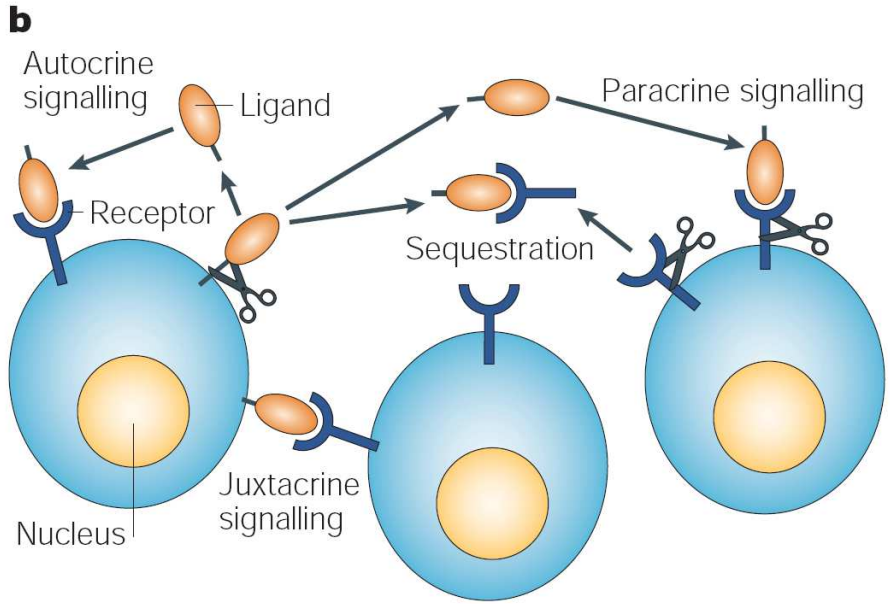
Directional Guidance cues involved in CNS neuronal migration in vivo and in vitro

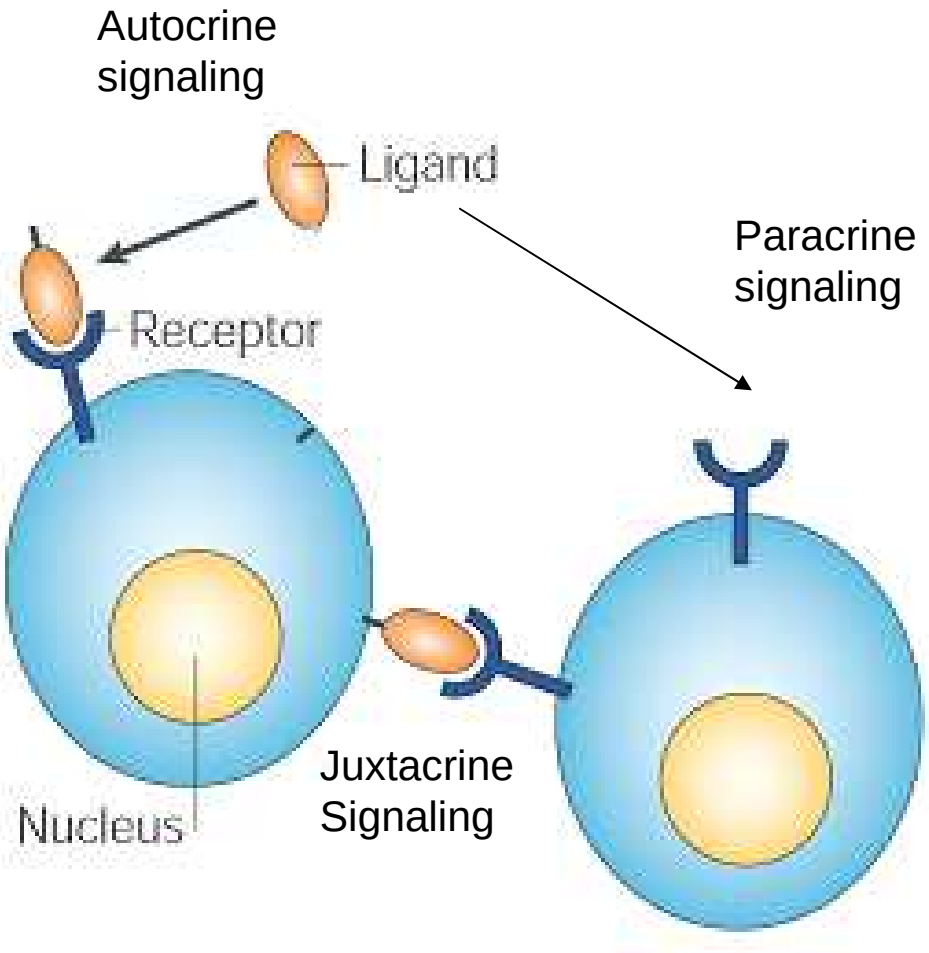
Ligands	Receptors	Defects in CNS neuronal migration in vivo	Neuronal migration in vitro
Slits	Robo	—	1. Slit repels postnatal SVZa cells[37] 2. Slit repels prenatal SVZ cells of GE[43]
Netrins	DCC	1. Abnormal pontine nuclei in DCC and netrin-1 mutants[46]	1. Netrin-1 attracts pontine nuclei[11]
	Unc-5h	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]

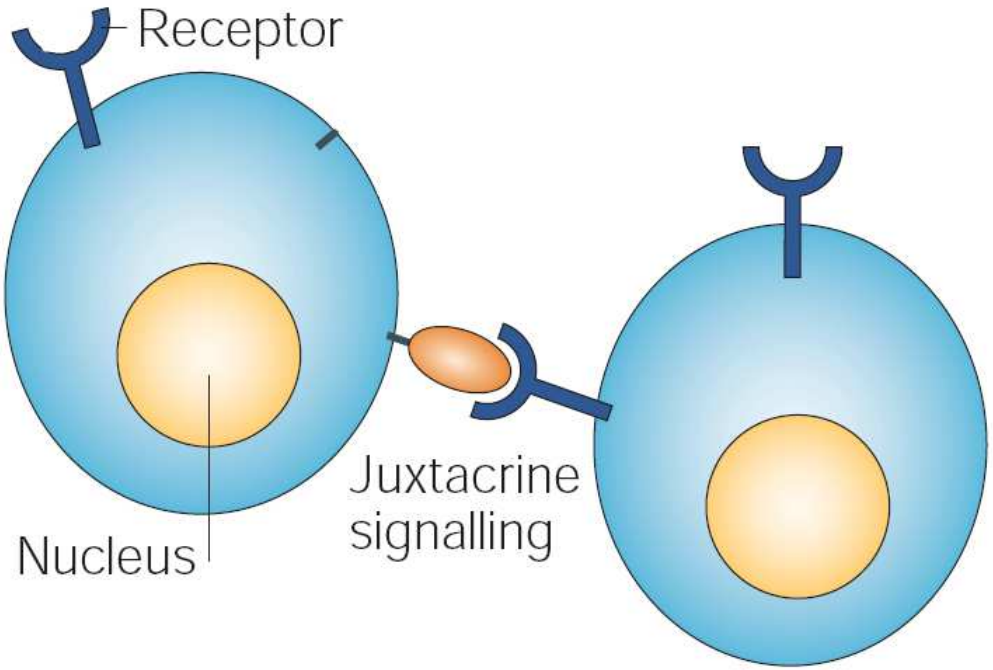
*Unc-5h3/RCM mutant mice showed abnormal development of cerebellum. However, it is still unclear that the defect is primarily caused by migration abnormality or other reasons.

Ligando o recettore?

- ligandi solubili e ligandi transmembrana
- recettori transmembrana e recettori in grado di rilasciare domini solubili e frammenti citoplasmatici
- esempi di recettori e ligandi in grado di svolgere entrambe le funzioni





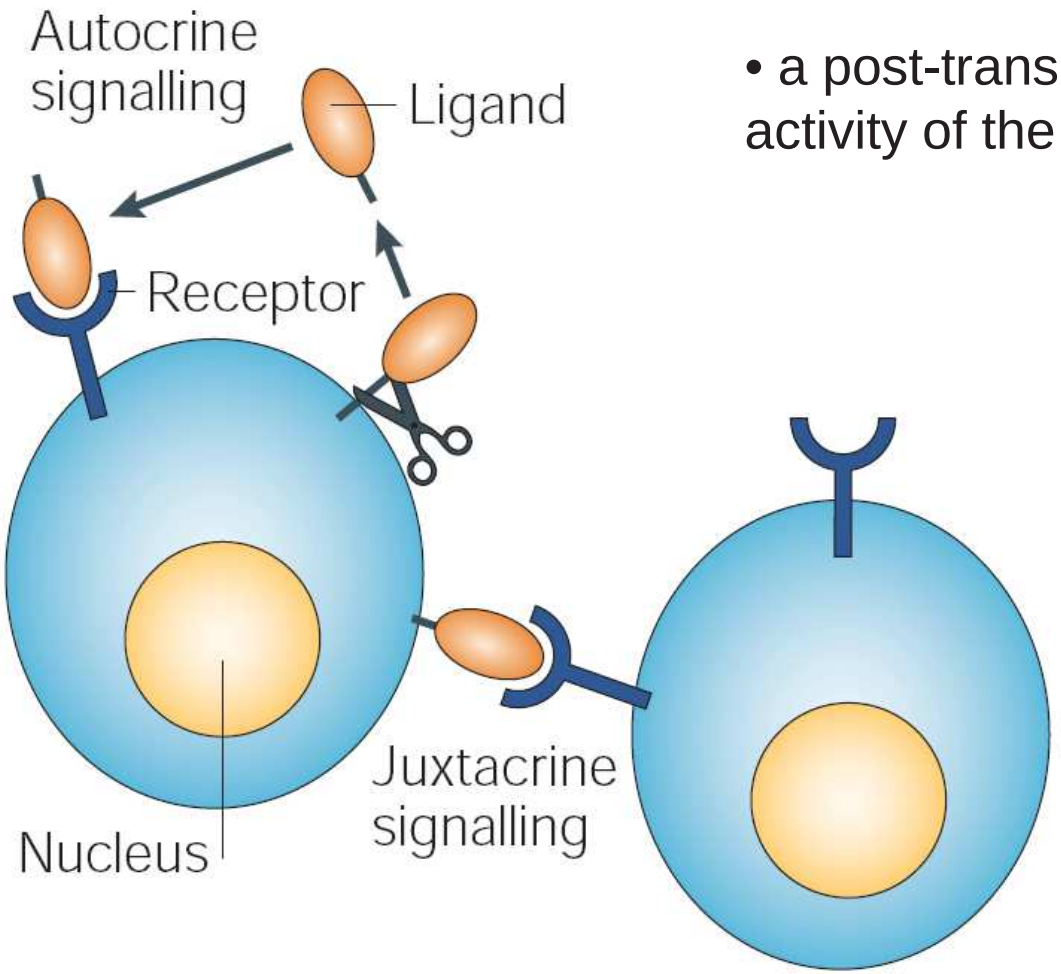


• a membrane-anchored ligand, in the absence of shedding, might only engage its receptor in a juxtacrine fashion

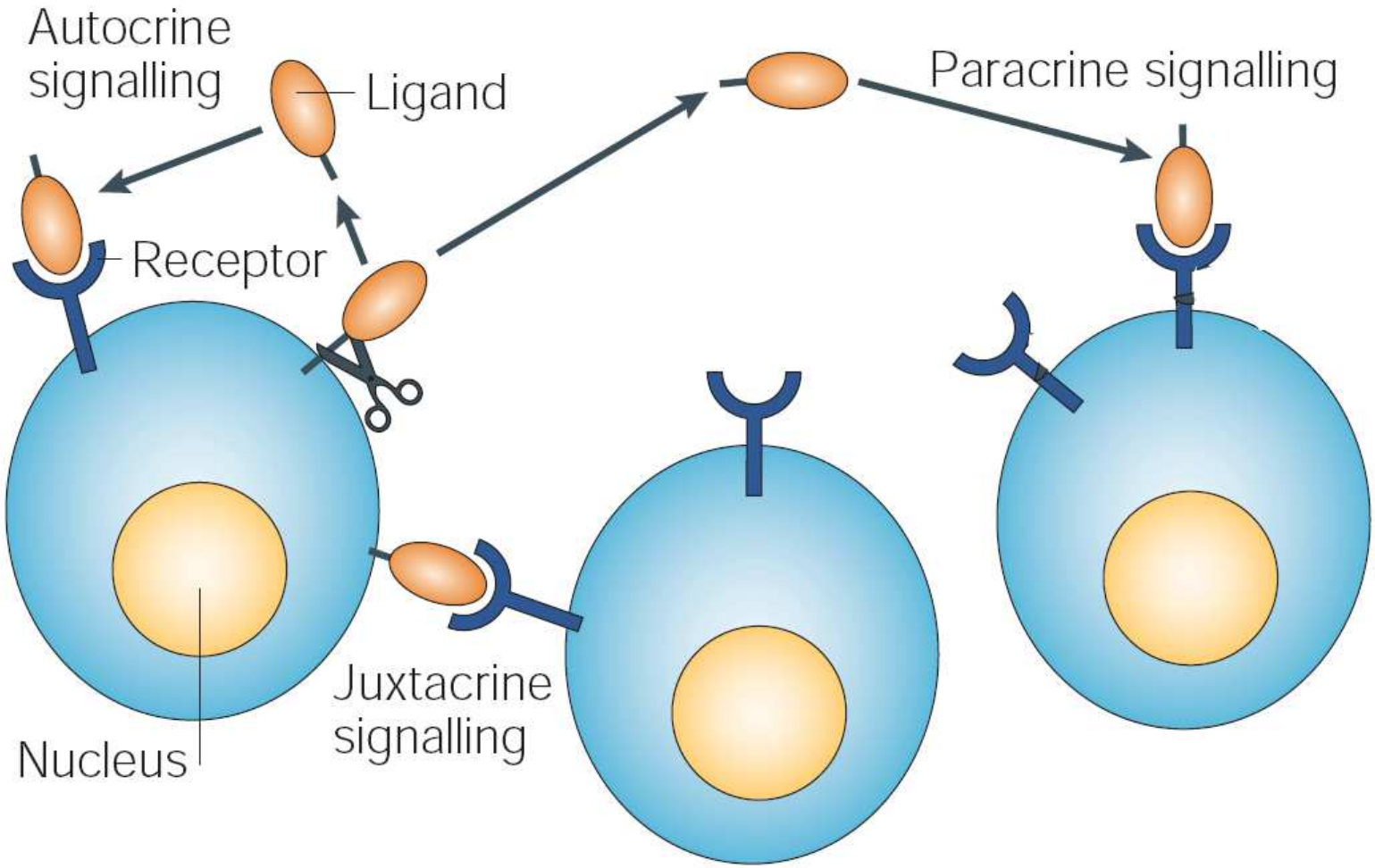
• autocrine fashion could be possible, if there aren't impediments to autocrine receptor stimulation, such as improper orientation of the ligand and receptor

PROTEIN ECTODOMAIN SHEDDING

- proteolytic processing and release of membrane proteins
- a post-translational switch that regulates the activity of the cleaved substrate

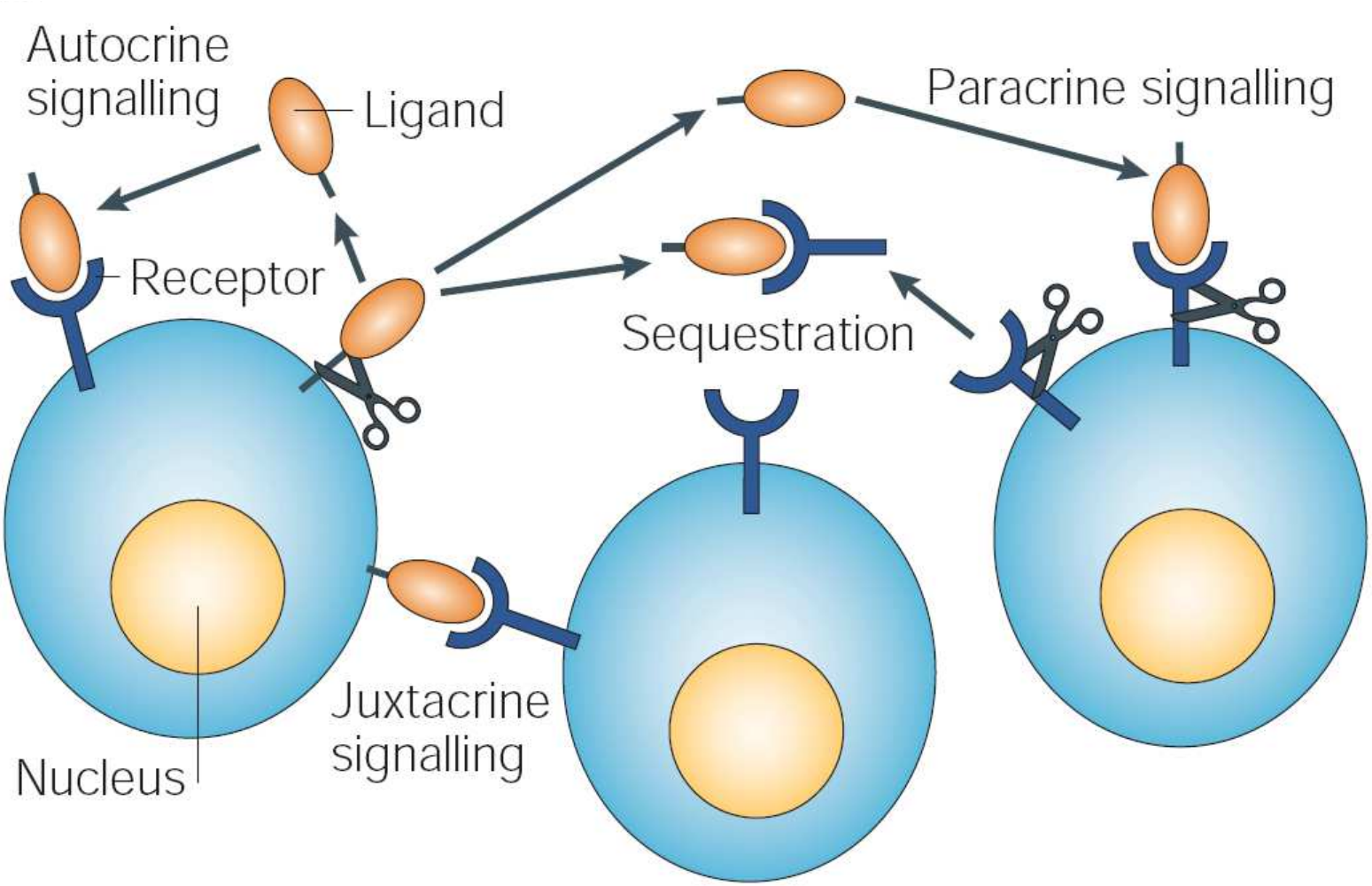


Ligand ectodomain shedding



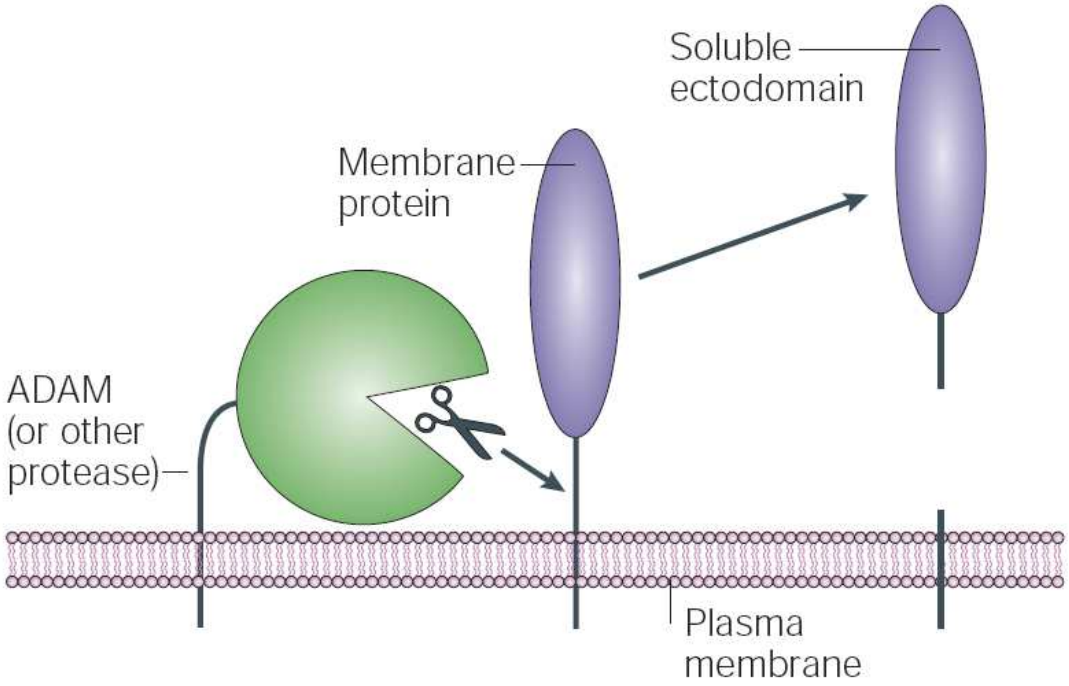
• for autocrine stimulation or to reach a receptor at a distance and to participate in paracrine signalling, a membrane-anchored ligand must be shed

Receptor ectodomain shedding



- receptors might also be shed, which could result in their activation or inactivation
- shedding might also produce a soluble decoy receptor that could sequester a ligand

Protein ectodomain shedding



• protein ectodomain shedding = the proteolytic release of the ectodomain of a membrane protein that is usually triggered by a cut adjacent to the plasma membrane

• ectodomain shedding affects many structurally and functionally diverse molecules, such as the pro-inflammatory cytokine TNFα, all EGFR ligands, receptors such as TNF receptor-I and -II, ErbB4-JMa, and a number of other proteins such as Delta, the amyloid precursor protein and L-selectin

• 2–4% of the proteins on the cell surface are subjected to ectodomain shedding

Published November 30, 1998

Removal of the Membrane-anchoring Domain of Epidermal Growth Factor Leads to Intracrine Signaling and Disruption of Mammary Epithelial Cell Organization

H. Steven Wiley,* Margaret F. Woolf,* Lee K. Opresko,* Patrick M. Burke,* Birgit Will,* Jeffrey R. Morgan,‡ and Douglas A. Lauffenburger§

*Division of Cell Biology and Immunology, Department of Pathology, University of Utah Medical School, Salt Lake City, Utah 84132; ‡Surgical Services, Massachusetts General Hospital, Harvard Medical School and the Shriners Burn Unit, Cambridge, Massachusetts 02139; and §Division of Bioengineering & Environmental Health, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Abstract. Autocrine EGF-receptor (EGFR) ligands are normally made as membrane-anchored precursors that are proteolytically processed to yield mature, soluble peptides. To explore the function of the membrane-anchoring domain of EGF, we expressed artificial EGF genes either with or without this structure in human mammary epithelial cells (HMEC). These cells require activation of the EGFR for cell proliferation. We found that HMEC expressing high levels of membrane-anchored EGF grew at a maximal rate that was not increased by exogenous EGF, but could be inhibited by anti–EGFR antibodies. In contrast, when cells expressed EGF lacking the membrane-anchoring domain (sEGF), their proliferation rate, growth at clonal densities, and receptor substrate phosphorylation were not affected by anti–EGFR antibodies. The sEGF was found to be colocalized with the EGFR within small cytoplasmic vesicles. It thus appears that removal of the membrane-anchoring domain converts autocrine to intracrine signaling. Significantly, sEGF inhibited the organization of HMEC on Matrigel, suggesting that spatial restriction of EGF access to its receptor is necessary for organization. Our results indicate that an important role of the membrane-anchoring domain of EGFR ligands is to restrict the cellular compartments in which the receptor is activated.

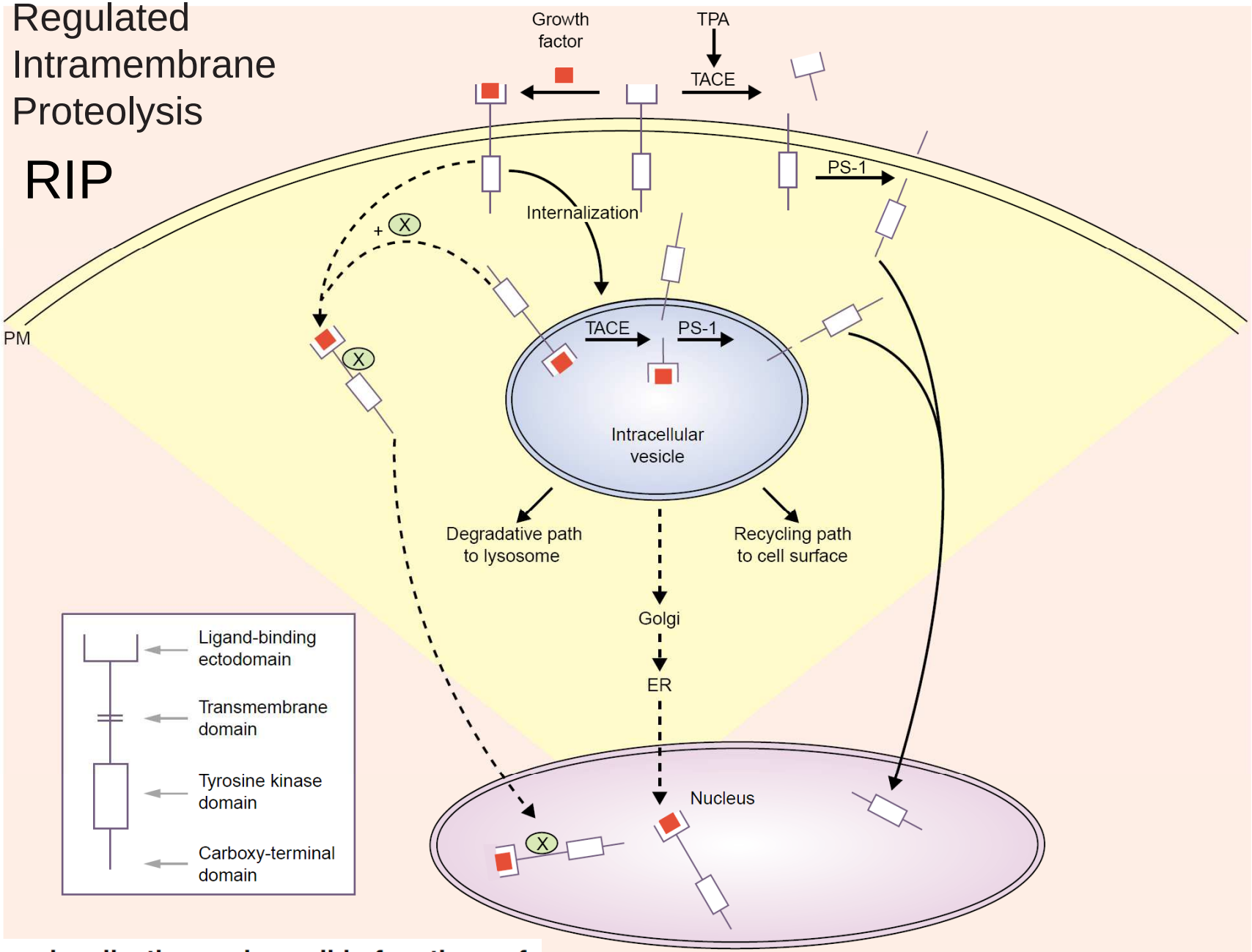
Key words: epidermal growth factor • autocrine • intracrine • receptors • epithelium

Regulated Intramembrane Proteolysis - RIP

- ectodomain shedding can activate both receptors and ligands
- a membrane-proximal cleavage by an ADAM triggers a second (presenilin-dependent) cleavage, which is referred to as Regulated Intramembrane Proteolysis (RIP)
- RIP releases the cytoplasmic domain from its membrane anchor, and allows it to enter the nucleus and participate in the transcriptional regulation of specific target genes

Regulated Intramembrane Proteolysis

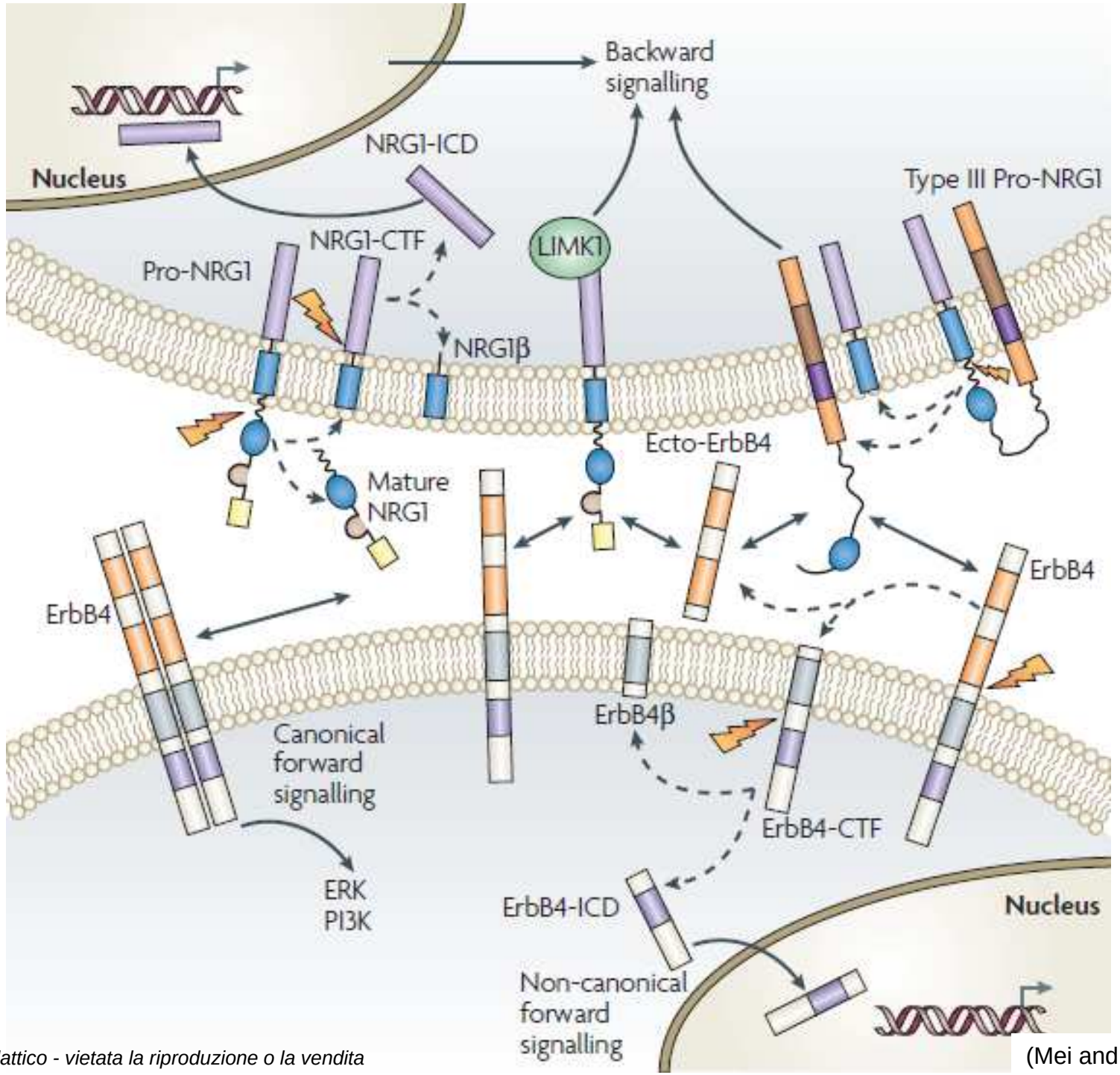
RIP



Nuclear localization and possible functions of receptor tyrosine kinases

Graham Carpenter

Current Opinion in Cell Biology



(Mei and Xiong, 2008)

Durante lo sviluppo,
come fanno le cellule a sapere dove e quando andare?
la coppia recettore-ligando Eph-ephrin
gioca un ruolo importante in questo fenomeno

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.

Eph- ephrin

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.

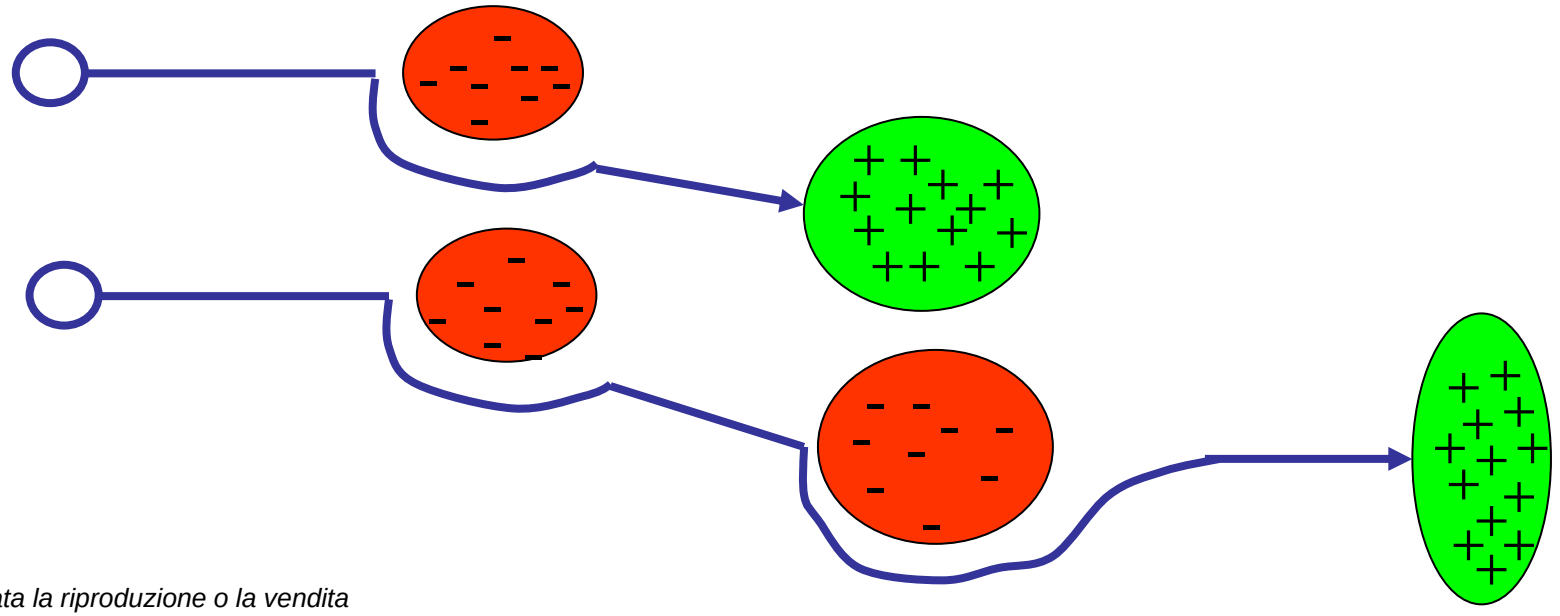
- come fanno i vertebrati - partendo da una singola cellula - a svilupparsi in un embrione e quindi in un individuo adulto?

- la proliferazione cellulare ed il differenziamento sono due processi critici durante lo sviluppo

- la migrazione cellulare e l'adesione giocano un ruolo chiave nella formazione delle strutture anatomiche

- la capacità di una cellula di migrare verso la sua corretta destinazione dipende fortemente dalla segnalazione mediata dalle proteine esposte in membrana

- i recettori Eph ed i ligandi ephrin giocano un ruolo importante nel guidare una cellula o un assone verso la sua destinazione



I recettori esposti sulla superficie della cellula hanno un'influenza sulla migrazione cellulare e sullo sviluppo

- durante la migrazione le cellule, o gli assoni, si devono muovere per distanze considerevoli, lunghe migliaia di volte la lunghezza di una singola cellula

- è fondamentale che le cellule migranti raggiungano il loro bersaglio in maniera precisa

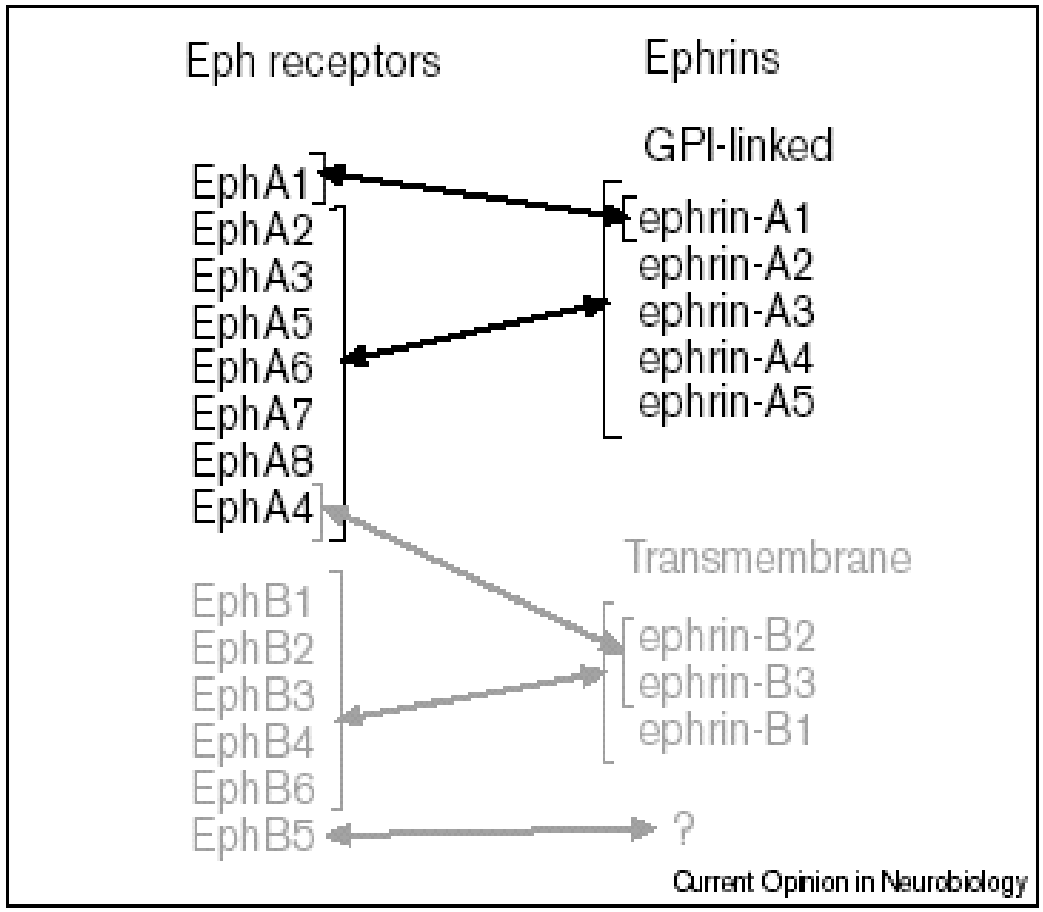
- i recettori di membrana guidano le cellule migranti verso la loro destinazione

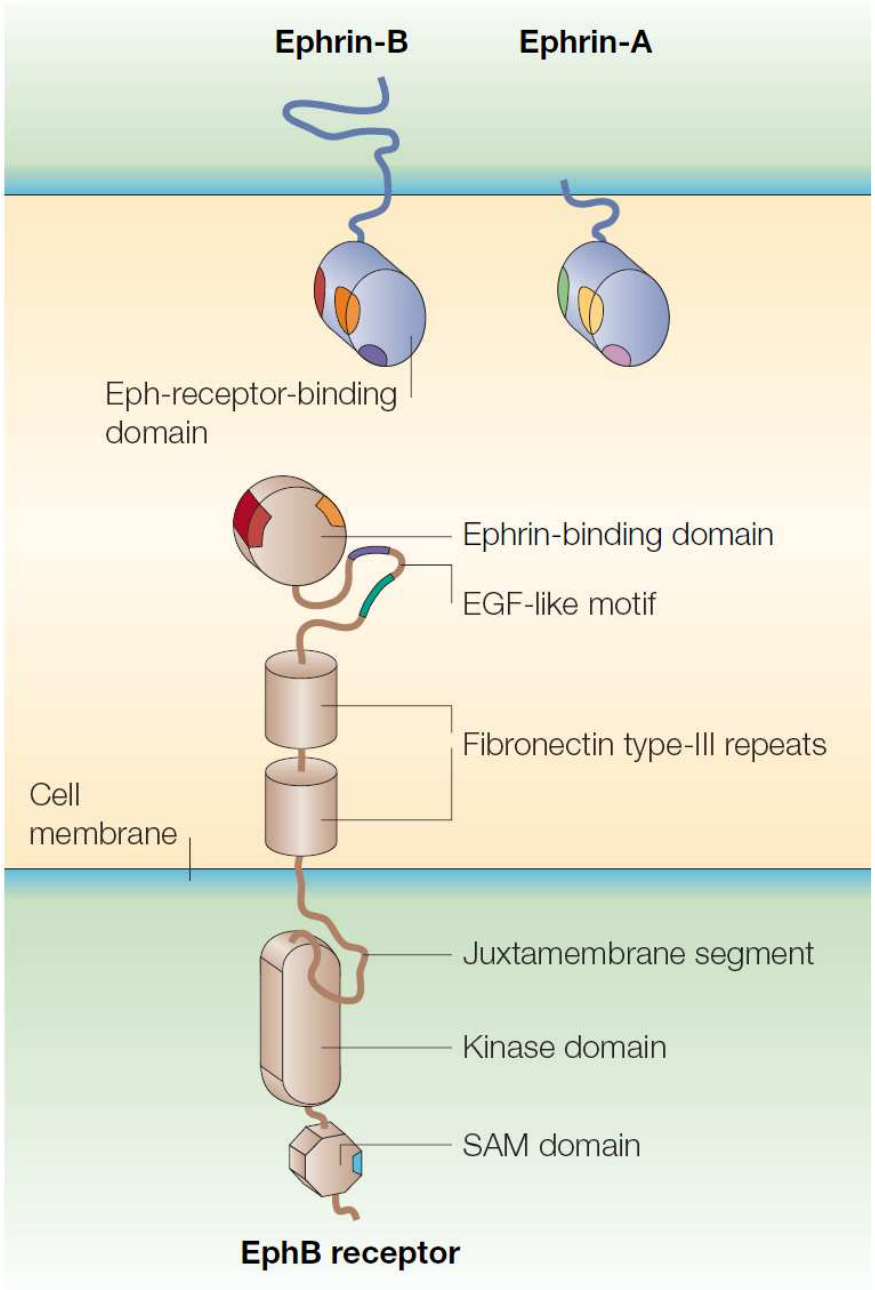
- interagendo con le strutture del citoscheletro, i trasduttori intracellulari del segnale possono istruire la cellula migrante in modi diversi, quali "gira intorno", "sta ferma", "continua per questa strada"

- usando diversi recettori di membrana, tutti sensibili a ligandi diversi e tutti in grado di attivare vie di segnalazione ben precise, la cellula ha un sistema di navigazione che le consente di migrare per distanze relativamente grandi verso bersagli molto precisi

- il sistema di navigazione più ampio usato dalla cellula durante questo processo altamente complesso e dinamico è la segnalazione attraverso i recettori Eph ed i loro ligandi ephrine

recettori Eph e ligandi ephrin





- i ligandi ephrin sono espressi sulla superficie delle cellule

- l’attivazione dei recettori Eph avviene solo in seguito alle interazioni cellula-cellula

- il contatto è necessario perché avvenga la segnalazione e consente istruzioni spaziali altamente specifiche, più di quanto non sia possibile ottenere con ligandi solubili
(è più facile muoversi nell’oscurità usando il tatto, piuttosto che l’odorato o l’udito)

Segnalazione ephrin-Eph

- la segnalazione fra Eph ed ephrin segue il modello classico dei RTK.

- in seguito al contatto cellula-cellula c'è legame forte fra recettori Eph e ligandi ephrin.

- In assenza di ligando le tirosine non fosforilate del recettore Eph interagiscono strettamente col dominio tirosina kinasi impedendogli di svolgere la sua attività

- In seguito all'interazione col ligando, i recettori Eph interagiscono, si transfosforilano e fosforilano altre tirosina kinasi appartenenti alla famiglia Src.

-Eph fosforila substrati a valle e attiva la trasduzione del segnale

- Il semplice legame di ephrin al recettore Eph trasforma la regione intracellulare del recettore Eph da uno stato cataliticamente inattivo (off) ad uno cataliticamente attivo (on) con conseguente attivazione della trasduzione del segnale

Una scoperta inattesa porta alla comprensione della segnalazione bidirezionale Eph-ephrin

- in seguito all'interazione Eph-ephrin, la segnalazione avviene solo dentro la cellula che esprime il recettore Eph o si attiva una via di segnalazione anche dentro la cellula che esprime ephrin?

- lo studio pioneristico che ha dimostrato questo fenomeno è stato effettuato nel 1996 nel laboratorio di Tony Pawson al Toronto's Mount Sinai Hospital

- i ricercatori stavano studiando cervelli di topi privi di EphB2 (KO completo) per determinare se EphB2 giocava un ruolo nello sviluppo delle strutture neurali.

Cell. 1996 Jul 12;86(1):35-46.

Nuk controls pathfinding of commissural axons in the mammalian central nervous system.

Henkemeyer M, Orioli D, Henderson JT, Saxton TM, Roder J, Pawson T, Klein R.

Programme in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada.

Abstract

Eph family receptor tyrosine kinases have been proposed to control axon guidance and fasciculation. To address the biological functions of the Eph family member Nuk, two mutations in the mouse germline have been generated: a protein null allele (Nuk1) and an allele that encodes a Nuk-beta gal fusion receptor lacking the tyrosine kinase and C-terminal domains (Nuk(lacZ)). In Nuk1 homozygous brains, the majority of axons forming the posterior tract of the anterior commissure migrate aberrantly to the floor of the brain, resulting in a failure of cortical neurons to link the two temporal lobes. These results indicate that Nuk, a receptor that binds transmembrane ligands, plays a critical and unique role in the pathfinding of specific axons in the mammalian central nervous system.

PMID: 8689685 [PubMed - indexed for MEDLINE]

- tagliando e colorando sezioni di cervello, i ricercatori si accorsero che i topi KO per EphB2 (Nuk) mostravano una migrazione assonale alterata in un importante fascio di assoni chiamato commissura anteriore, che connette gli emisferi destro e sinistro del cervello

- i ricercatori si chiesero dove era cruciale la presenza di EphB2 per lo sviluppo normale della commissura anteriore

- per capire dove era espresso EphB2 in queste regioni, Mark Henkemeyer realizzò un topo mutante nel quale le regioni citoplasmatiche di EphB2 (contenenti le strutture necessarie per la segnalazione) erano state eliminate e sostituite con la beta-galattosidasi (*EphB2*-lacZ mice)

- posta nella regione citoplasmatica del recettore EphB2, la beta-galactosidase serviva come gene reporter per mostrare anatomicamente dove era espresso EphB2 e contemporaneamente inattivava la capacità di EphB2 di segnalare dentro la cellula

- cosa scoprì Henkemeyer dopo aver ottenuto questo topo?

- incredibilmente, scoprì che topi omozigoti per la mutazione *EphB2*lacZ non presentavano nessun difetto nella commissura anteriore

- EphB2 era capace dunque di segnalare anche senza il suo dominio intracellulare?

- attraverso l’analisi dell’espressione del gene reporter beta-galactosidase i ricercatori scoprirono che EphB2 non era espresso nella commissura anteriore

- EphB2 era espresso in una regione ventrale rispetto alla commissura anteriore, l’ipotalamo

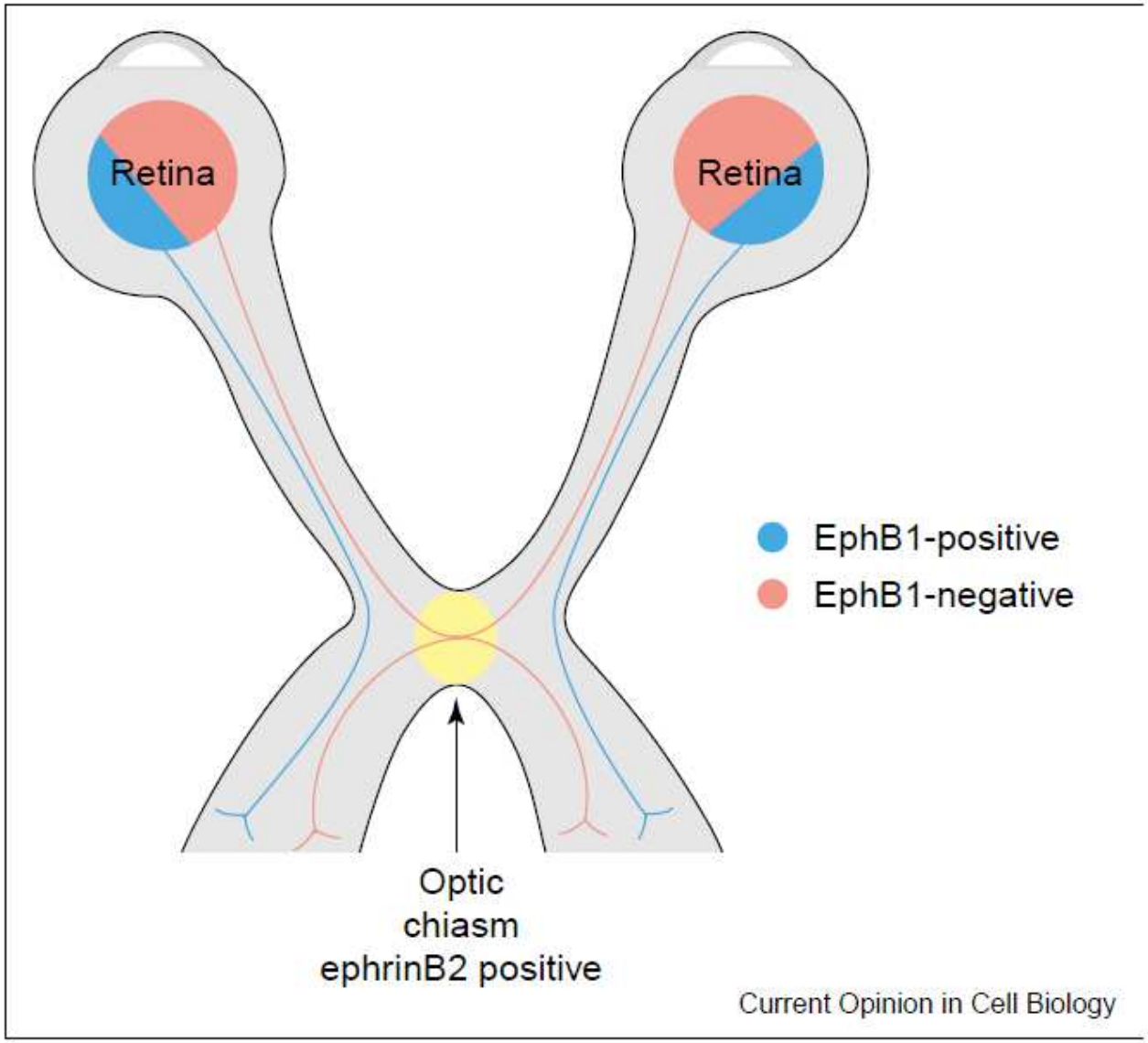
- scoprirono che ephrin-B2, il ligando, era espresso dagli assoni che devono passare dalla regione ricca di EphB2 per formare la commissura anteriore

- cosa significavano questi risultati?

- questi risultati indicavano che i recettori Eph potevano segnalare in qualche modo attraverso il loro dominio extracellulare, interagendo con le ephrin, perché il loro dominio extracellulare era sufficiente per lo sviluppo normale del cervello in questa regione

- questa nuova forma di segnalazione non richiedeva il dominio intracellulare dei recettori EphB2 e perciò suggeriva un nuovo ruolo, simile a ligando, del dominio extracellulare dei recettori Eph e, di conseguenza, un nuovo ruolo, simile a un recettore, per le ephrin

Midline guidance in the visual system



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.

- ora sappiamo che le ephrin hanno la capacità di segnalare dentro la cellula in seguito all'interazione coi recettori Eph

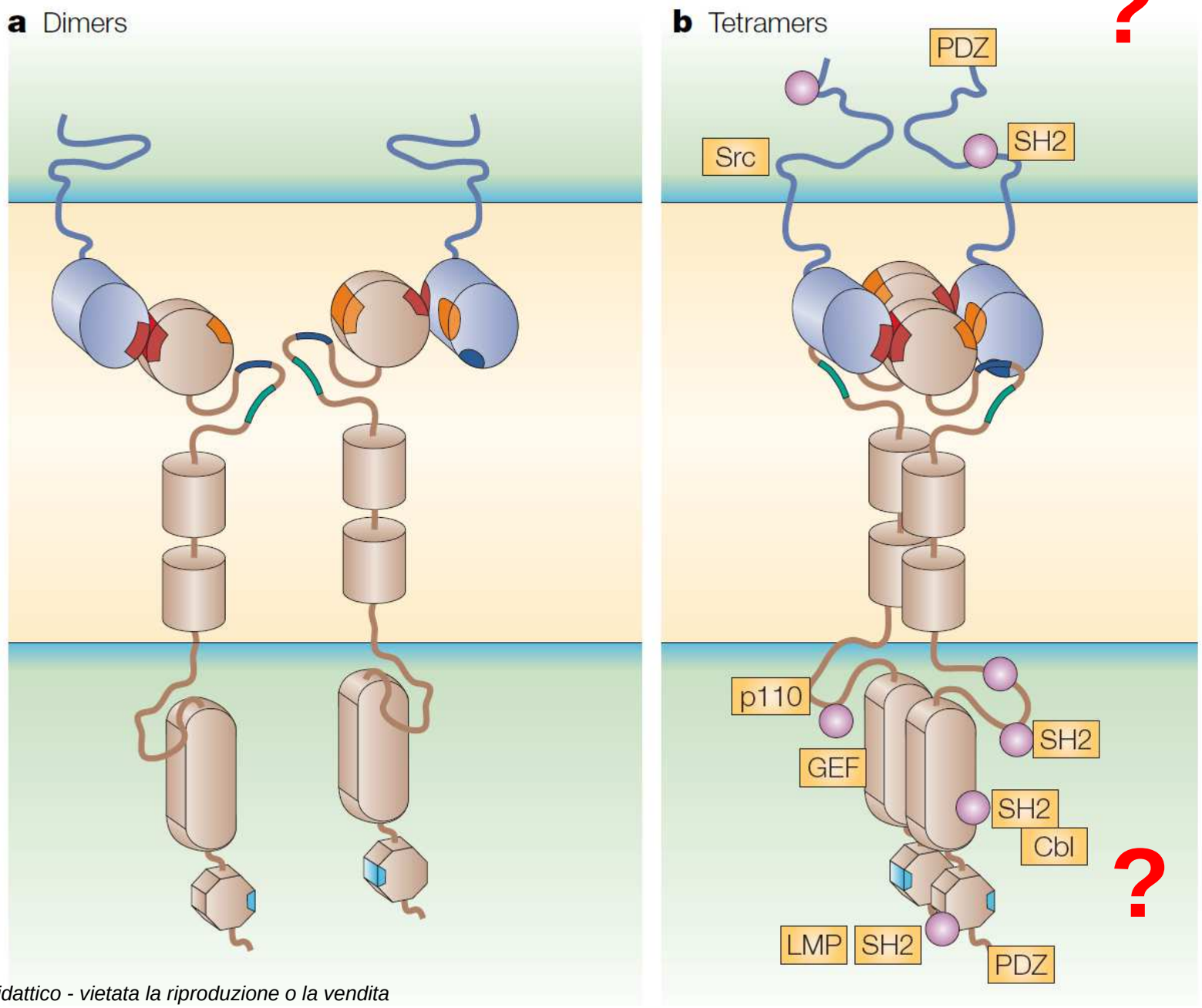
- il legame di Eph raggruppa e attiva le ephrine facendole interagire con le Src-family kinases (SFKs).

- nel caso delle ephrin A, le SFK si attivano e fosforilano tirosine di altre molecole presenti vicino alla membrana, attivando la trasduzione del segnale

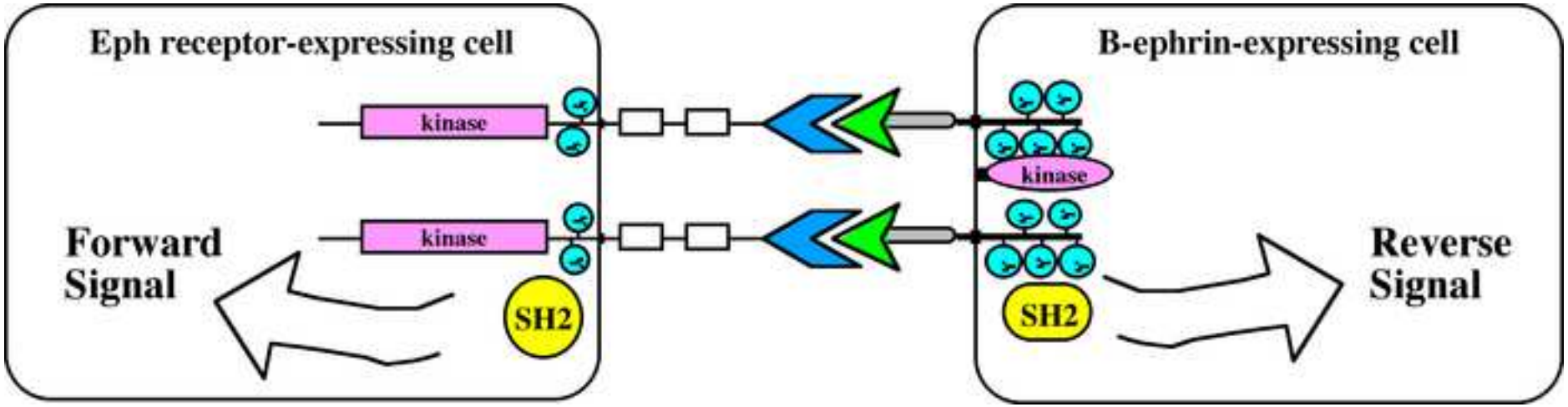
- nel caso delle ephrin B è stato dimostrato che la coda citoplasmatica viene fosforilata in tirosina diventando il substrato per la successiva trasduzione del segnale, anche se il dominio citoplasmatico è corto e privo di attività catalitica

- questi risultati inattesi cambiarono il modo con cui i ricercatori studiavano i recettori e vennero coniati nuovi modi per indicare in quale direzione procede la segnalazione fra cellule diverse: ***forward*** e ***reverse*** signalling

Protein interactions in Eph signalling



The Phenomenon of Bidirectional Signaling



Nature. 1996 Oct 24;383(6602):722-5.

Bidirectional signalling through the EPH-family receptor Nuk and its transmembrane ligands.

Holland SJ, Gale NW, Mbamalu G, Yancopoulos GD, Henkemeyer M, Pawson T.

Programme in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada.

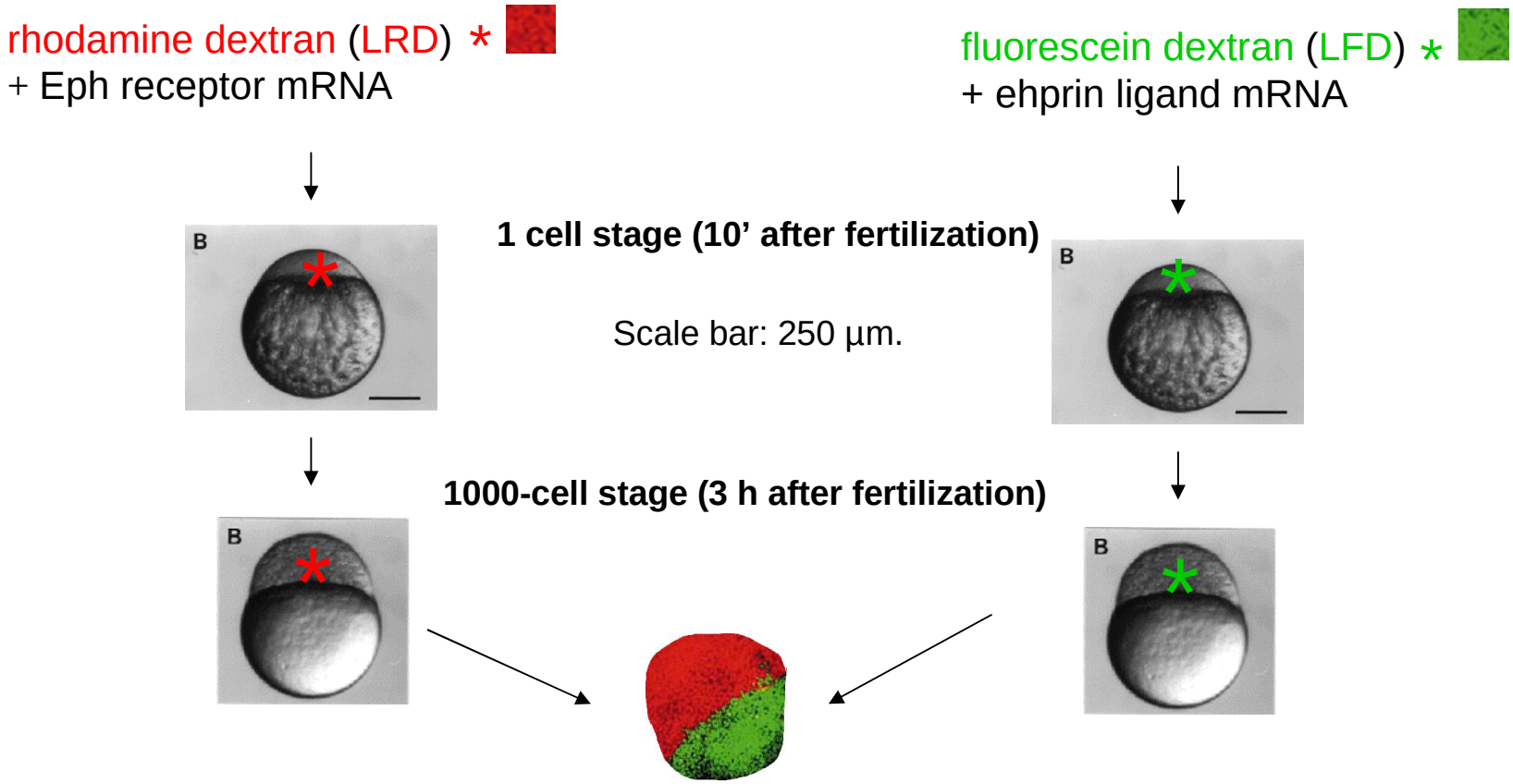
Abstract

Receptor tyrosine kinases of the EPH class have been implicated in the control of axon guidance and fasciculation, in regulating cell migration, and in defining compartments in the developing embryo. Efficient activation of EPH receptors generally requires that their ligands be anchored to the cell surface, either through a transmembrane (TM) region or a glycosyl phosphatidylinositol (GPI) group. These observations have suggested that EPH receptors can transduce signals initiated by direct cell-cell interaction. Genetic analysis of Nuk, a murine EPH receptor that binds TM ligands, has raised the possibility that these ligands might themselves have a signalling function. Consistent with this, the three known TM ligands have a highly conserved cytoplasmic region, with multiple potential sites for tyrosine phosphorylation. Here we show that challenging cells that express the TM ligands Elk-L or Htk-L with the clustered ectodomain of Nuk induces phosphorylation of the ligands on tyrosine, a process that can be mimicked both in vitro and in vivo by an activated Src tyrosine kinase. Co-culture of cells expressing a TM ligand with cells expressing Nuk leads to tyrosine phosphorylation of both the ligand and Nuk. These results suggest that the TM ligands are associated with a tyrosine kinase, and are inducibly phosphorylated upon binding Nuk, in a fashion reminiscent of cytokine receptors. Furthermore, we show that TM ligands, as well as Nuk, are phosphorylated on tyrosine in mouse embryos, indicating that this is a physiological process. EPH receptors and their TM ligands therefore mediate bidirectional cell signalling.

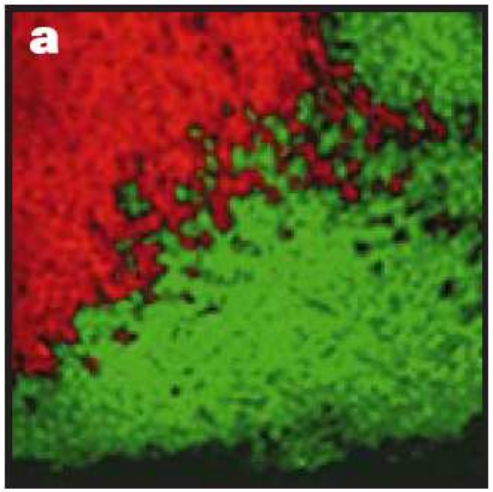
NATURE | VOL 400 | 1 JULY 1999 | www.nature.com

Eph receptors and ephrins restrict cell intermingling and communication

Georg Mellitzer, Qiling Xu & David G. Wilkinson

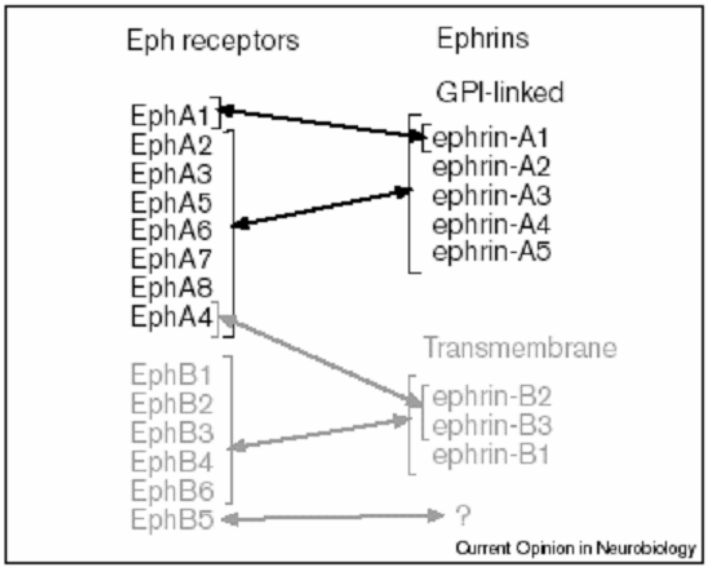


LRD /LFD

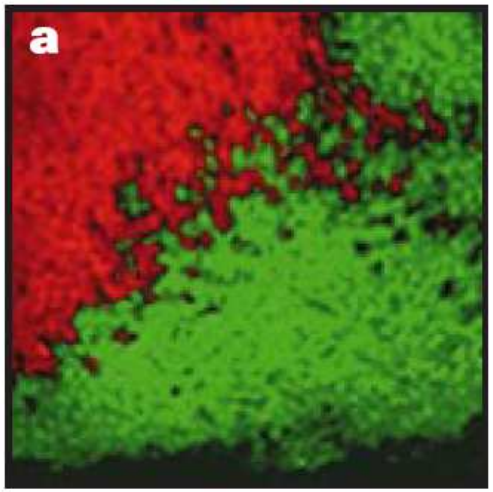


EphA4 /ephrin-B1

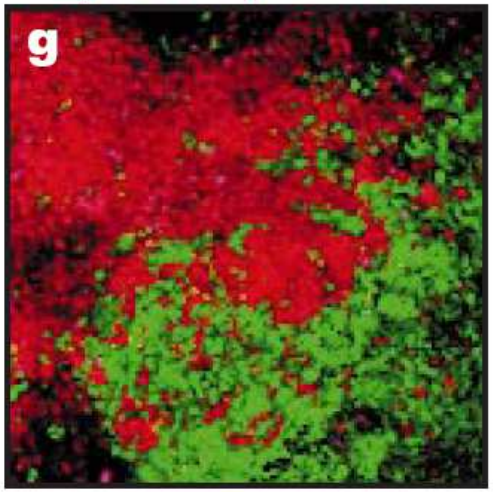
?

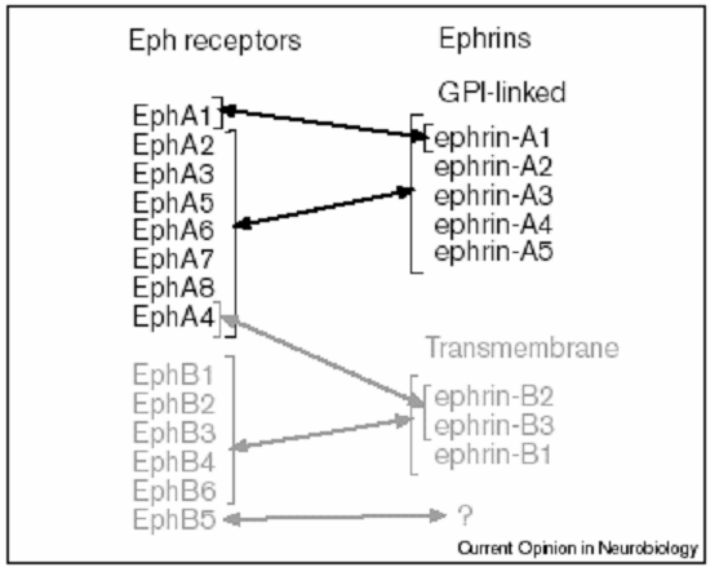


LRD /LFD



EphA4 /ephrin-B1

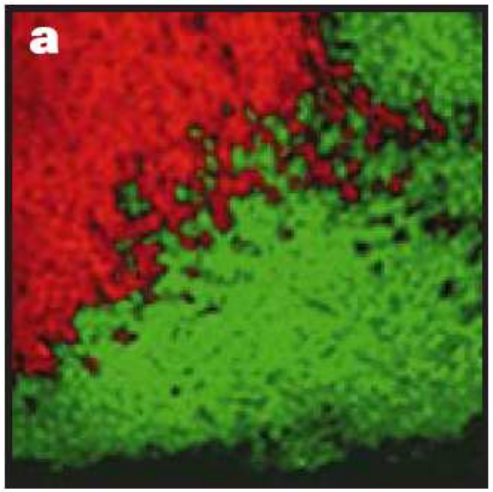




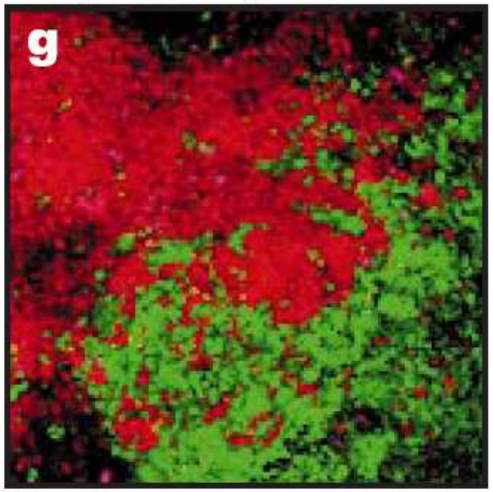
EphA4 /ephrin-B2

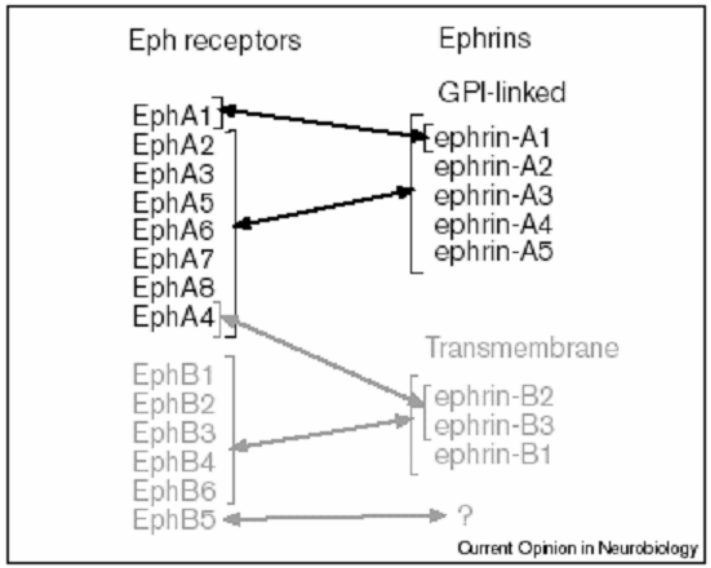
?

LRD /LFD

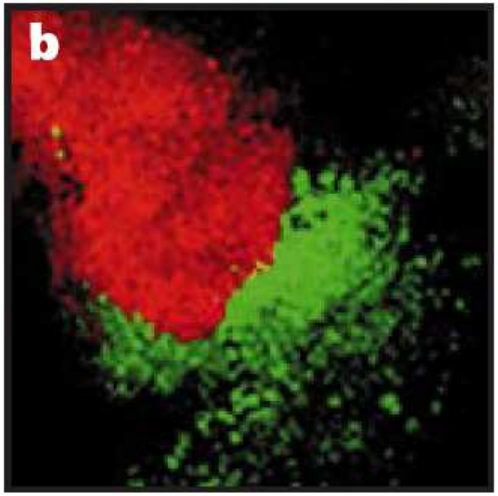


EphA4 /ephrin-B1





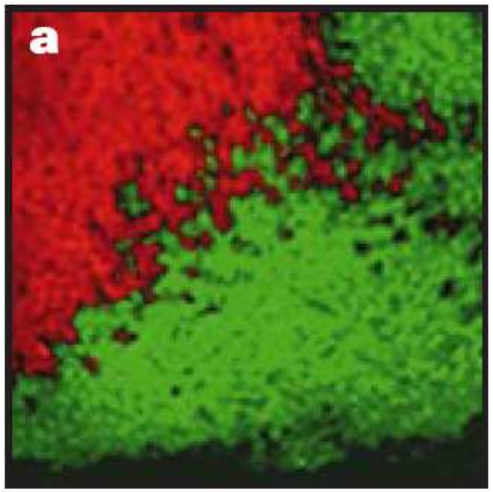
EphA4 /ephrin-B2



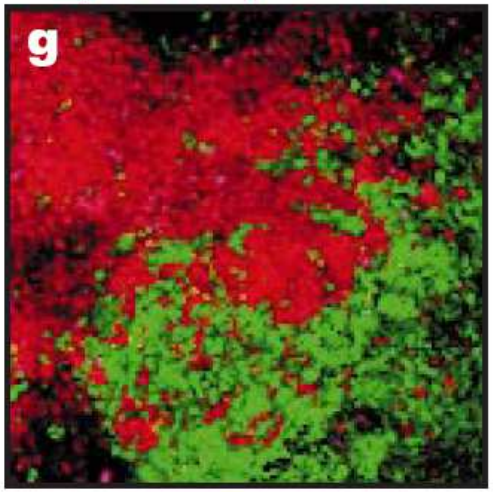
EphB2 /ephrin-B1

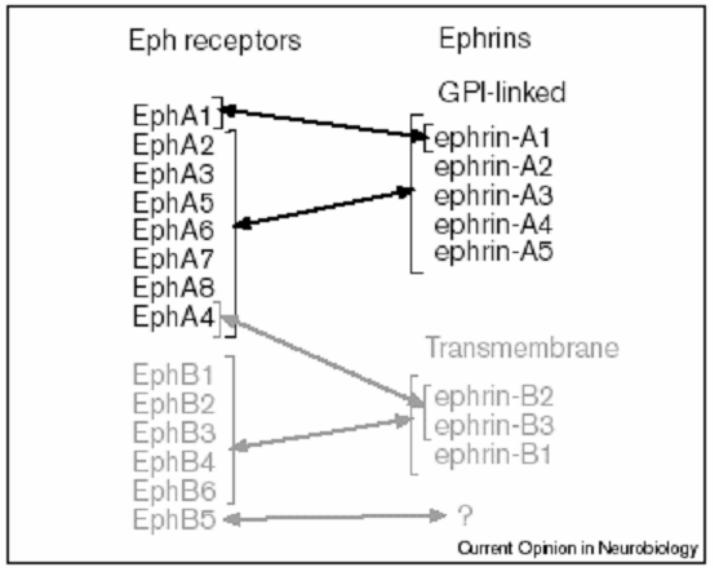
?

LRD /LFD

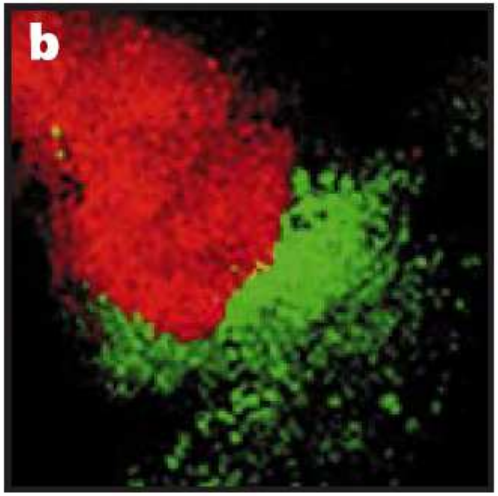


EphA4 /ephrin-B1

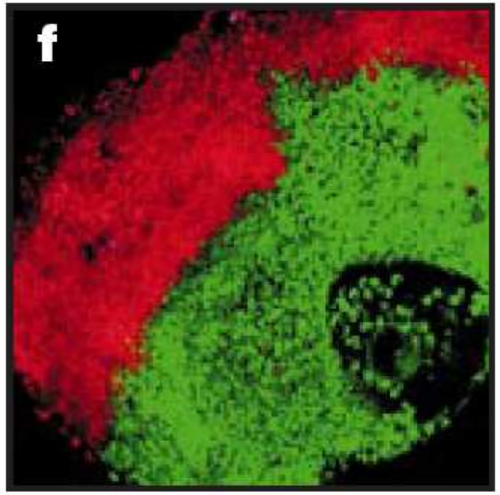




EphA4 /ephrin-B2



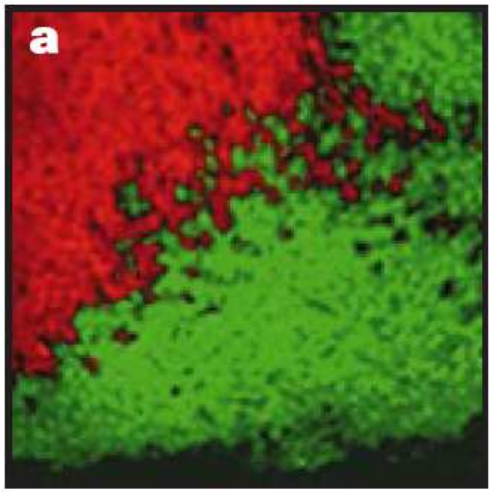
EphB2 /ephrin-B1



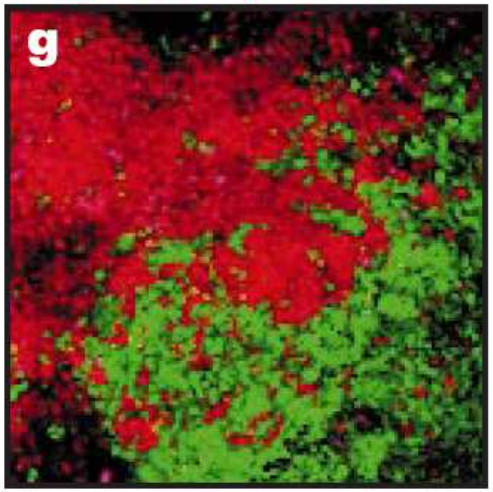
EphB2 /ephrin-B2

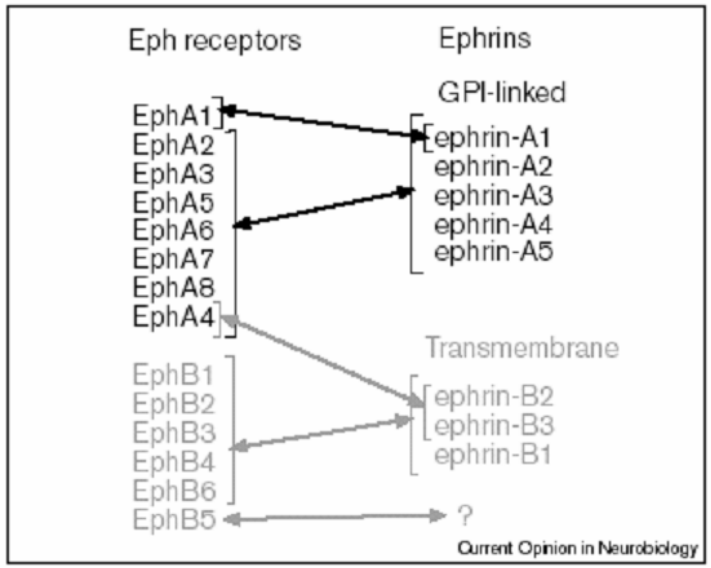
?

LRD /LFD

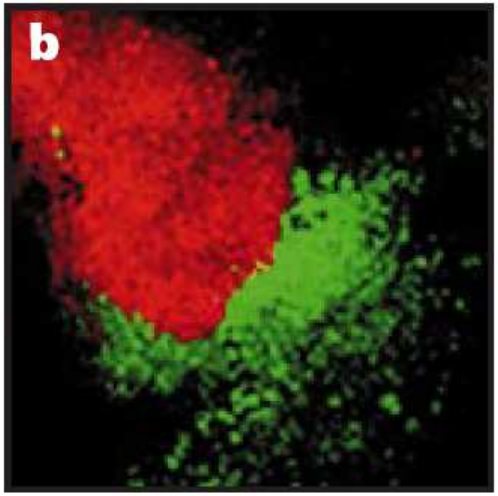


EphA4 /ephrin-B1

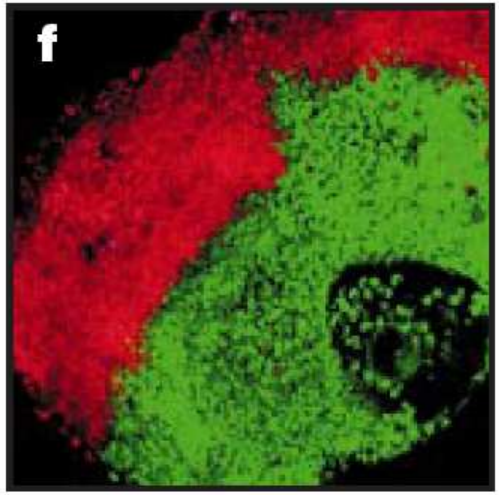




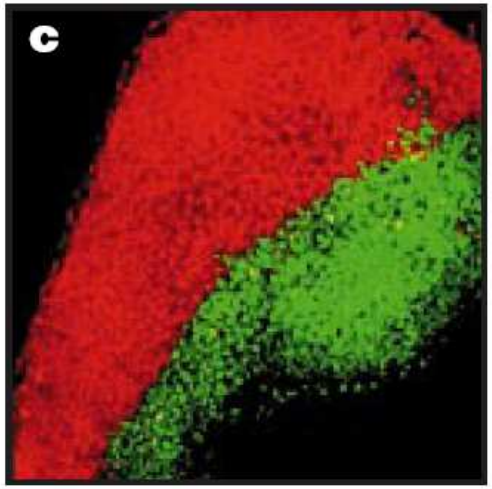
EphA4 /ephrin-B2



EphB2 /ephrin-B1

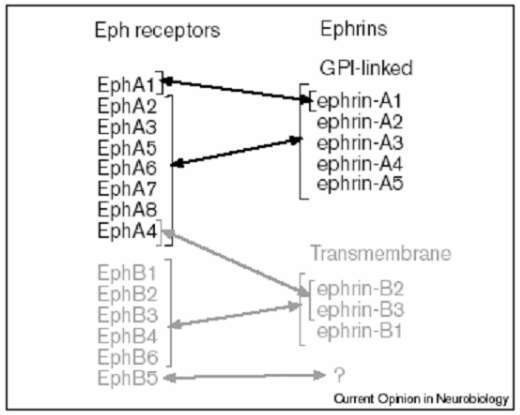


EphB2 /ephrin-B2

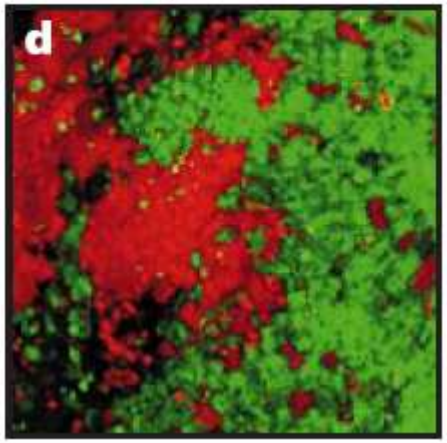


EphB2+EphA4 /
Δephrin-B2

?

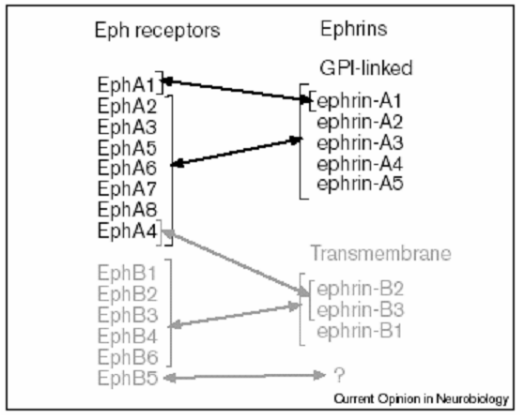


EphB2+EphA4 / Δephrin-B2

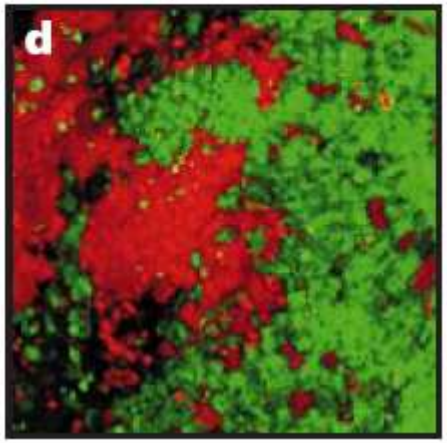


EphA4 /ephrinB1+ Δephrin-B2

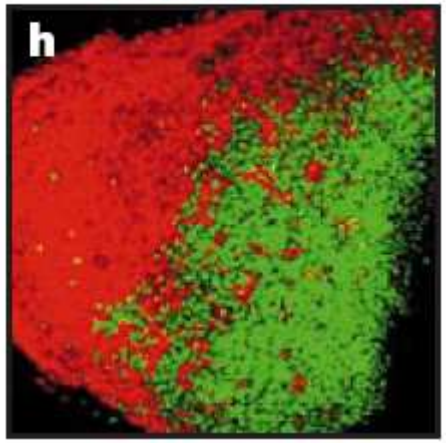
?

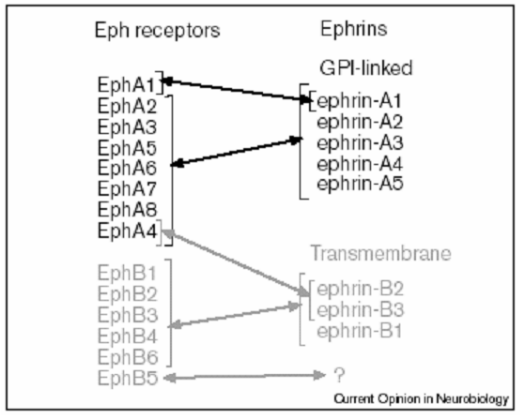


EphB2+EphA4 /
Δephrin-B2



EphA4 /ephrinB1+
Δephrin-B2

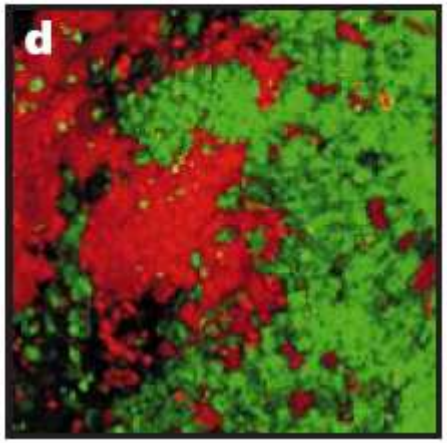




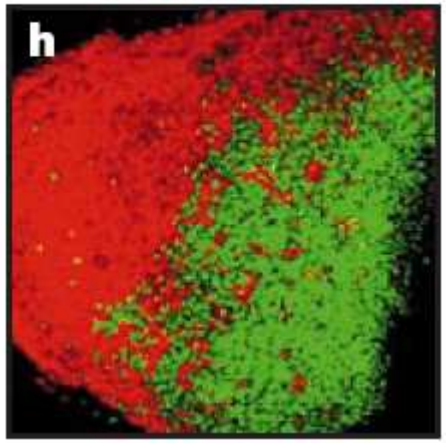
ΔEphB2 /ephrin-B2

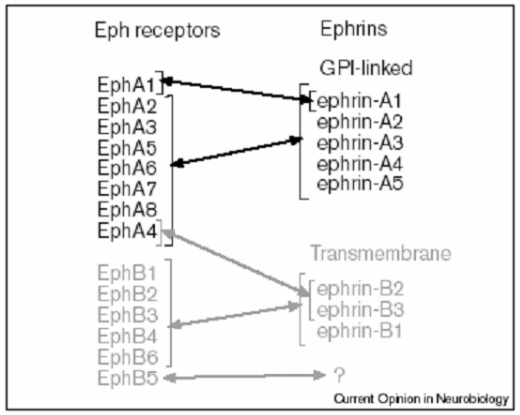
?

EphB2+EphA4 /
ΔephrinB2

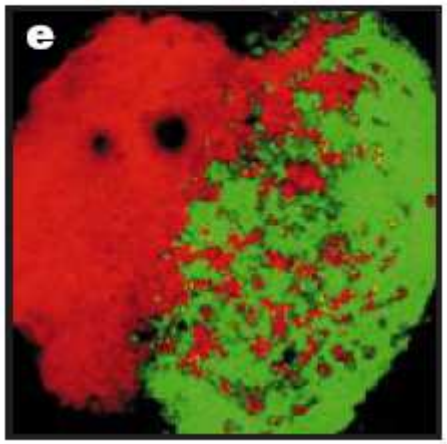


EphA4 /ephrinB1+
ΔephrinB2





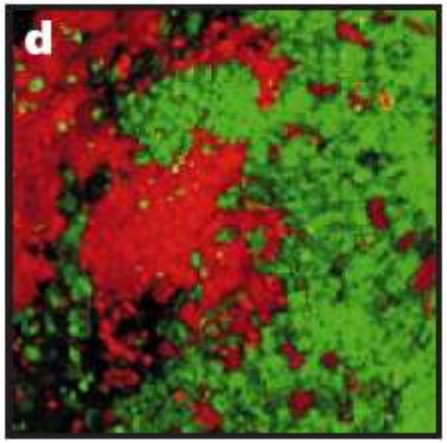
ΔEphB2 /ephrin-B2



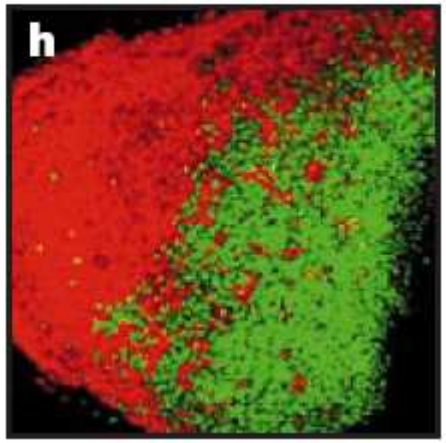
EphA4+ ΔEphB2
/ephrin-B1

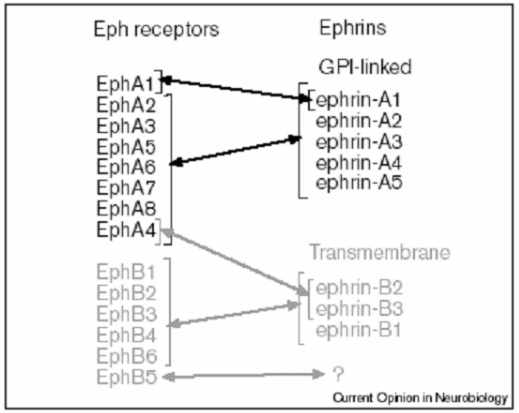
?

EphB2+EphA4 /
ΔephrinB2

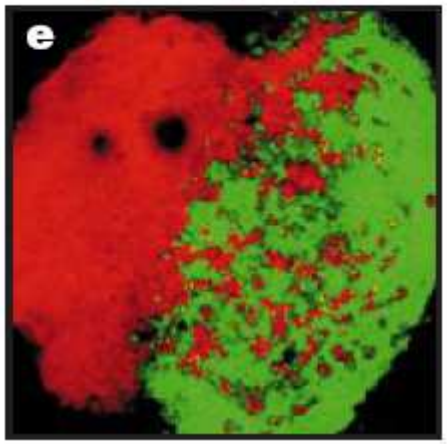


EphA4 /ephrinB1+
ΔephrinB2

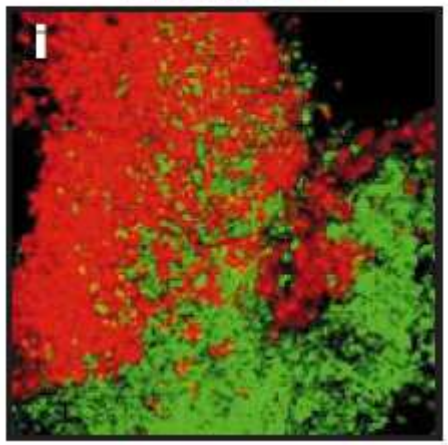


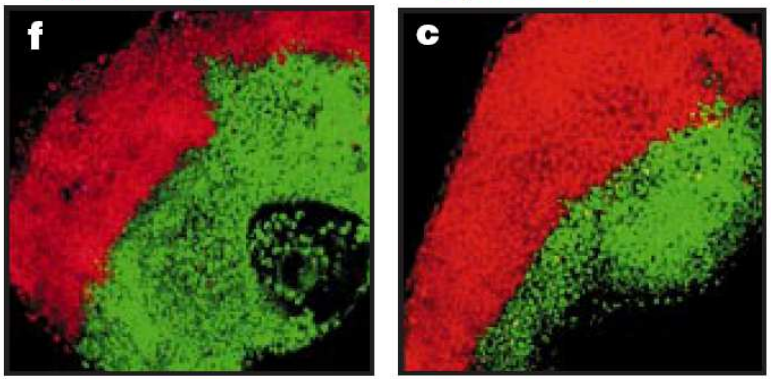


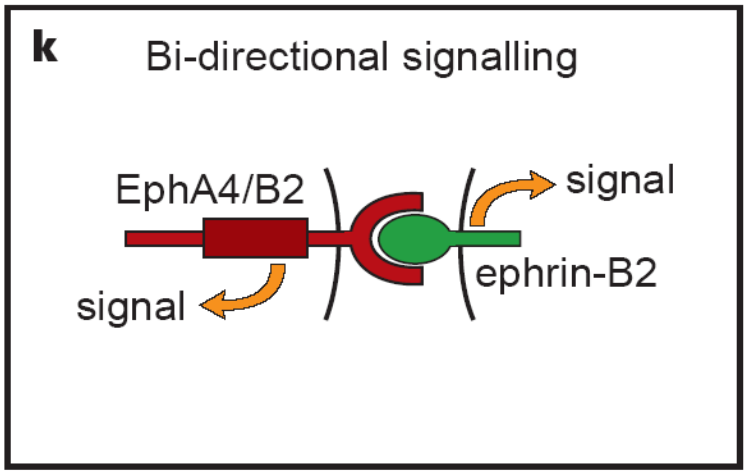
ΔEphB2 /ephrin-B2

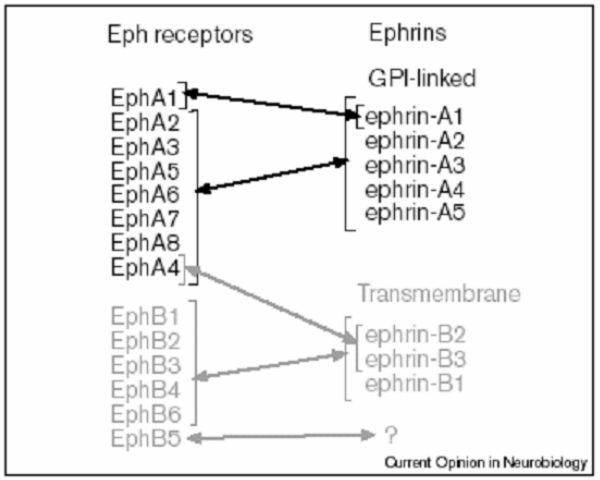


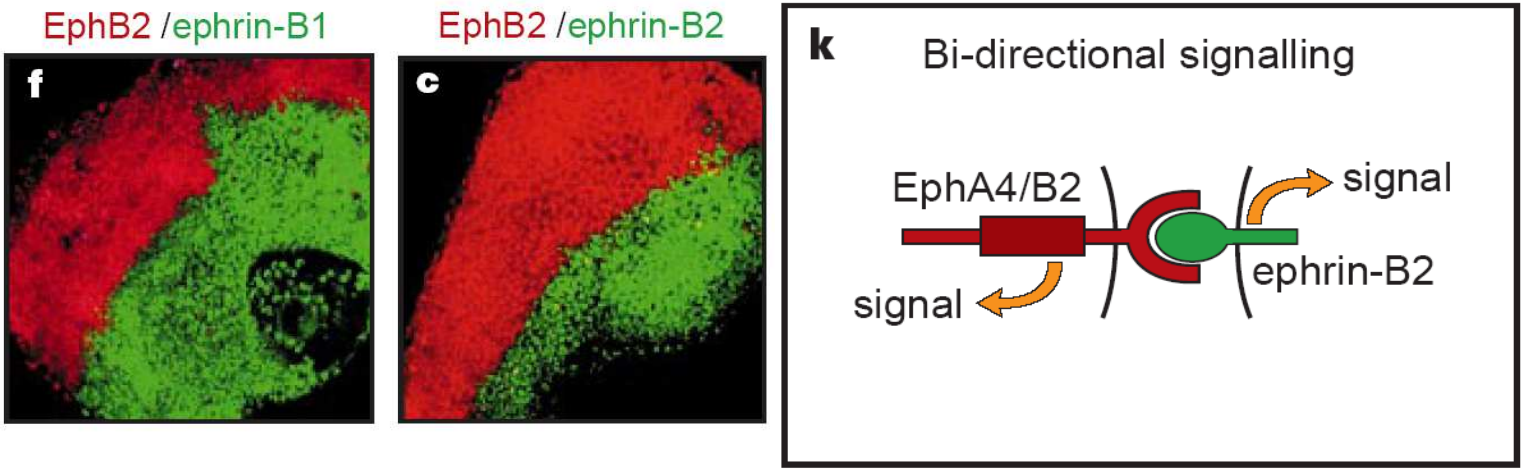
EphA4+ ΔEphB2
/ephrin-B1

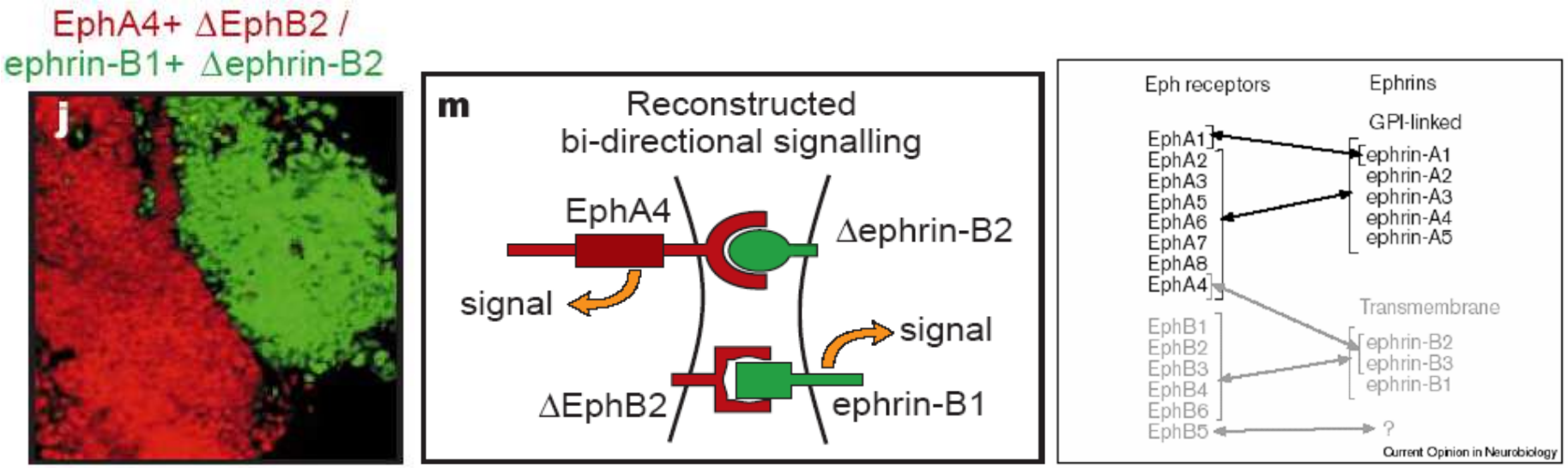




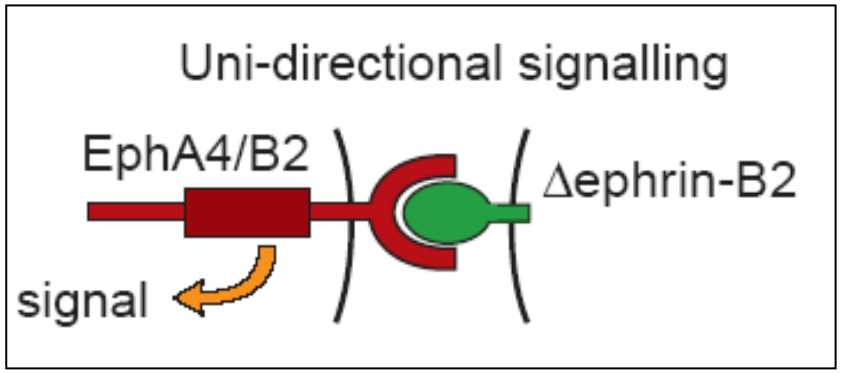


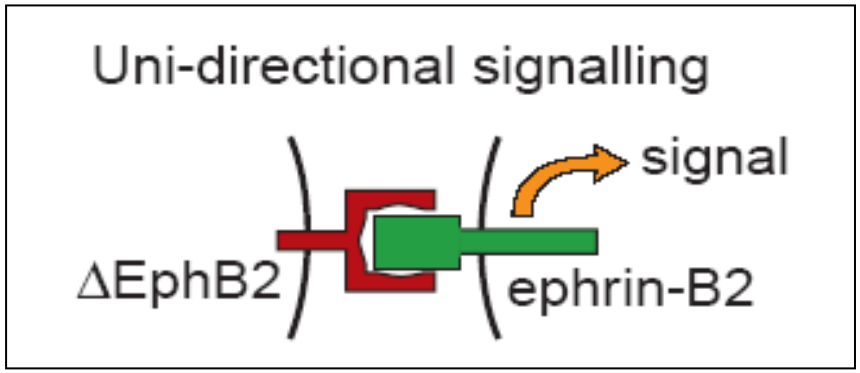




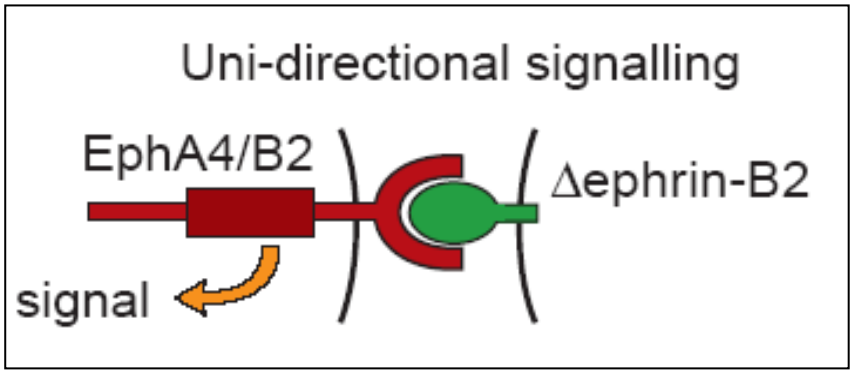


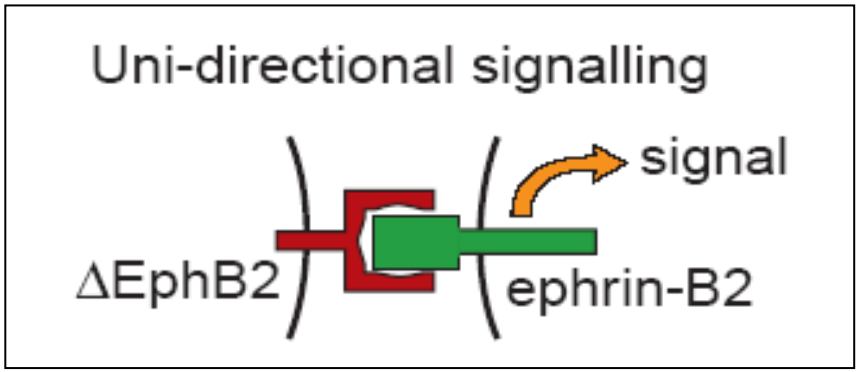
-> se la segnalazione è monodirezionale, ossia solo *forward* o solo *reverse*, non si ha il segnale repulsivo che inibisce il rimescolamento

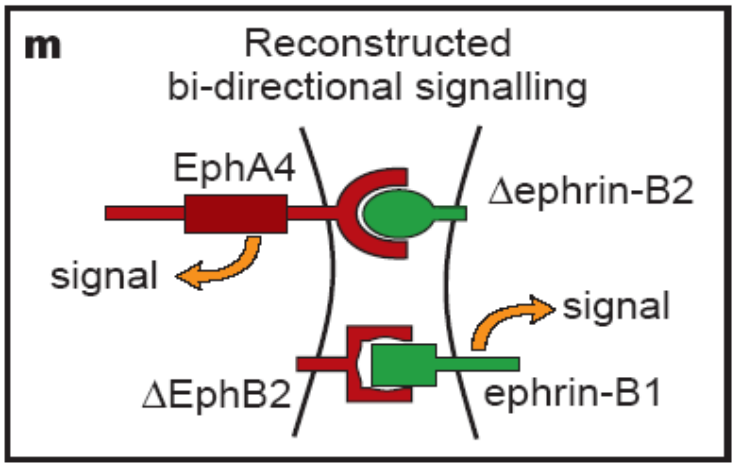


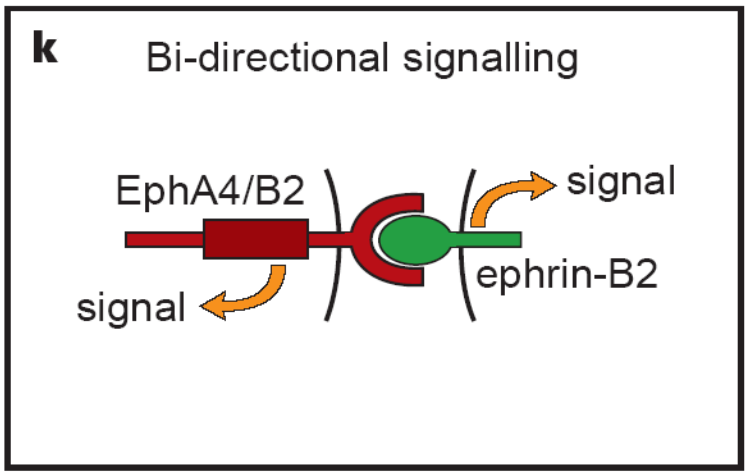


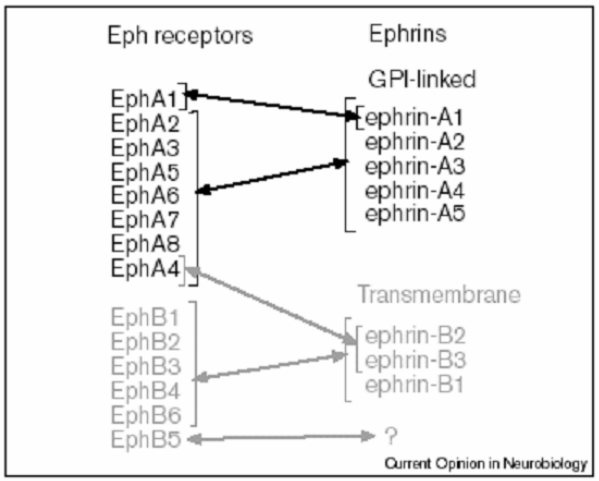
- come si può ricreare un segnale bidirezionale per verificare che la segnalazione bidirezionale sia la responsabile del non rimescolamento?

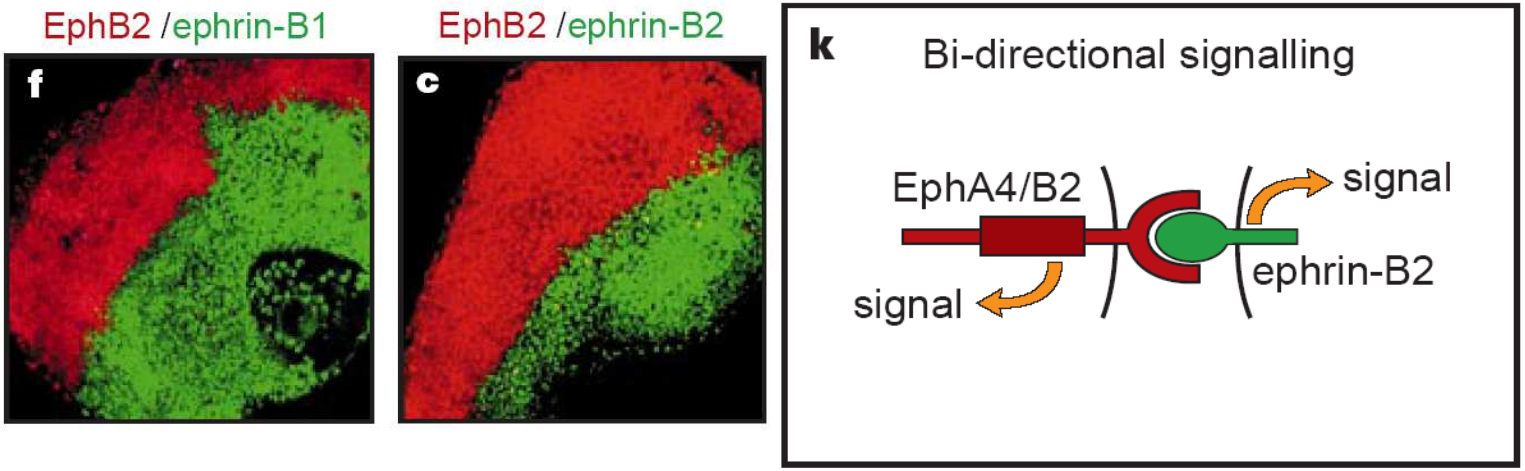


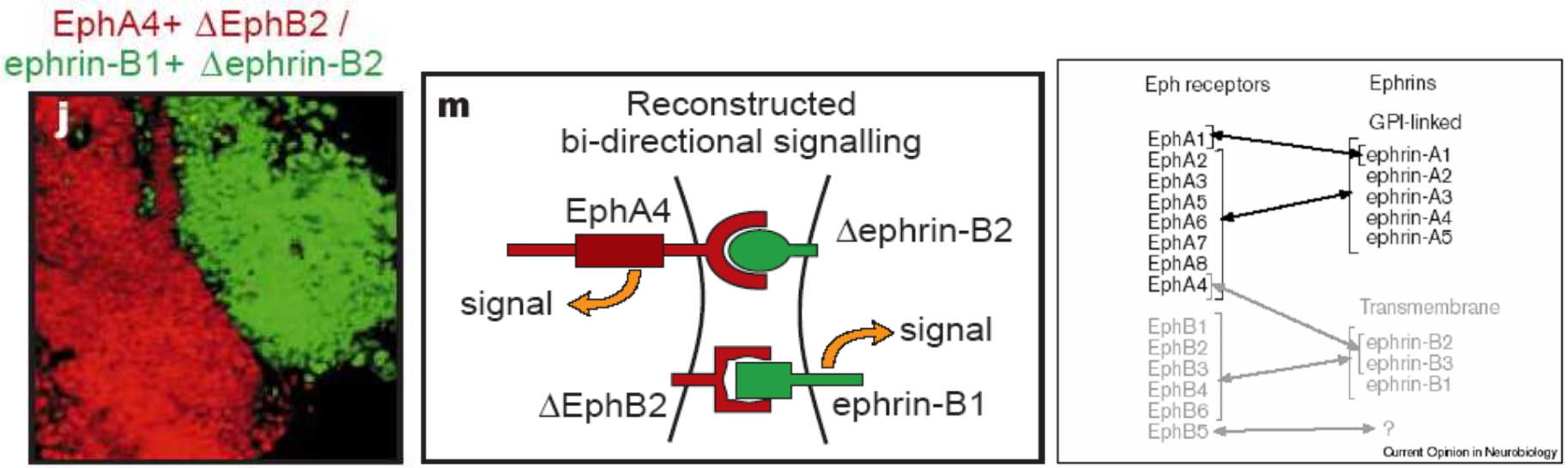








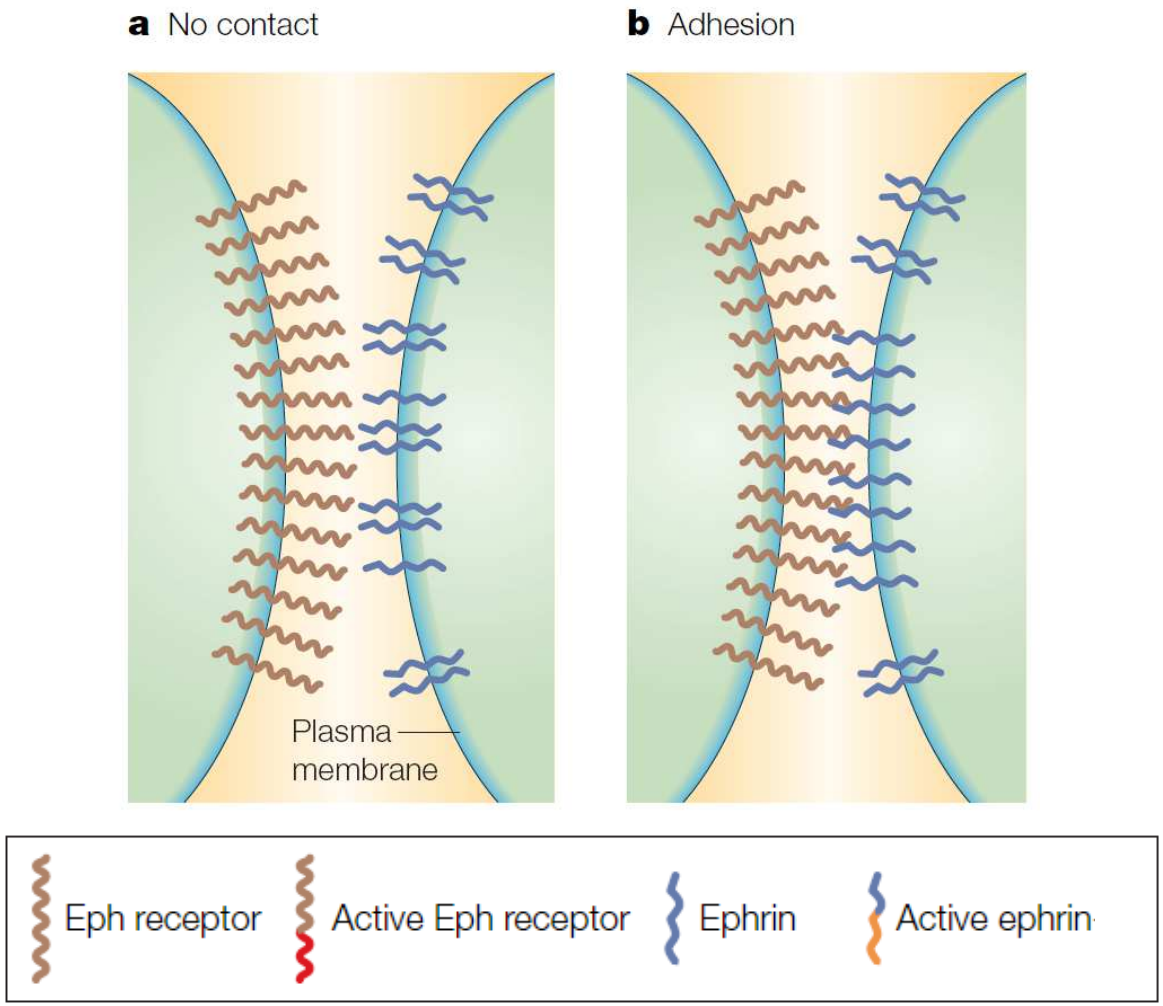




Paradoxes of Eph signalling

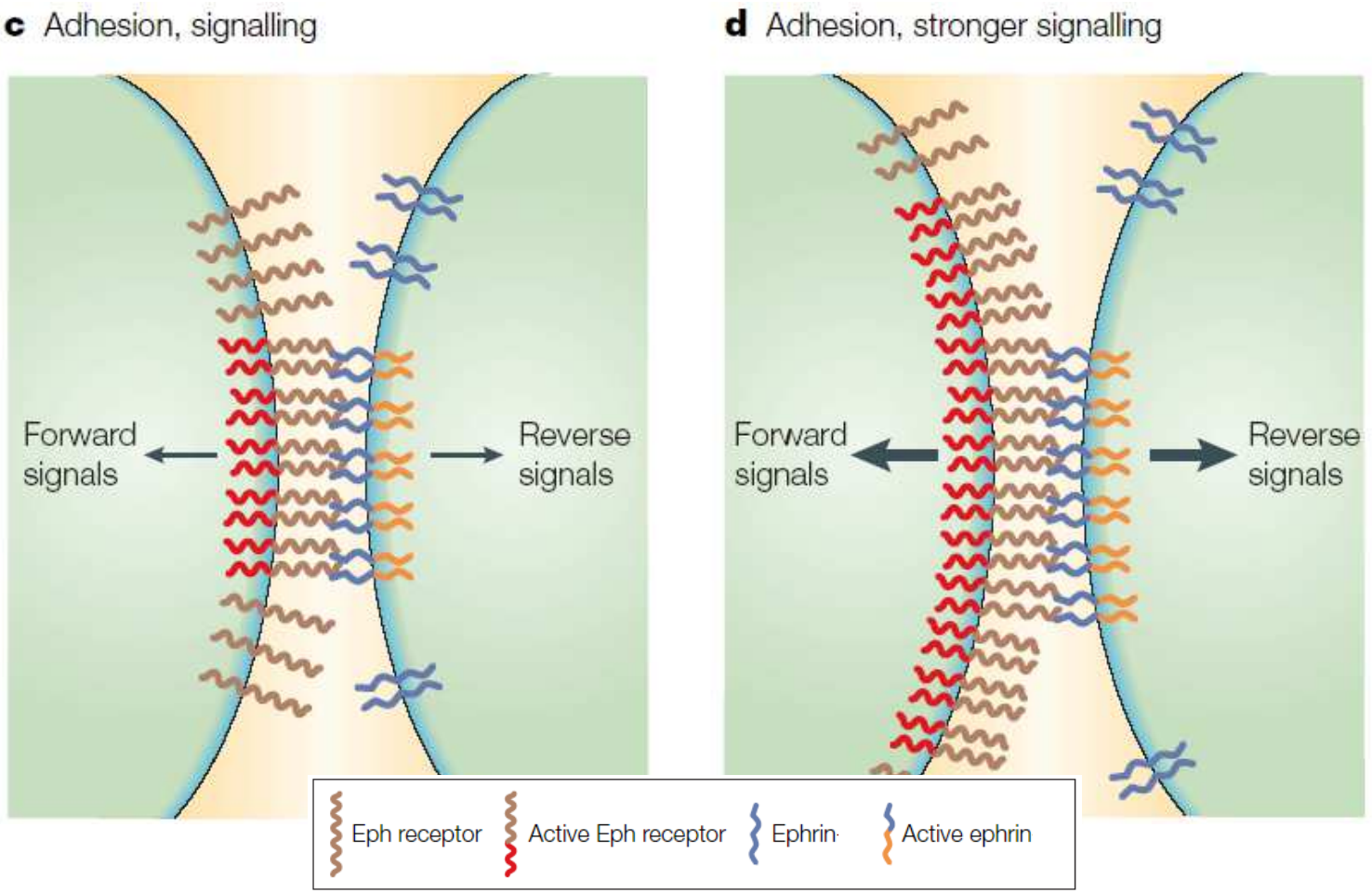
- **Paradox 1.** The interaction between Eph receptors and ephrins requires cell–cell contact and mediates strong cell adhesion, but often the ensuing signals induce the separation of the two cells.

Steps in cell-contact-dependent Eph bidirectional signalling

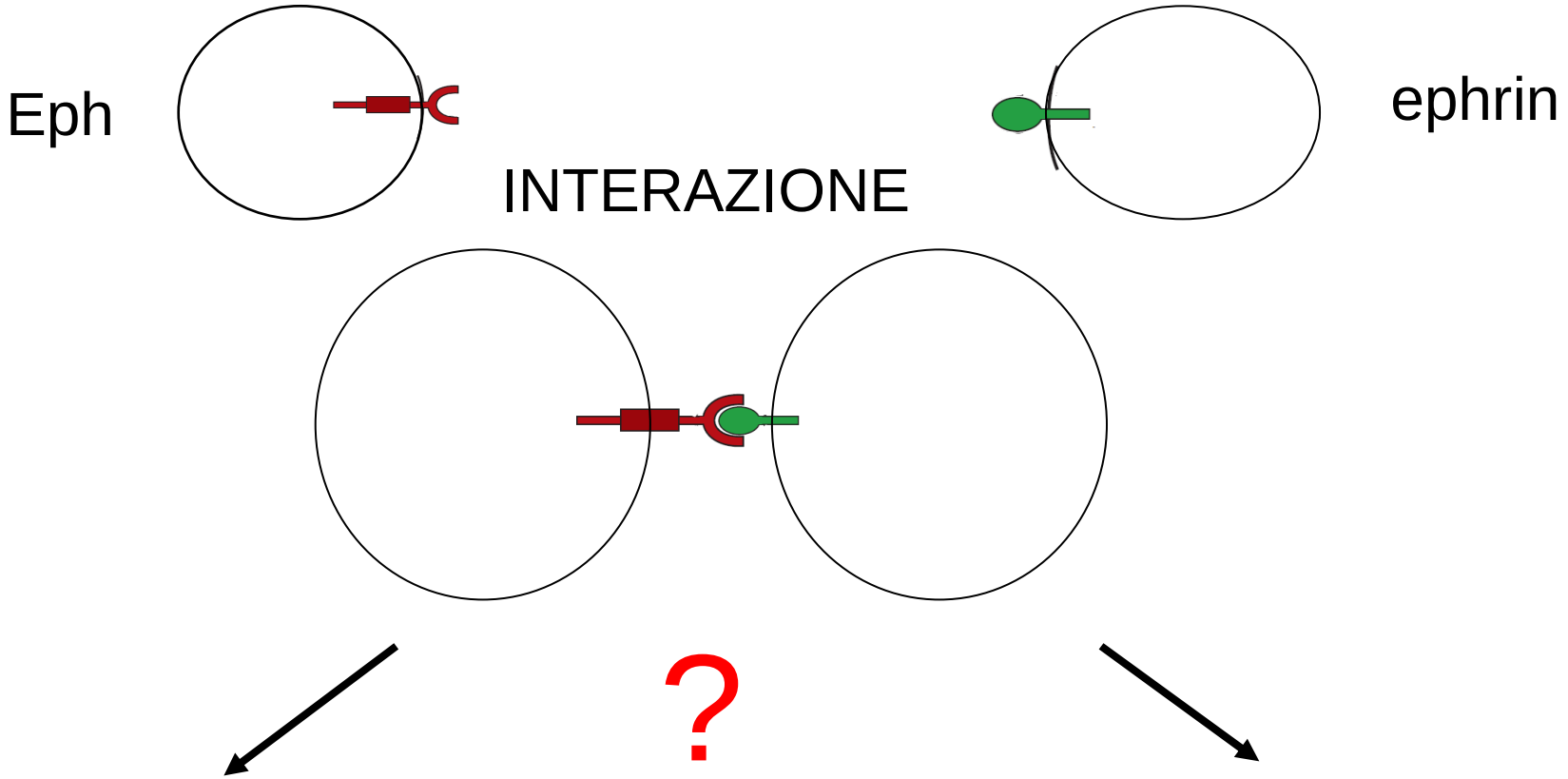


a,**b -** Eph receptors and ephrins on opposed cell surfaces mediate cell adhesion on cell contact.

Steps in cell-contact-dependent Eph bidirectional signalling



c - tetramerization leads to tyrosine phosphorylation and signalling.
d - the tetrameric complexes can further grow into larger clusters that, in the Eph receptor-expressing cells, can extend beyond the region of contact through homophilic interactions between Eph receptors. The degree of clustering might regulate signal intensity and the nature of the signals.



Come fanno due cellule a separarsi dopo l’interazione fra recettore e ligando?

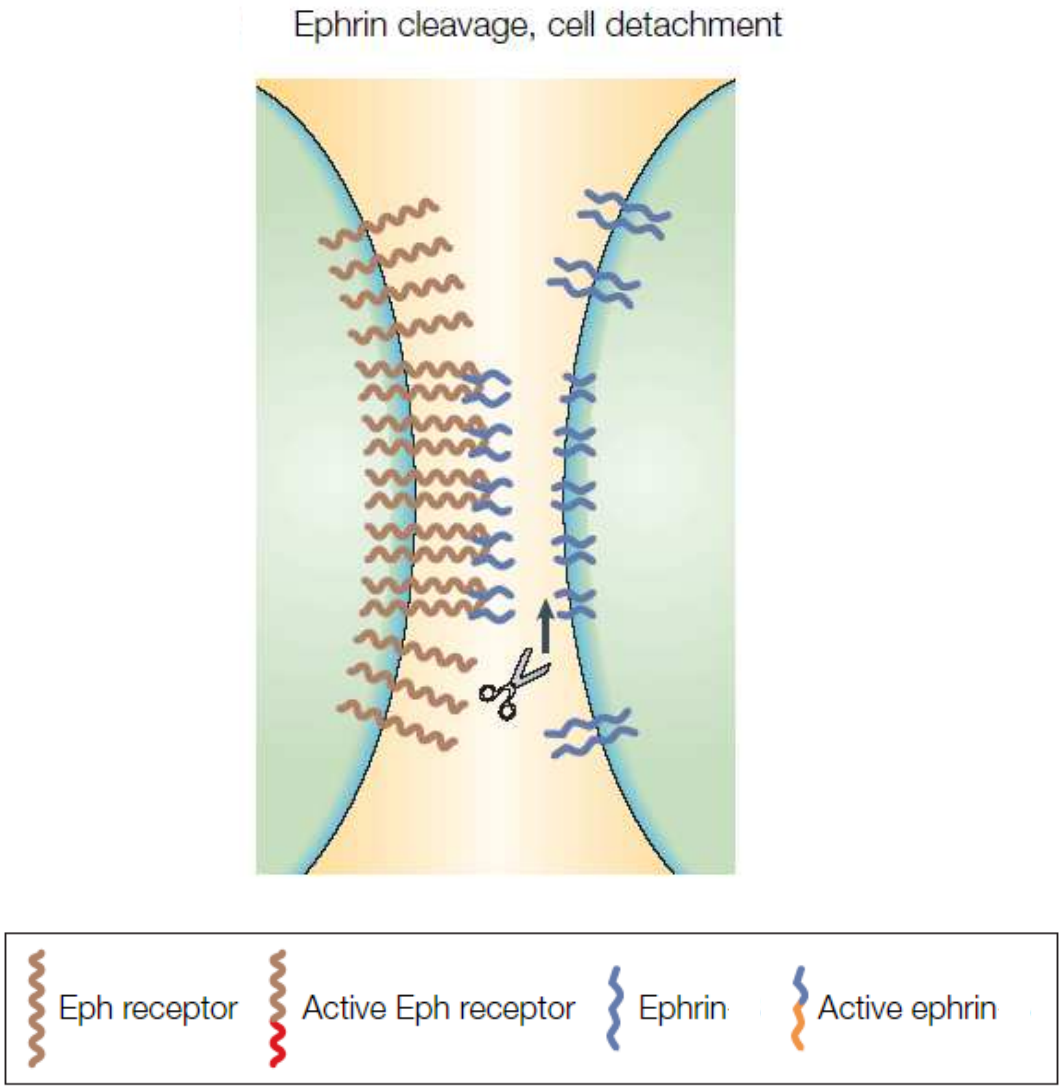
• many axon guidance molecules, including ephrins, netrins, semaphorins and slits, elicit repulsive responses when bound to their receptors; some of these factors are diffusible and growth cones respond to concentration gradients, whereas others, including the ephrins, are membrane-bound and repulsion 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

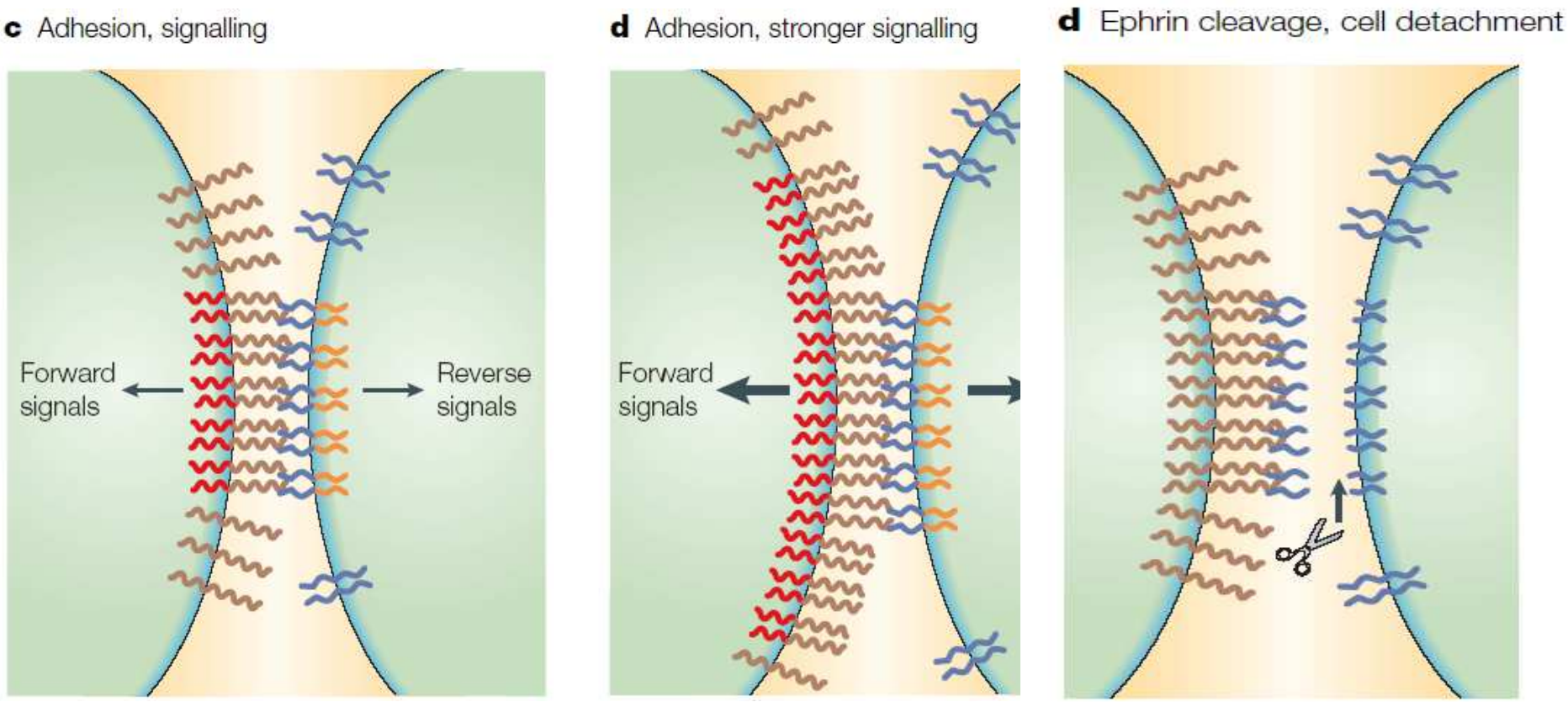
→ one mechanism that may remove ligand–receptor complexes from the cell surface is **PROTEOLYTIC CLEAVAGE**

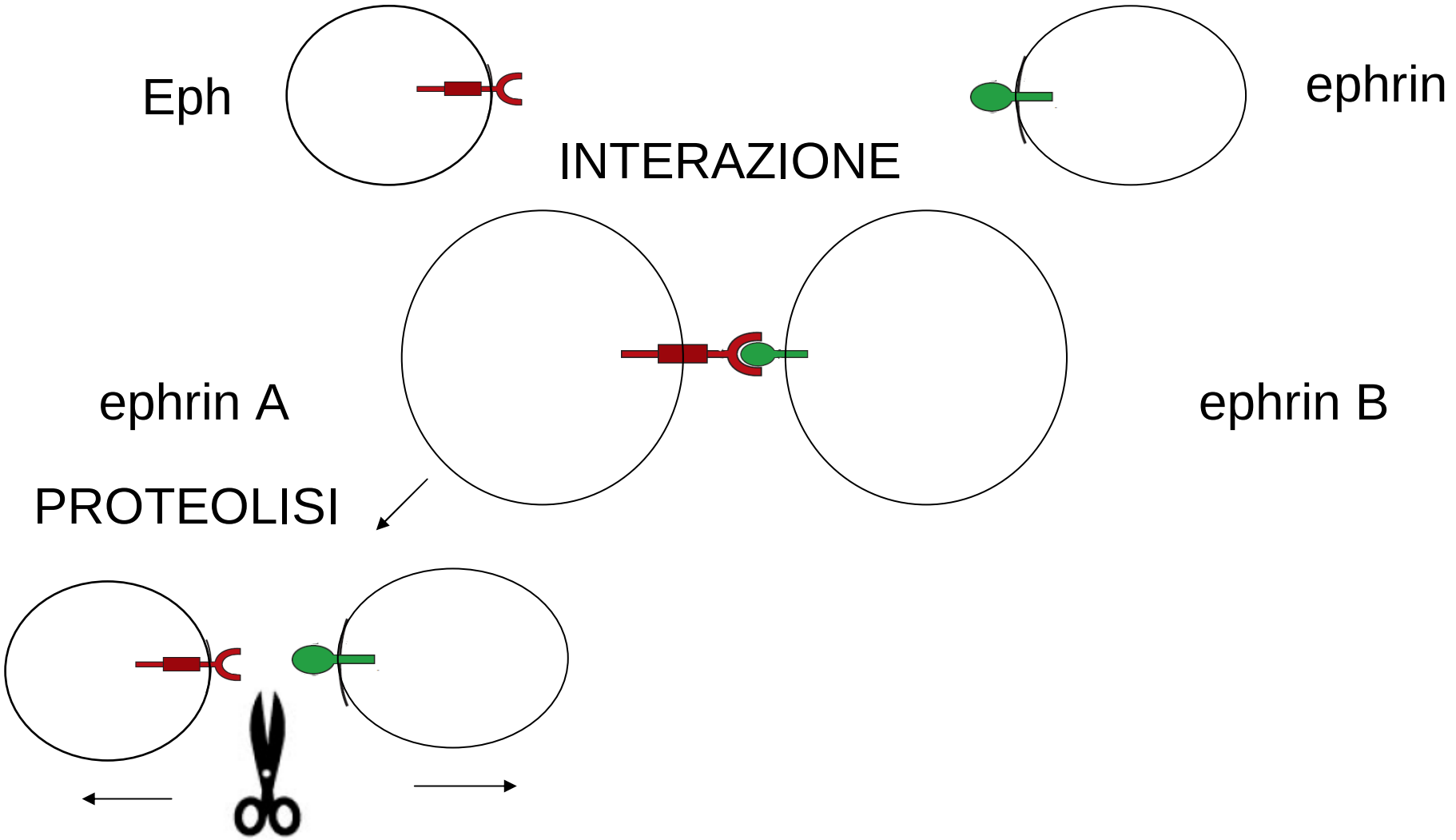
Mechanisms of Eph signal attenuation and termination.



Cleavage of the ephrin by a protease also allows cell separation following Eph–ephrin engagement.

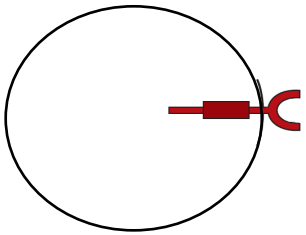
PROTEOLYTIC CLEAVAGE

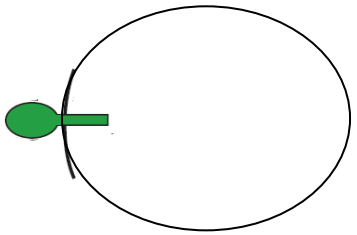




RISULTATO → REPULSIONE

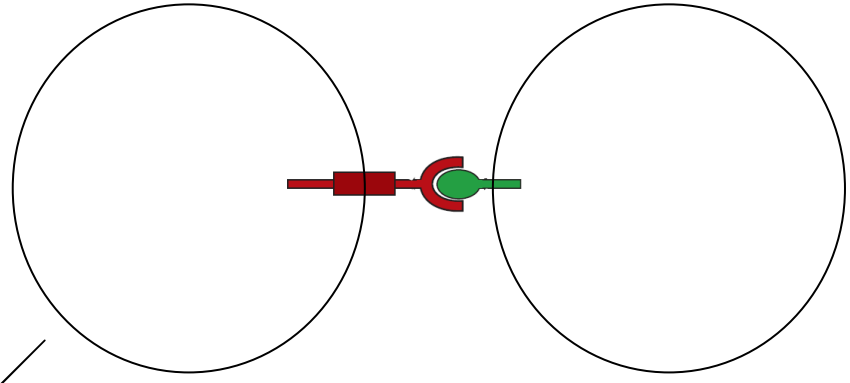
Eph





ephrin

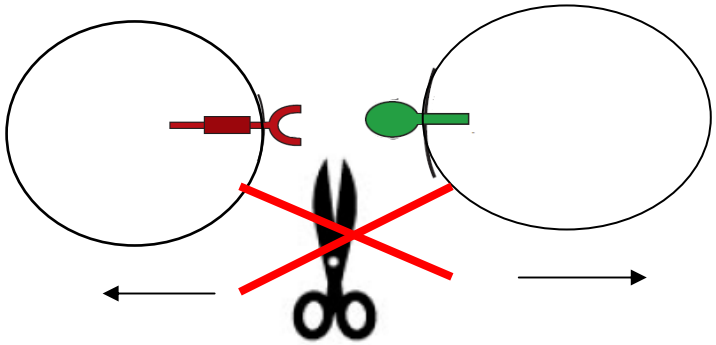
INTERAZIONE



ephrin A

ephrin B

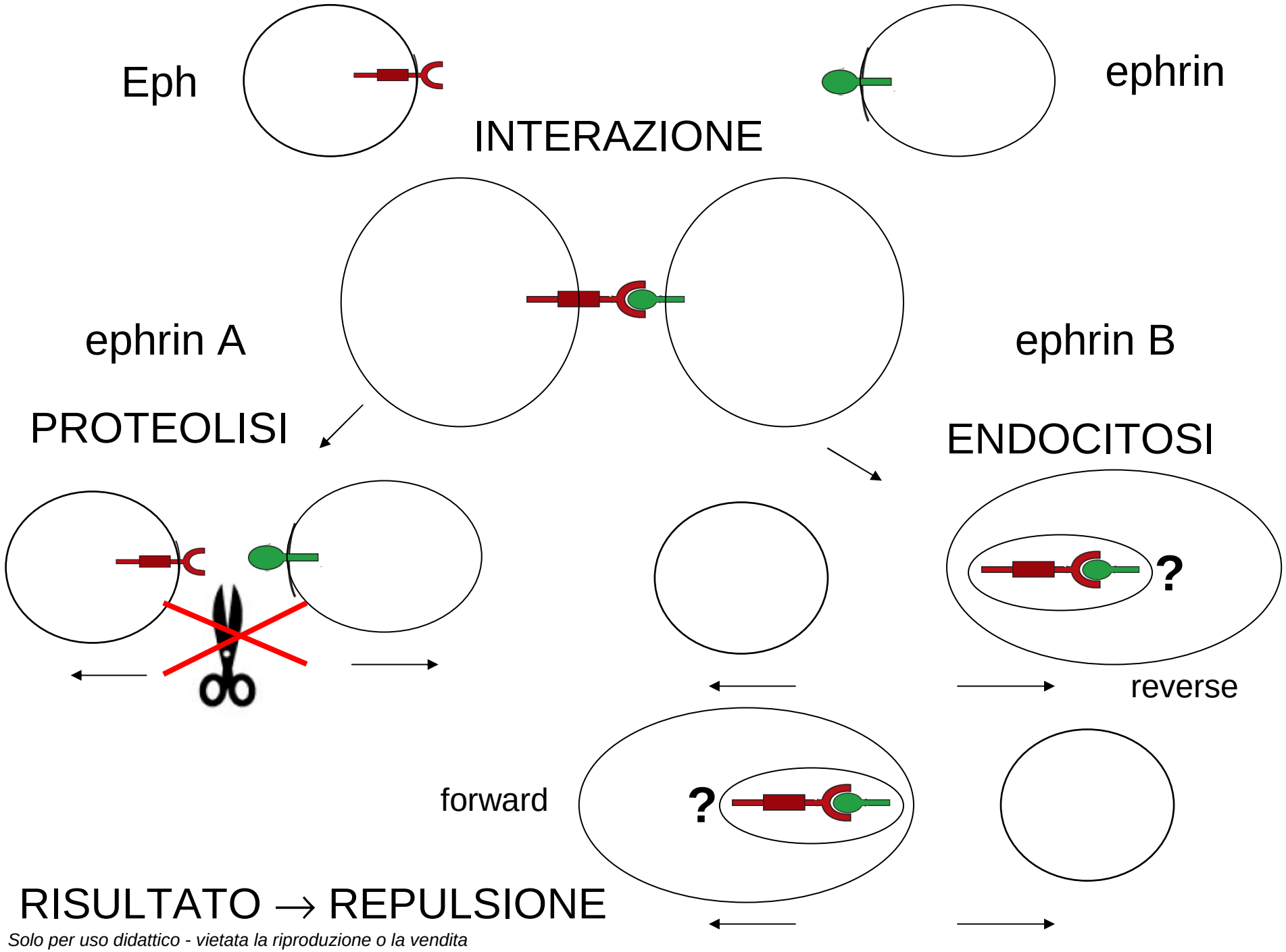
PROTEOLISI



?

Se non avviene il taglio enzimatico, come fanno le due cellule a separarsi?

RISULTATO → REPULSIONE



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

Trans-endocytosis

• endocytosis of protein complexes involving the intercellular (trans) interaction of two transmembrane proteins is unusual and rarely documented in the literature

• in *Drosophila melanogaster* the seven transmembrane ligand, Boss, is internalized into the R7 photo-receptor precursor cell after trans-interaction with the sevenless (sev) tyrosine kinase receptor
- the entire Boss protein enters the sev-expressing cell and endocytosis occurs only in forward direction

• the receptor patched-1 (Ptc-1) is able to retrieve membrane-bound forms of sonic hedgehog (Shh) from adjacent cells, a process that is uni-directional

• Notch receptor binding to its membrane-anchored ligand, Delta, triggers proteolytic shedding of the Notch ectodomain and endocytosis of the Notch-Delta protein complex into the Delta-expressing cell. Notch endocytosis into the Notch-expressing cell also occurs but after a second cleavage event. In this case endocytosis is bi-directional, but involves proteolytic cleavage of one of the proteins.

EphB–ephrinB bi-directional endocytosis terminates adhesion allowing contact mediated repulsion

Manuel Zimmer[1], Amparo Palmer[1], Jenny Köhler[1] and Rüdiger Klein[1,2]

NATURE CELL BIOLOGY VOLUME 5 | NUMBER 10 | OCTOBER 2003

- Eph receptors and their membrane-associated ephrin ligands mediate cell–cell repulsion to guide migrating cells and axons
- repulsion requires that the ligand–receptor complex be removed from the cell surface, for example by PROTEOLYTIC PROCESSING of the ephrin ectodomain
- cell contact-induced EphB–ephrinB complexes are rapidly ENDOCYTOSED during the retraction of cells and neuronal growth cones
- ENDOCYTOSIS occurs in a bi-directional manner that comprises of full-length receptor and ligand complexes
- ENDOCYTOSIS is sufficient to promote cell detachment and seems necessary for axon withdrawal during growth cone collapse
- this is a mechanism for the termination of adhesion and the promotion of cell repulsion after intercellular (trans) interaction between two transmembrane proteins