

Axon guidance

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Complexity of neural circuits



Kirksville Osteopathic College, 1920s



Complexity of neural circuits



Kirkville Osteopathic College, 1920s

- billions of neurons in adult humans (located in CNS and PNS)
- many different types of neurons, each with their own synaptic targets
- axon tracts with lengths of micrometers to over one meter
- unique axon tracts with specific turns, midline crosses etc.
- simultaneous formation of many different axon tracts (both ascending and descending)
-

Mechanisms of neural circuit development



- Neurite formation & polarity
- Neurite growth
- **Axon guidance**
- Axon fasciculation & pruning
- Target innervation
- Synapse formation
-

Neural circuit disease



Autism



ALS



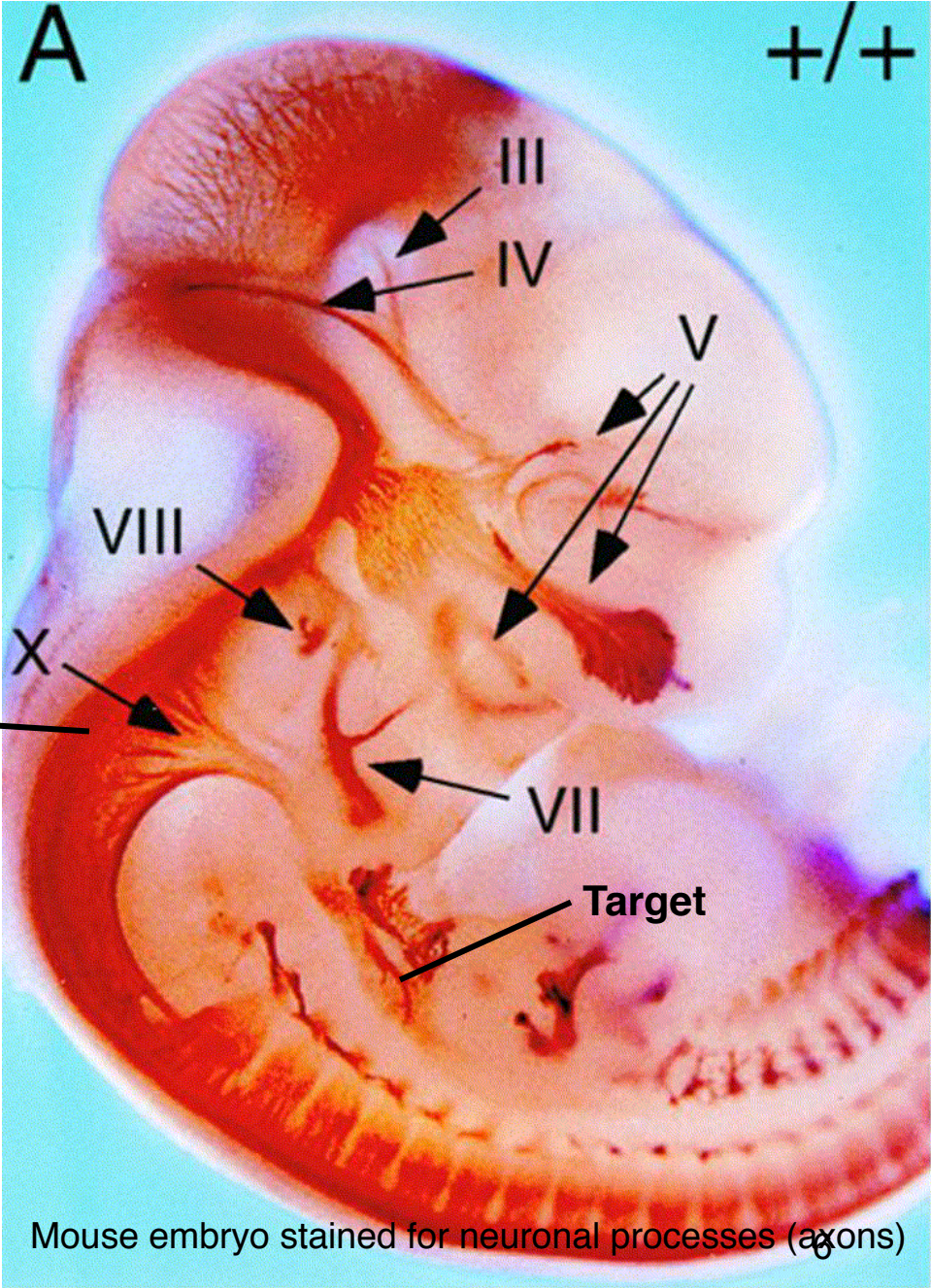
Epilepsy



Schizophrenia

Axon guidance

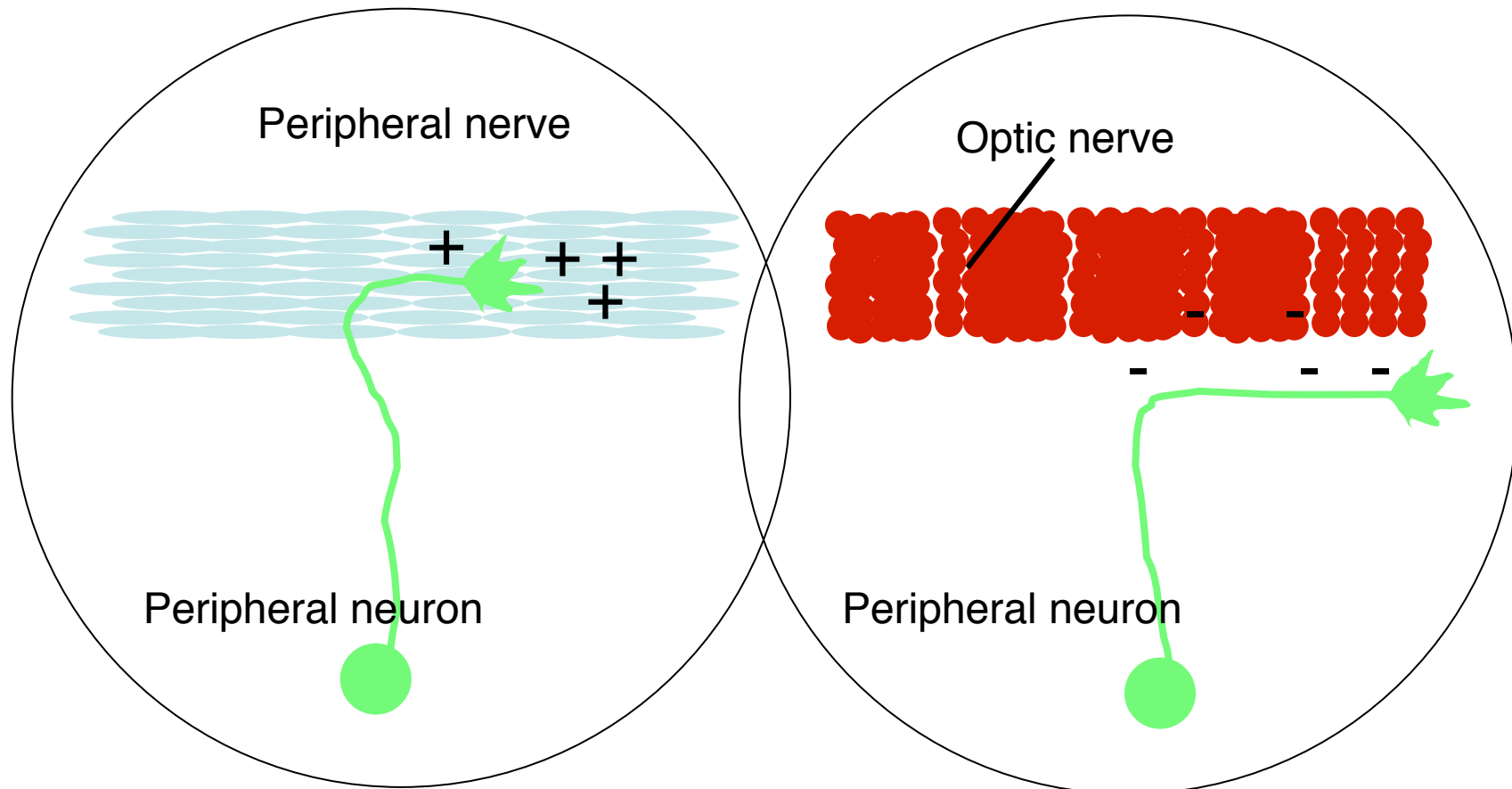
Neuronal cell bodies



“Process during which axonal processes find their way from the neuronal cell body to target structures elsewhere in or outside the nervous system”

Mouse embryo stained for neuronal processes (axons)

Identification of guidance activities



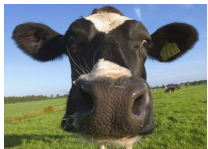
Axons are attracted by certain tissues but repelled by others

Identification of guidance activities and axon guidance proteins: an example of a biochemical purification

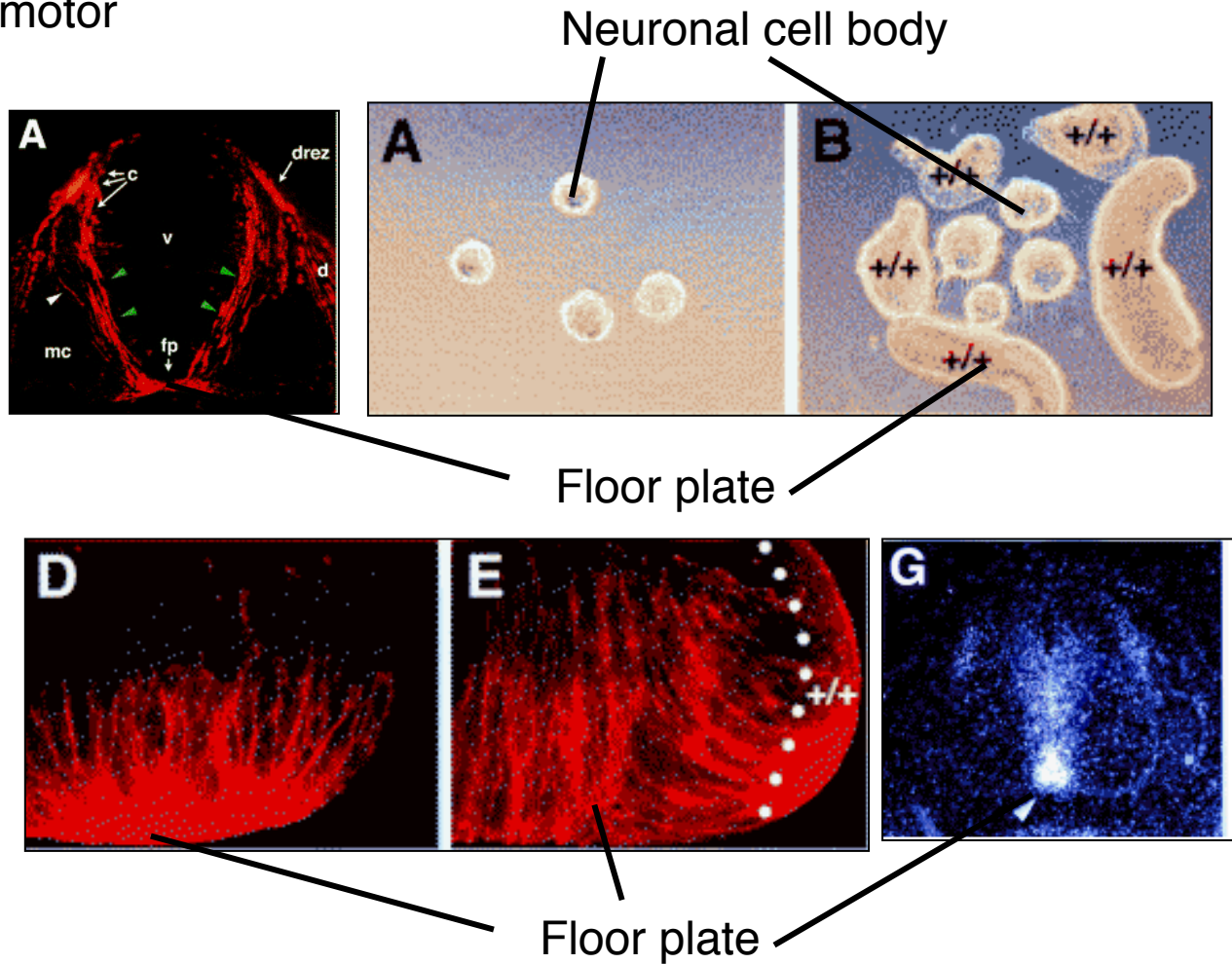
Model system: spinal cord
commissural axons (locomotor activity)

In vitro assays
(secreted attractant)

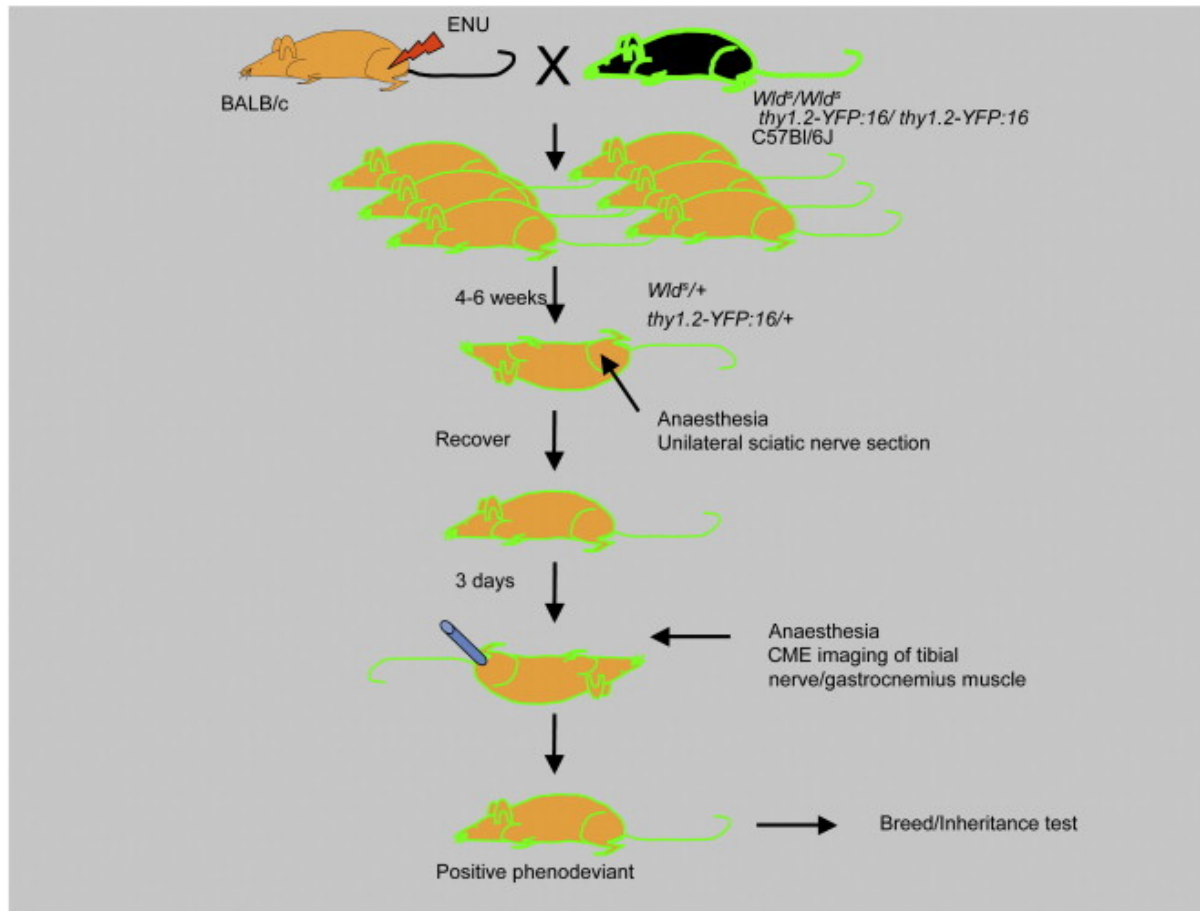
Biochemical identification
(cow)



Identification protein and
gene sequence
(Netrin)



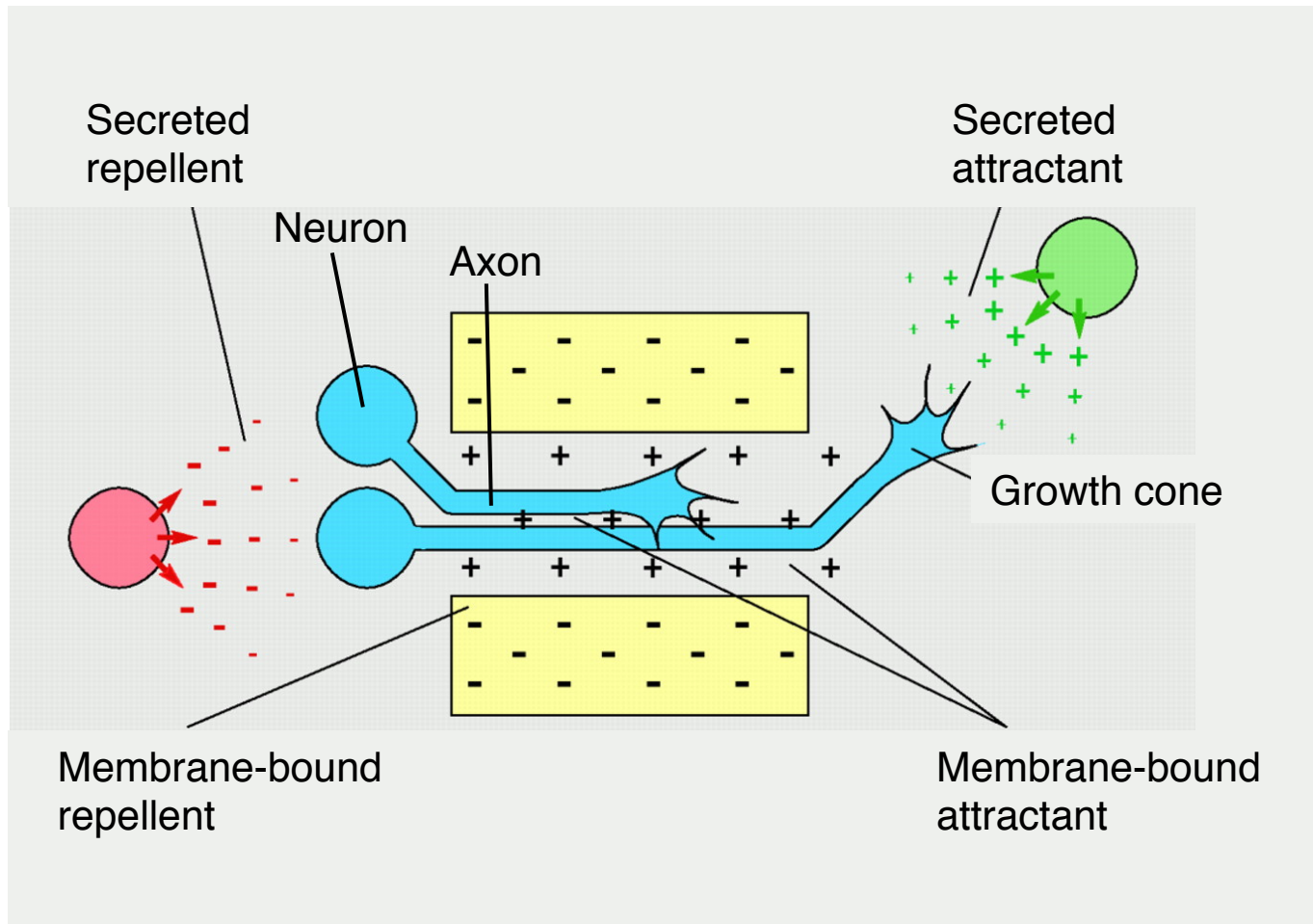
Genetic approaches



Model organisms:

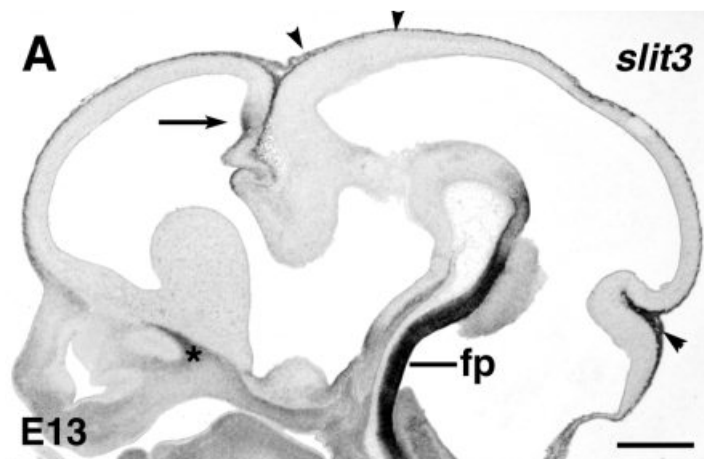
- C. Elegans
- Drosophila
- Mouse

Principles of axon guidance

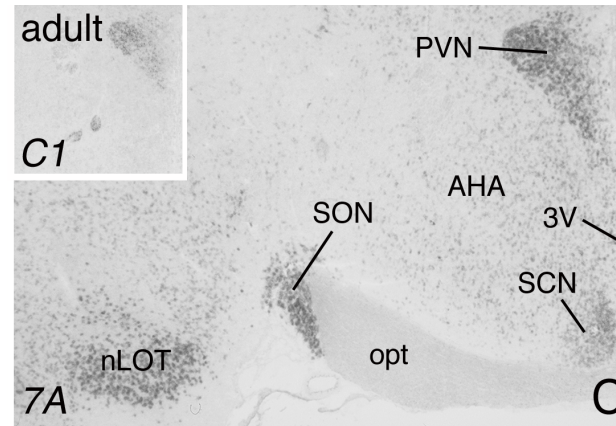


Tessier-Lavigne & Goodman, 1996, Science

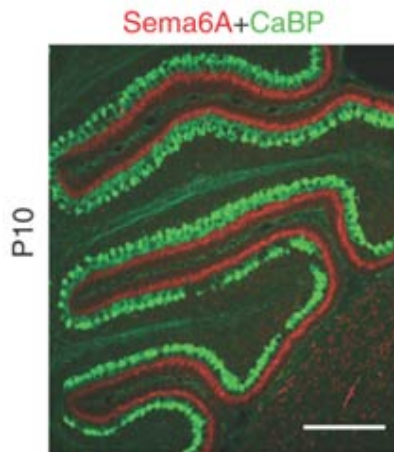
Axon guidance proteins are present and function in every neural system



Marillat et al., 2005

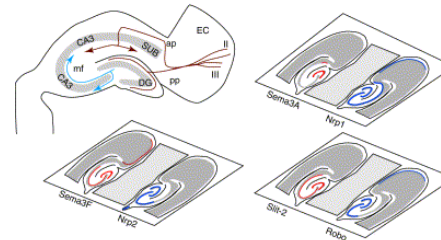


Pasterkamp, 2007

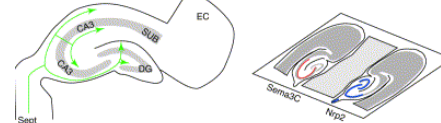


Kerja et al., 2006

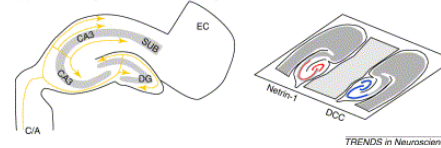
(a) Entorhinal and mossy fiber projection



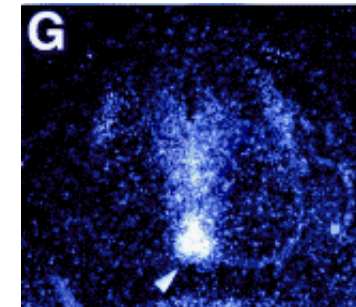
(b) Septal projection



(c) Excitatory commissural projection

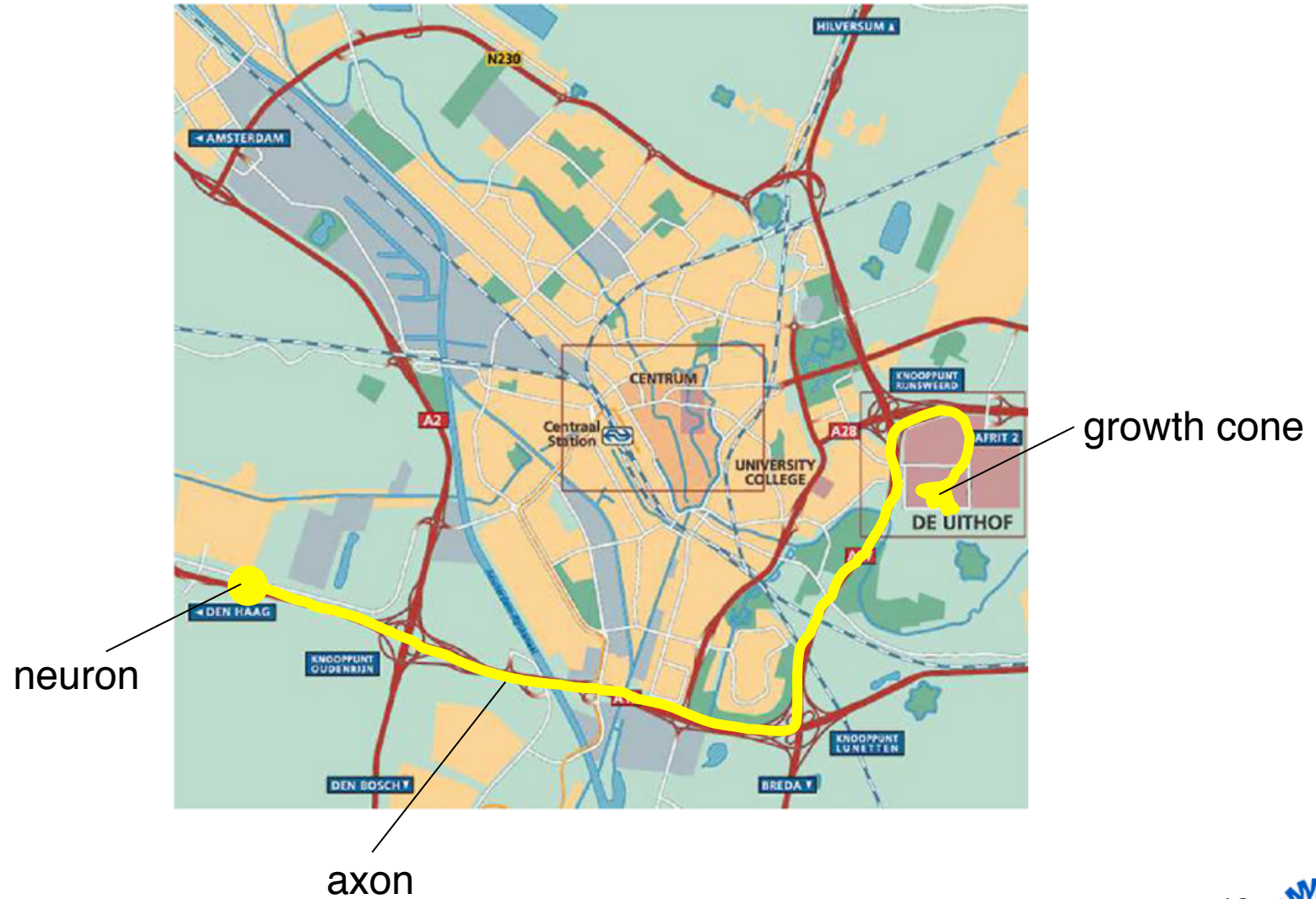


Skutella & Nitsch, 2001



Kennedy et al., 1994

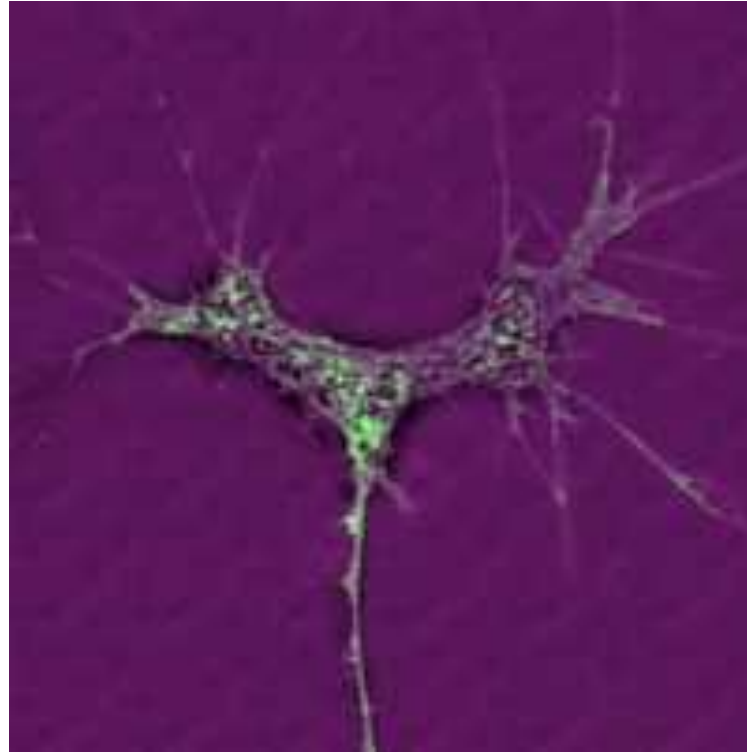
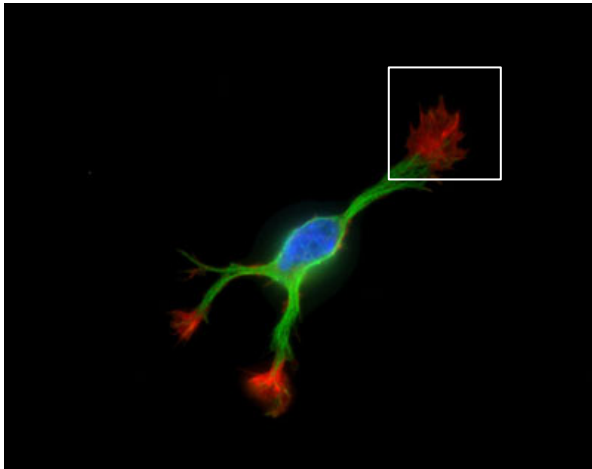
Axon guidance proteins form a molecular road map



Overview

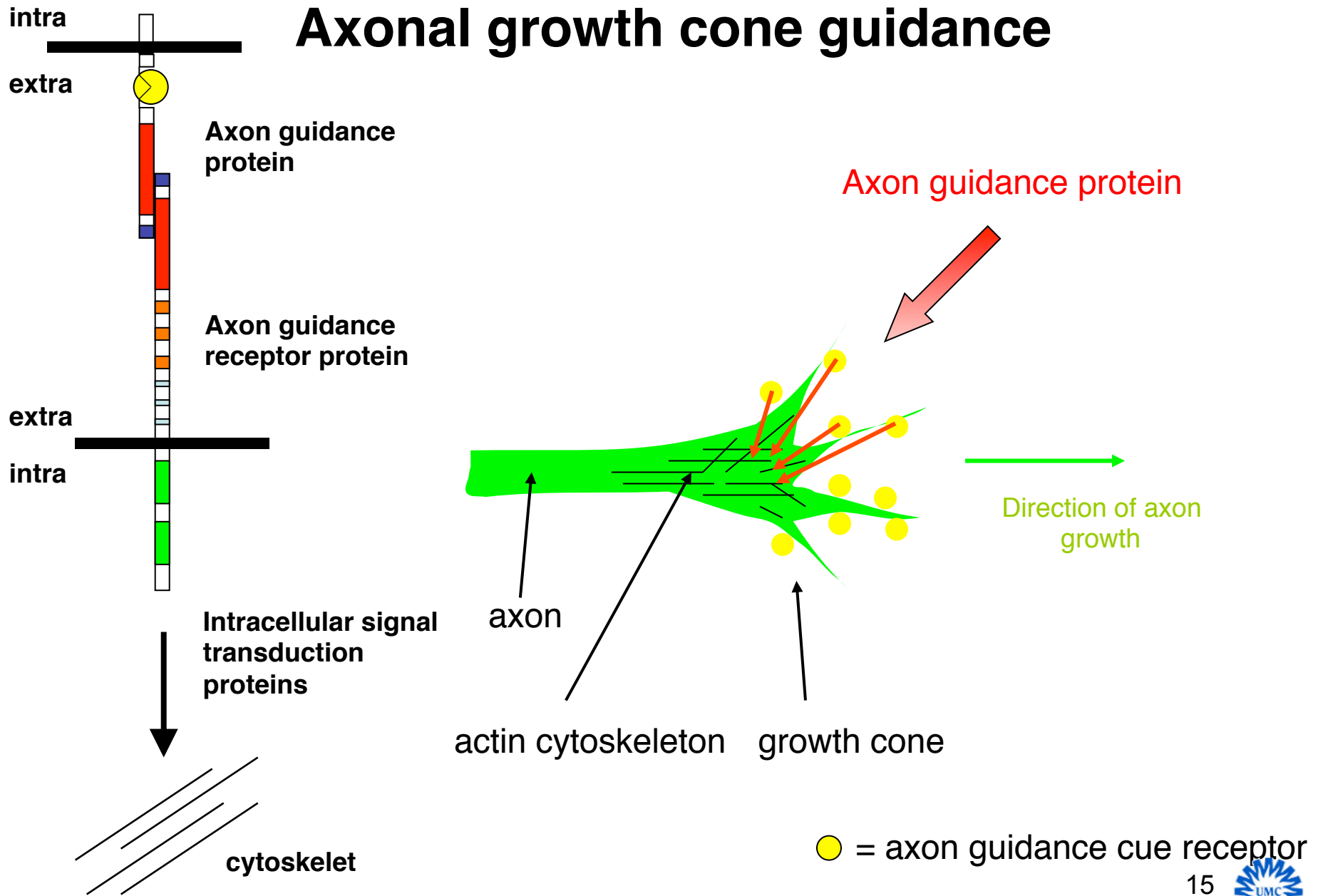
- Growth cones as integrators of positional information
- Which families of axon guidance proteins and receptors have been identified?
- What are the principles of axon guidance (in vitro and in vivo examples)?
- How are axon guidance receptors regulated?
- What does research on axon guidance look like?

Growth cones are the eyes of growing axons

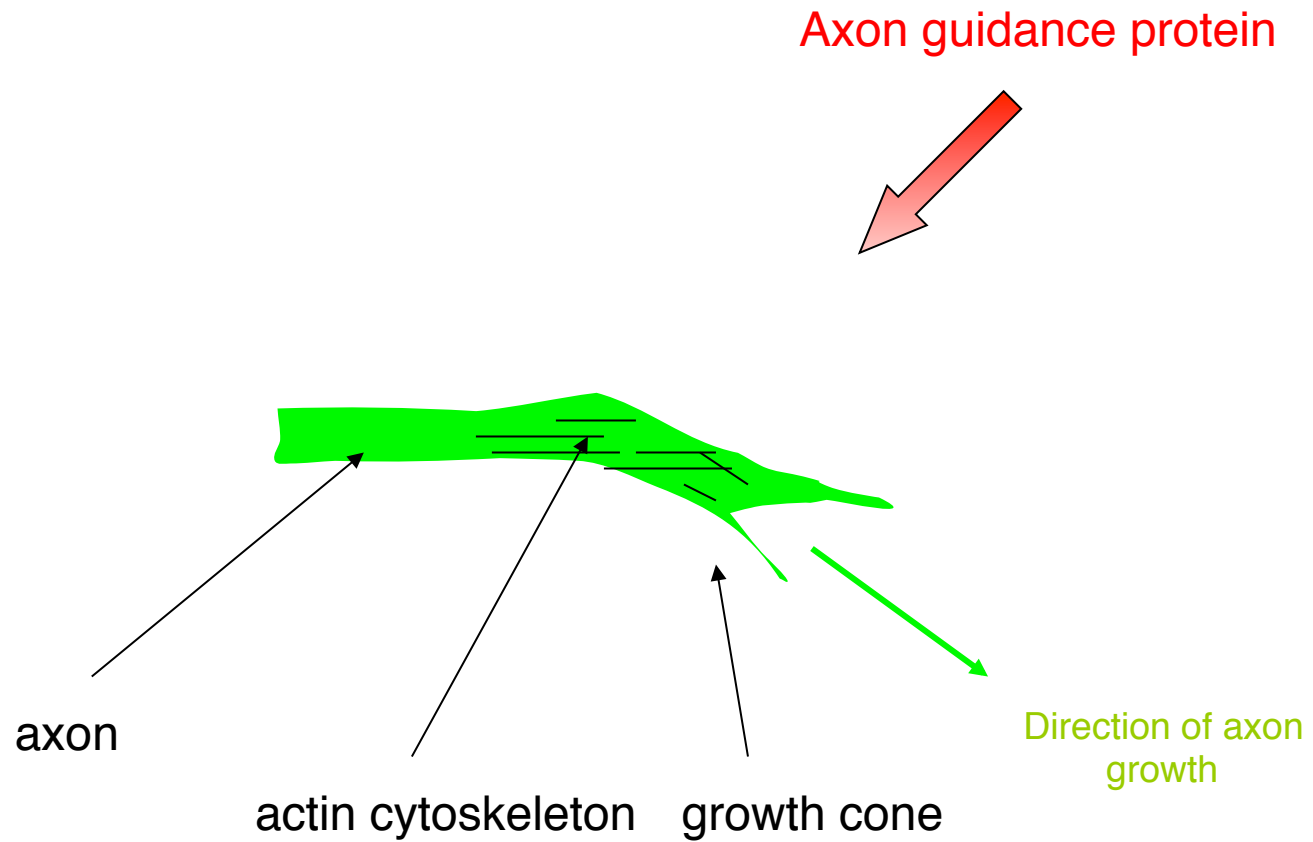


Growth cones continuously sample the outside environment for axon guidance cues and react to these cues by morphological changes that affect the direction of axonal growth

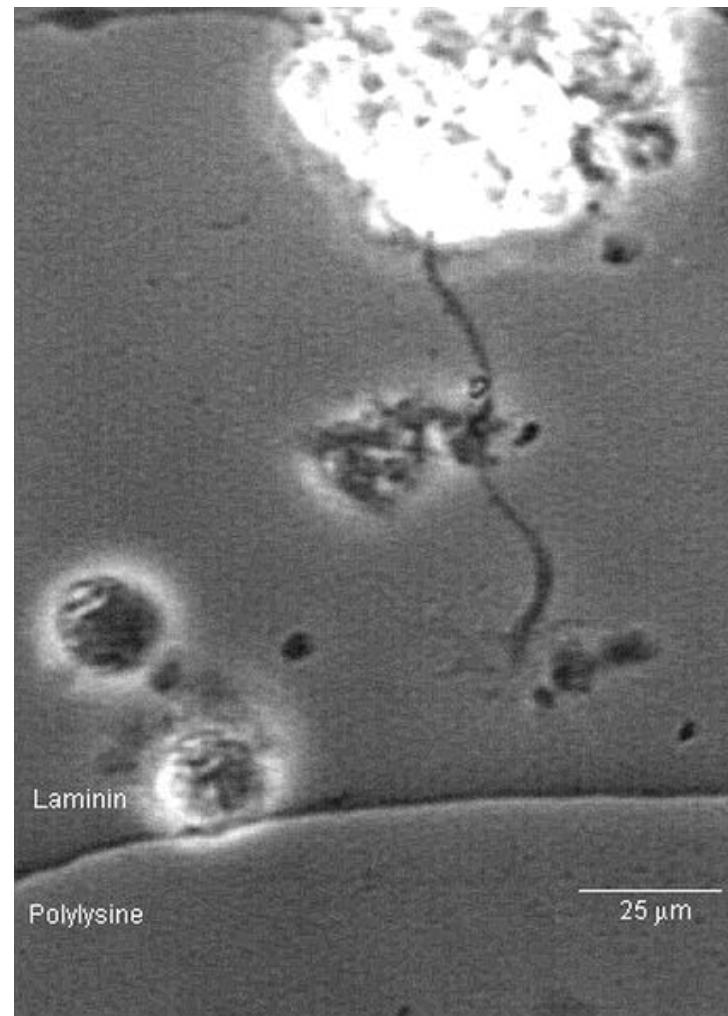
Axonal growth cone guidance



Axonal growth cone guidance



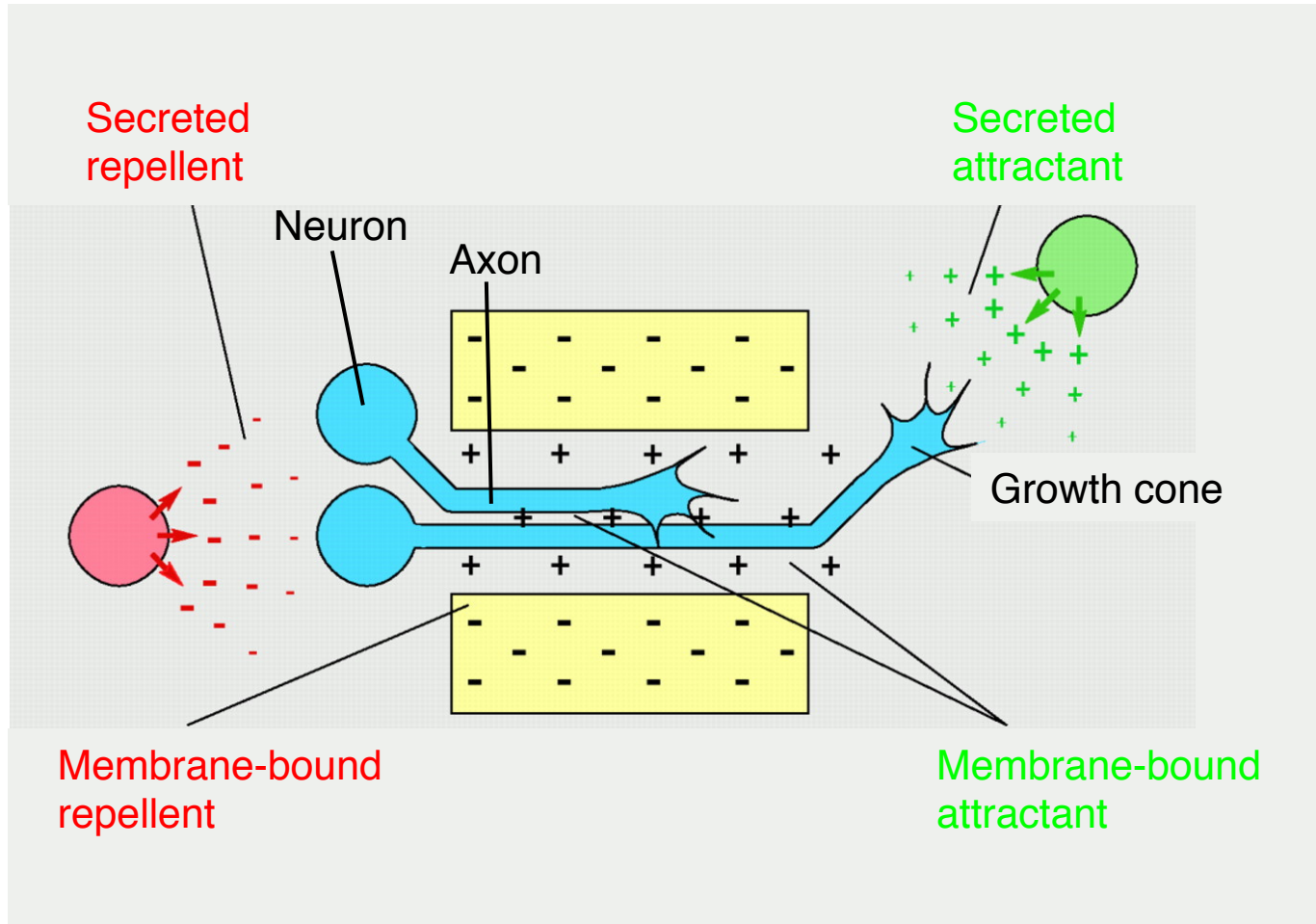
Growth cones sense the molecular environment



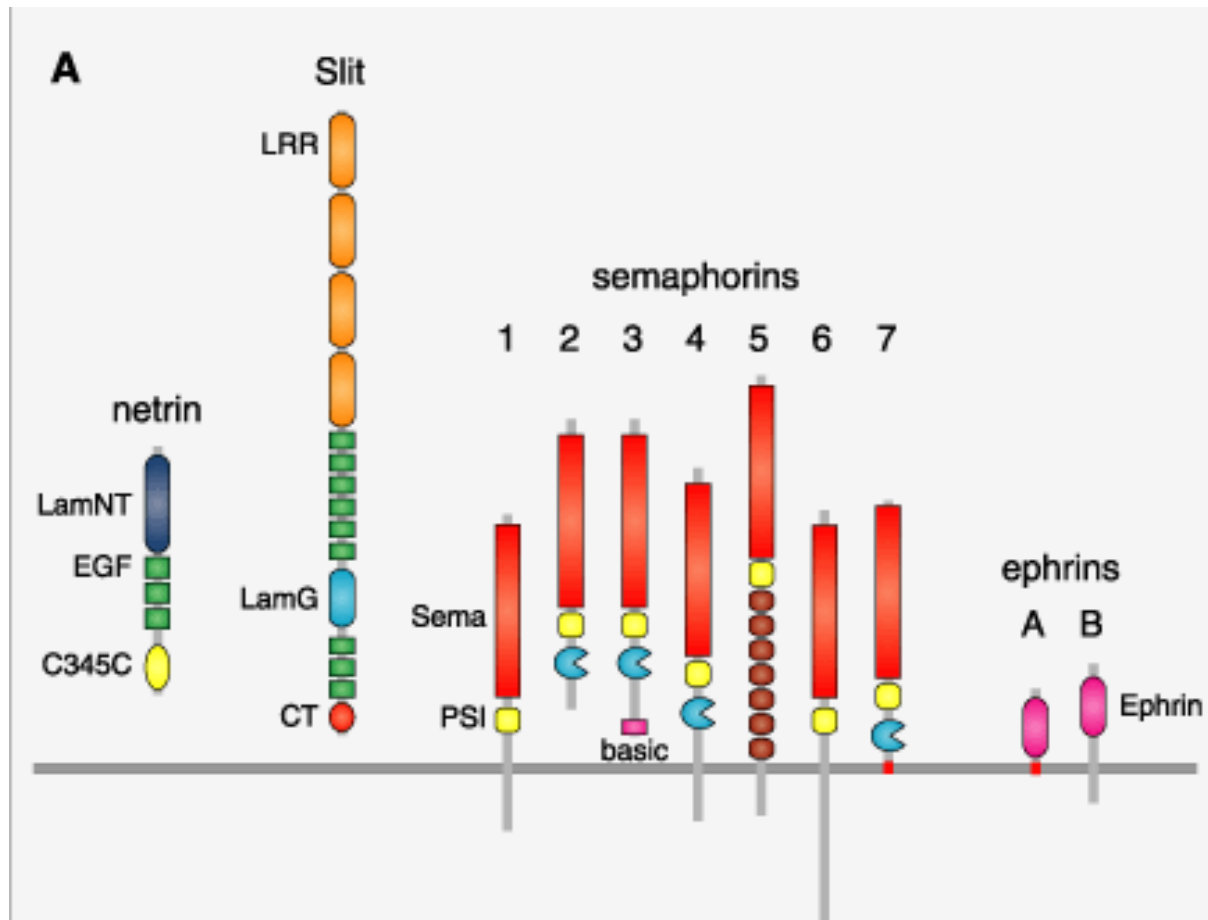
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Principles of axon guidance



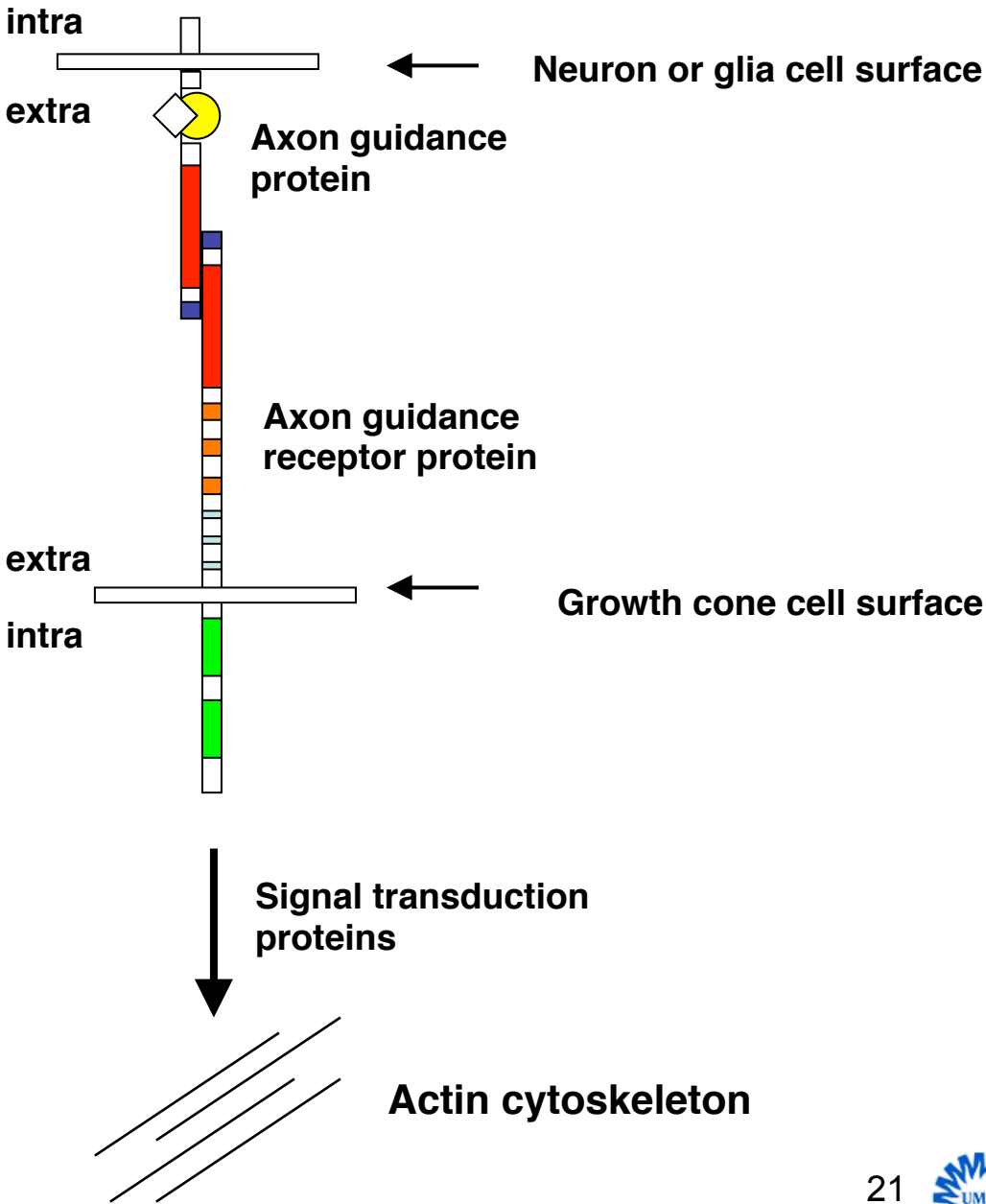
Canonical axon guidance proteins



Fifth family: RGMs

Schmidt, Van den Berg, Pasterkamp, 2009

Specialized receptors detect axon guidance cues



Axon guidance receptors

Netrin

DCC, UNC-5

Slit

Robo1, 2, and 3

Semaphorin

Plexin

Plexin, Met

Plexin, OTK

Plexin, Neuropilin, L1

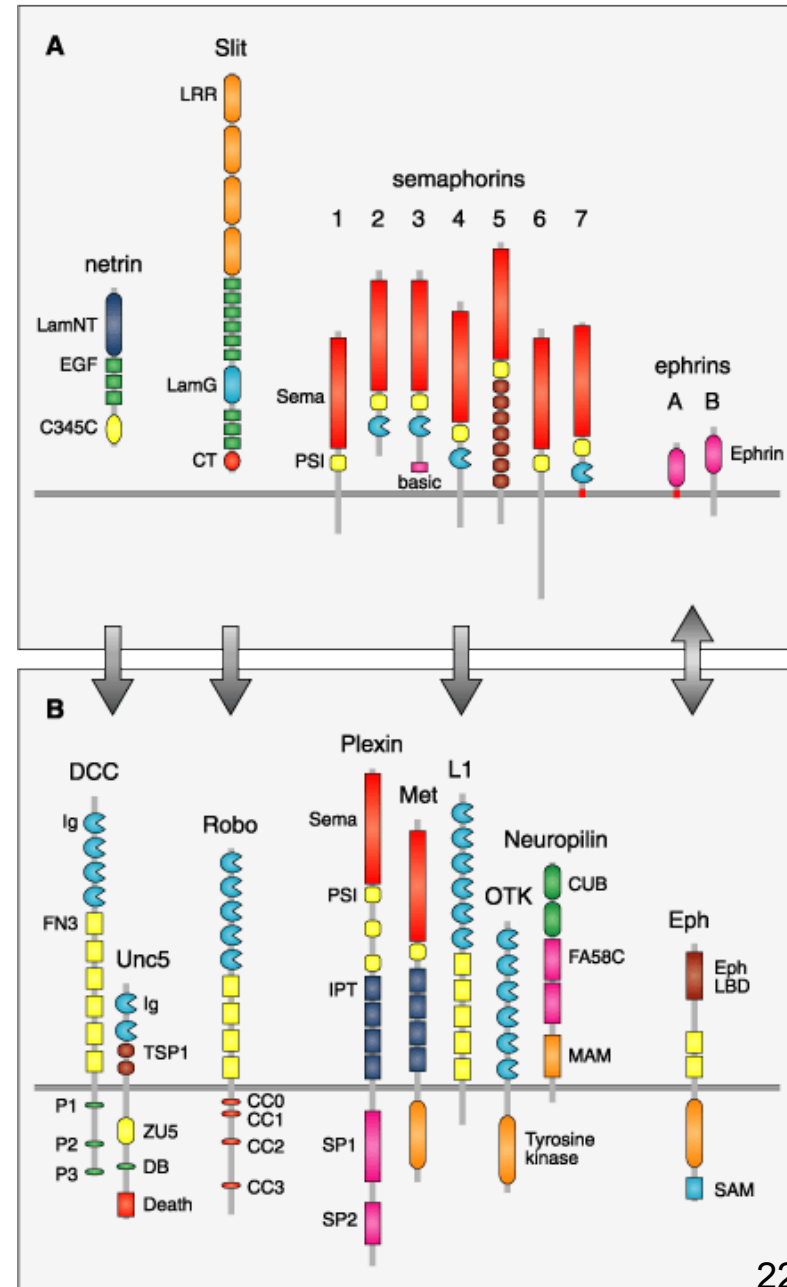
Integrin

Ephrin

EphA and B

RGM

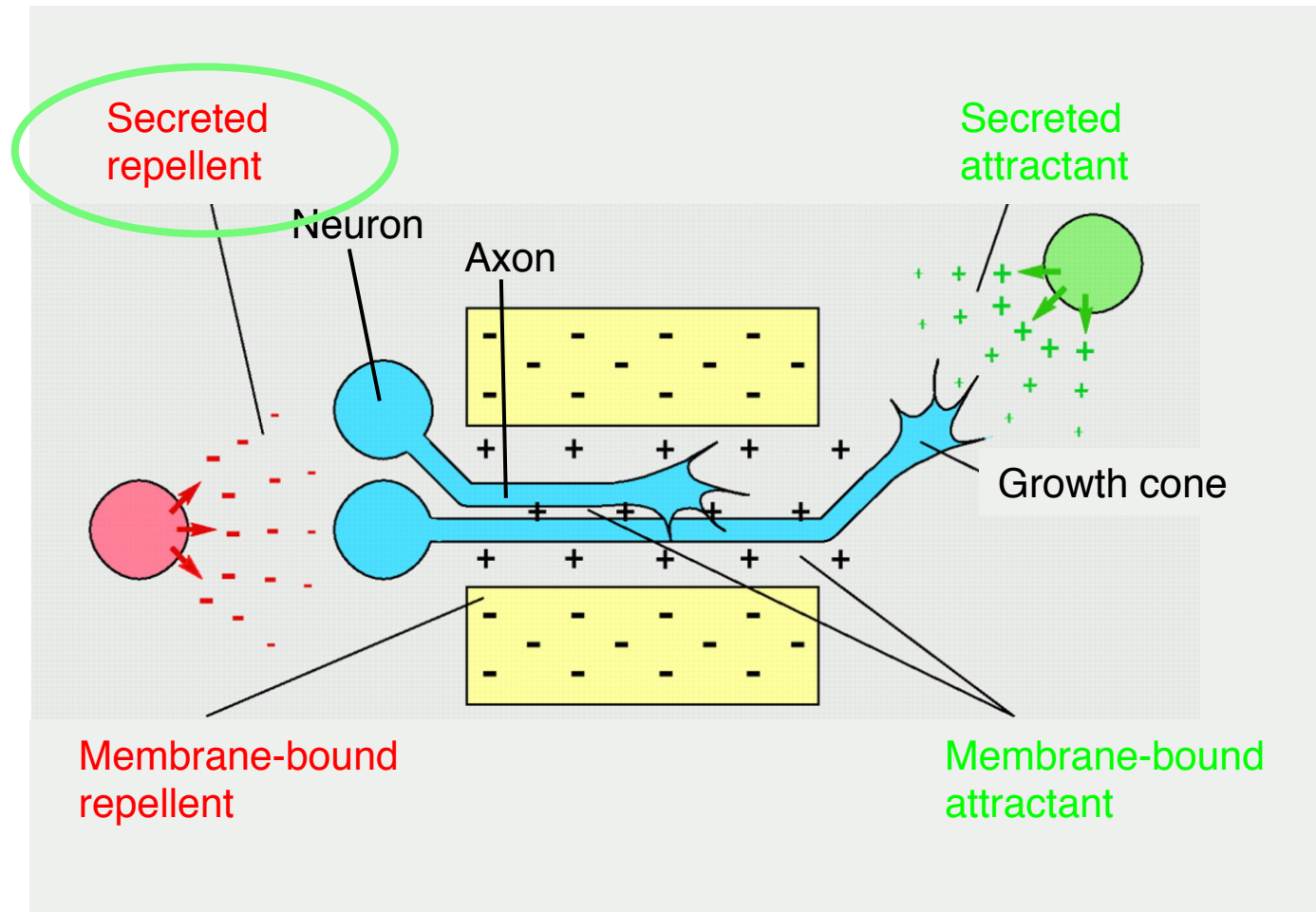
Neogenin



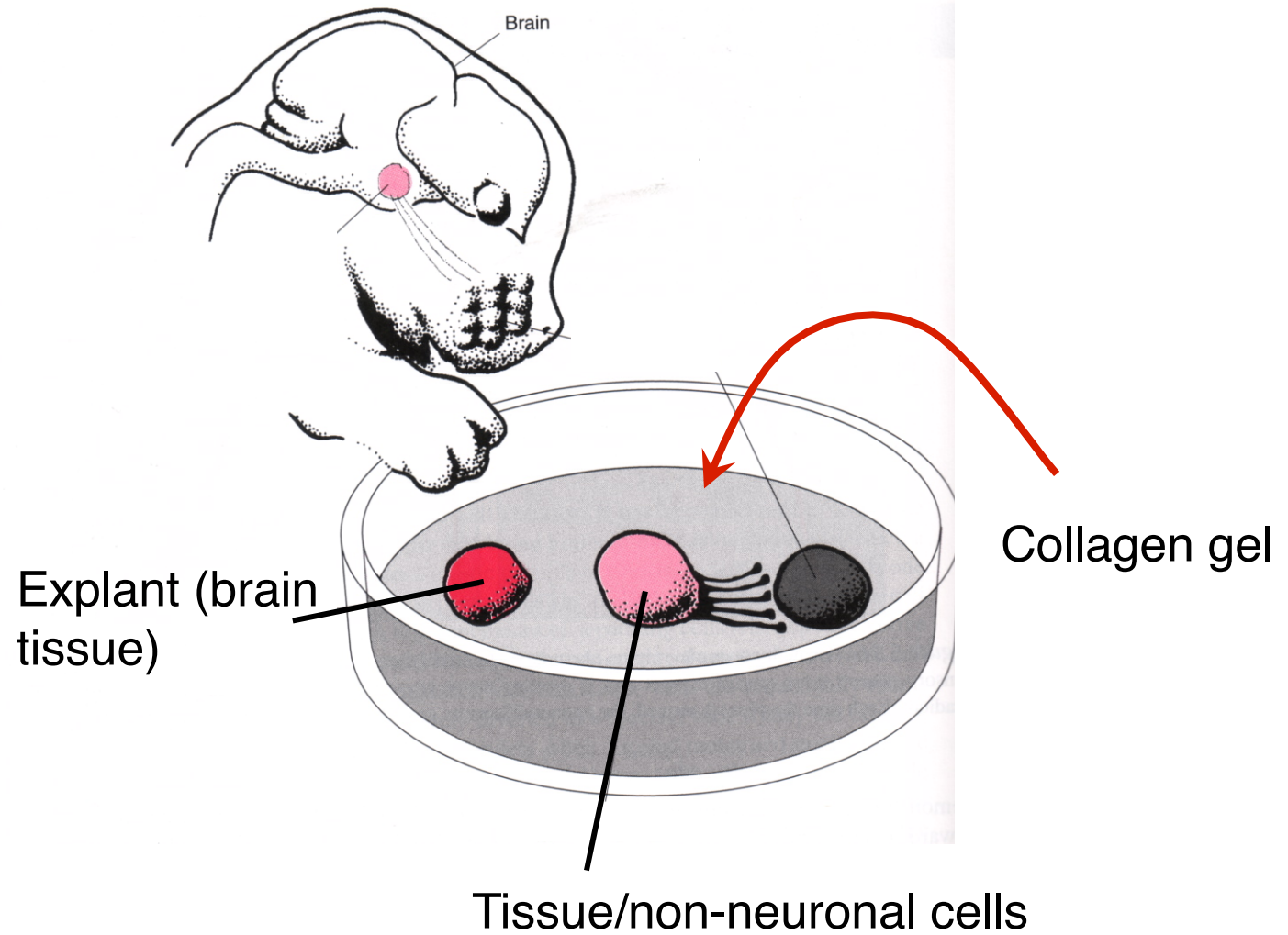
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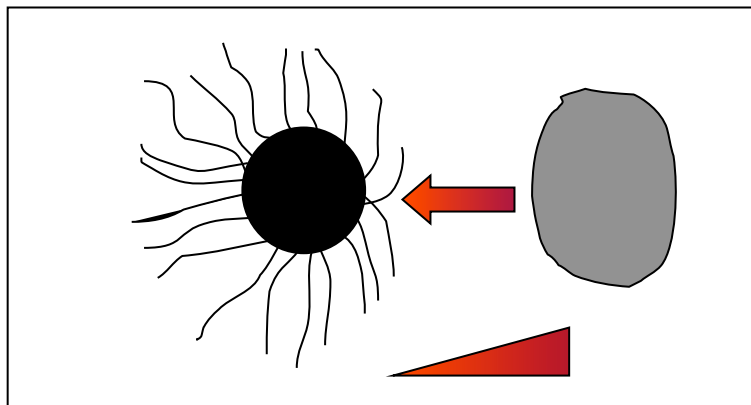
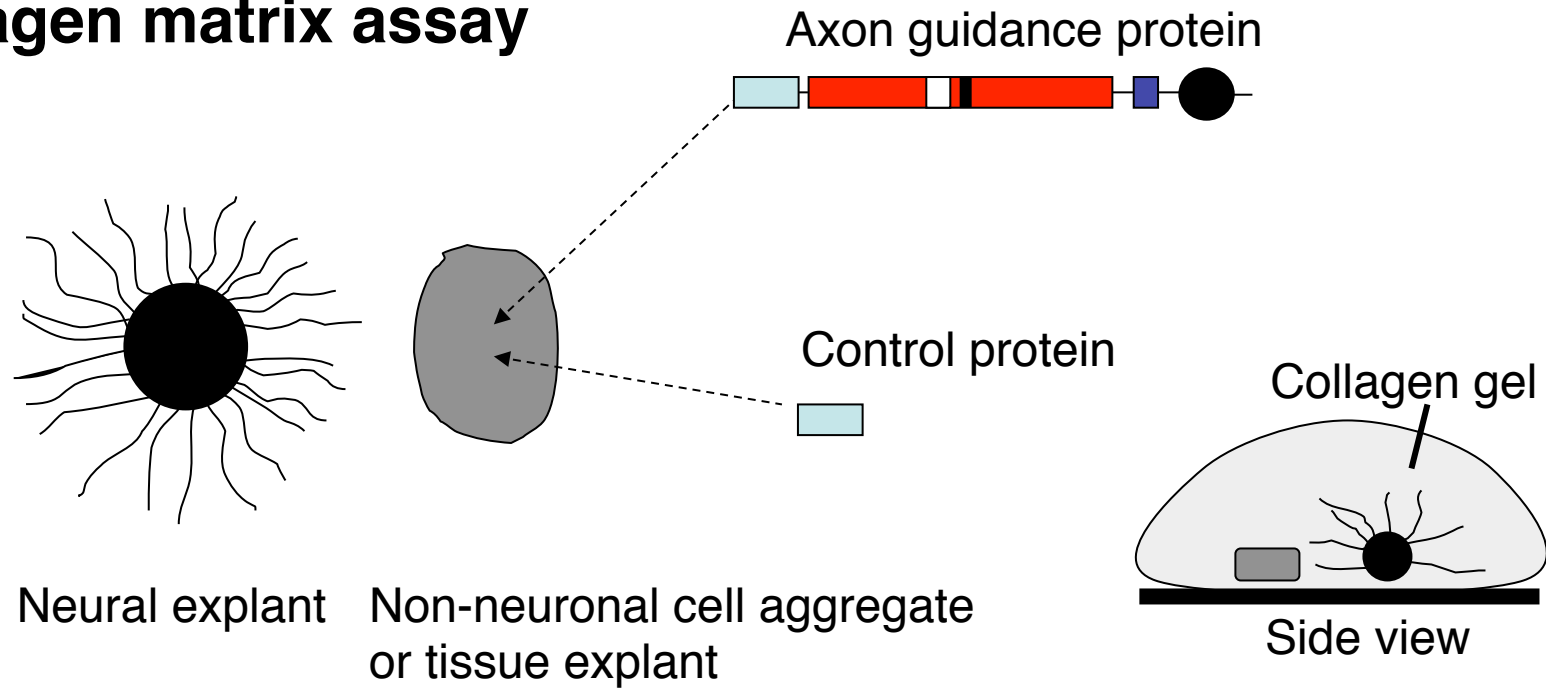
Principles of axon guidance



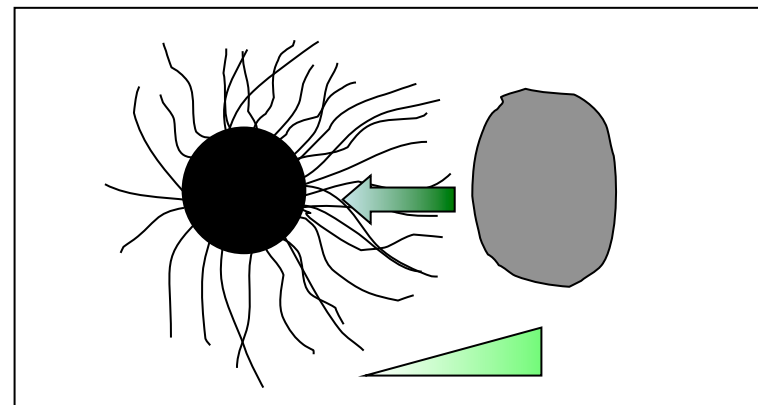
Collagen matrix assay



Collagen matrix assay



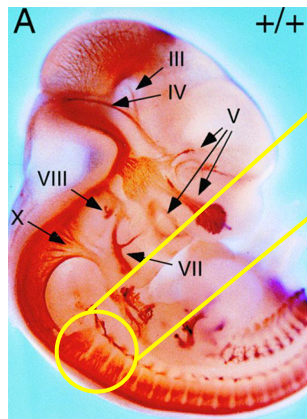
Repulsion



Attraction

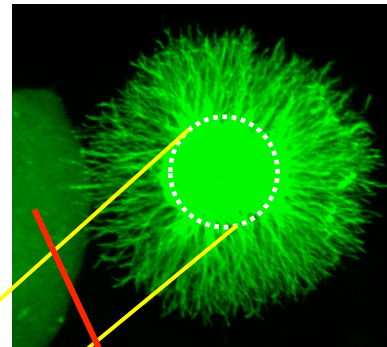
Chemorepulsion

In vitro
(collagen matrix assay)



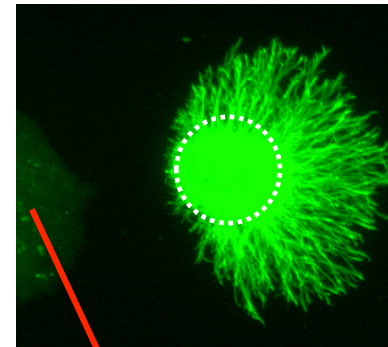
Sensory neurons

control



CON

+ Sema3A



Sema3A

Terman et al., 2002

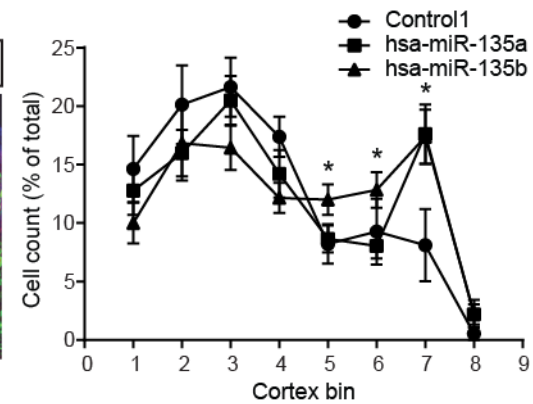
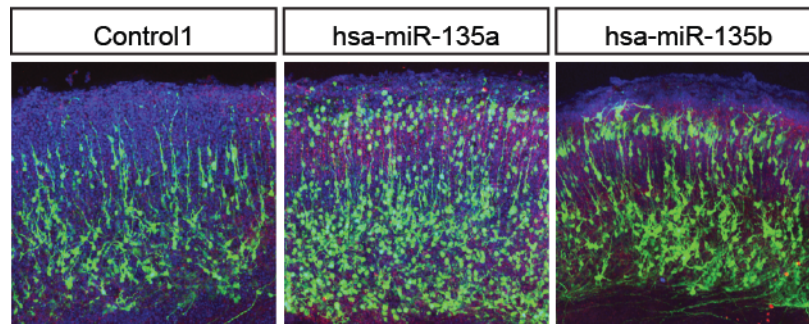
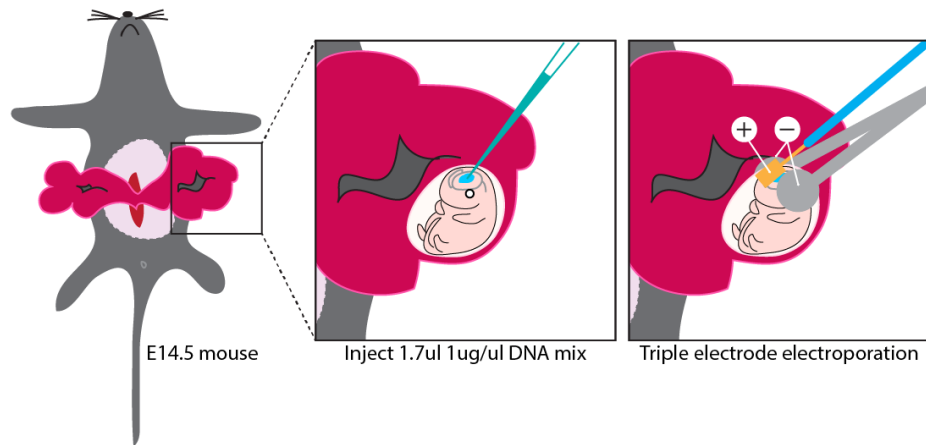
In vivo analysis axon guidance

Knockout mice: Genetically modified mice in which a single gene has been inactivated through homologous recombination events. These mice lack a specific gene product (protein) and can be used to study the role(s) of individual proteins in physiological processes, such as axon guidance.

Knockin mice: Similar to knockout mice. However, in this case another sequence has been inserted in the targeted gene.

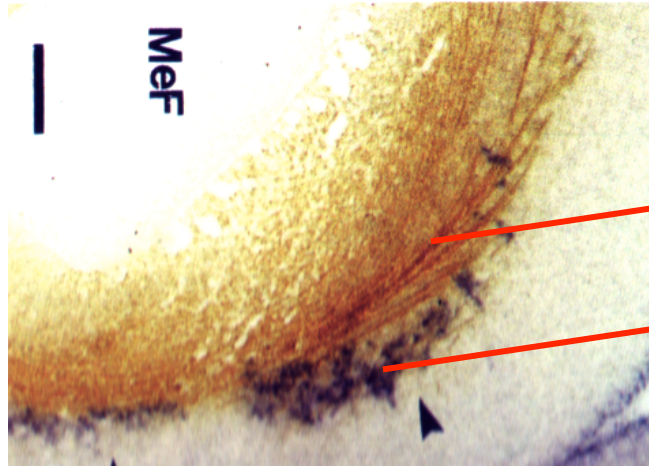
Transgenic mice: Mice in which genes have been added to the genome. The mice have an additional copy of a particular gene or express a fluorescent gene under the control of a promoter.

In utero electroporation



Chemorepulsion

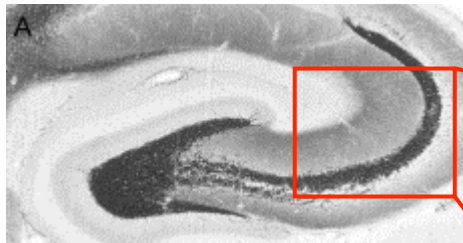
In vivo
(mouse knockout)



Axon (brown)

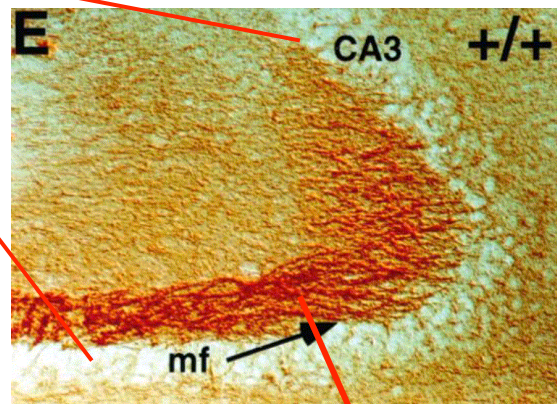
Sema3A (purple)

Giger et al, 1996

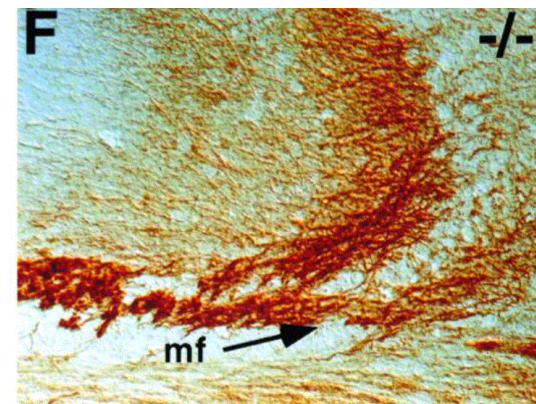


Hippocampus

Sema3



No Sema3

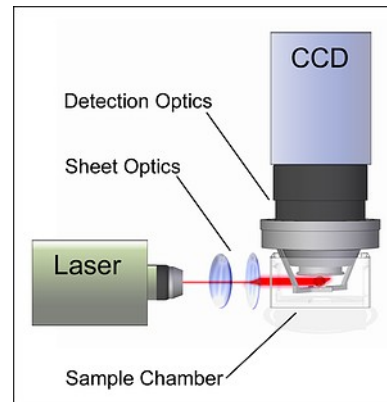
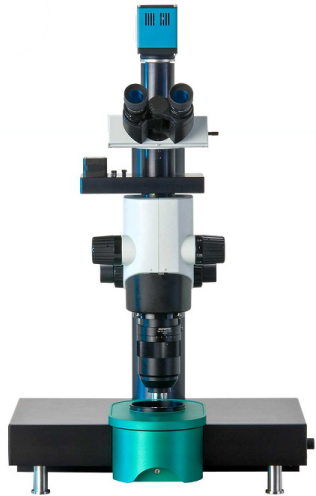


Axon (brown)

Giger et al, 1998

New microscopic approaches

Research approach:

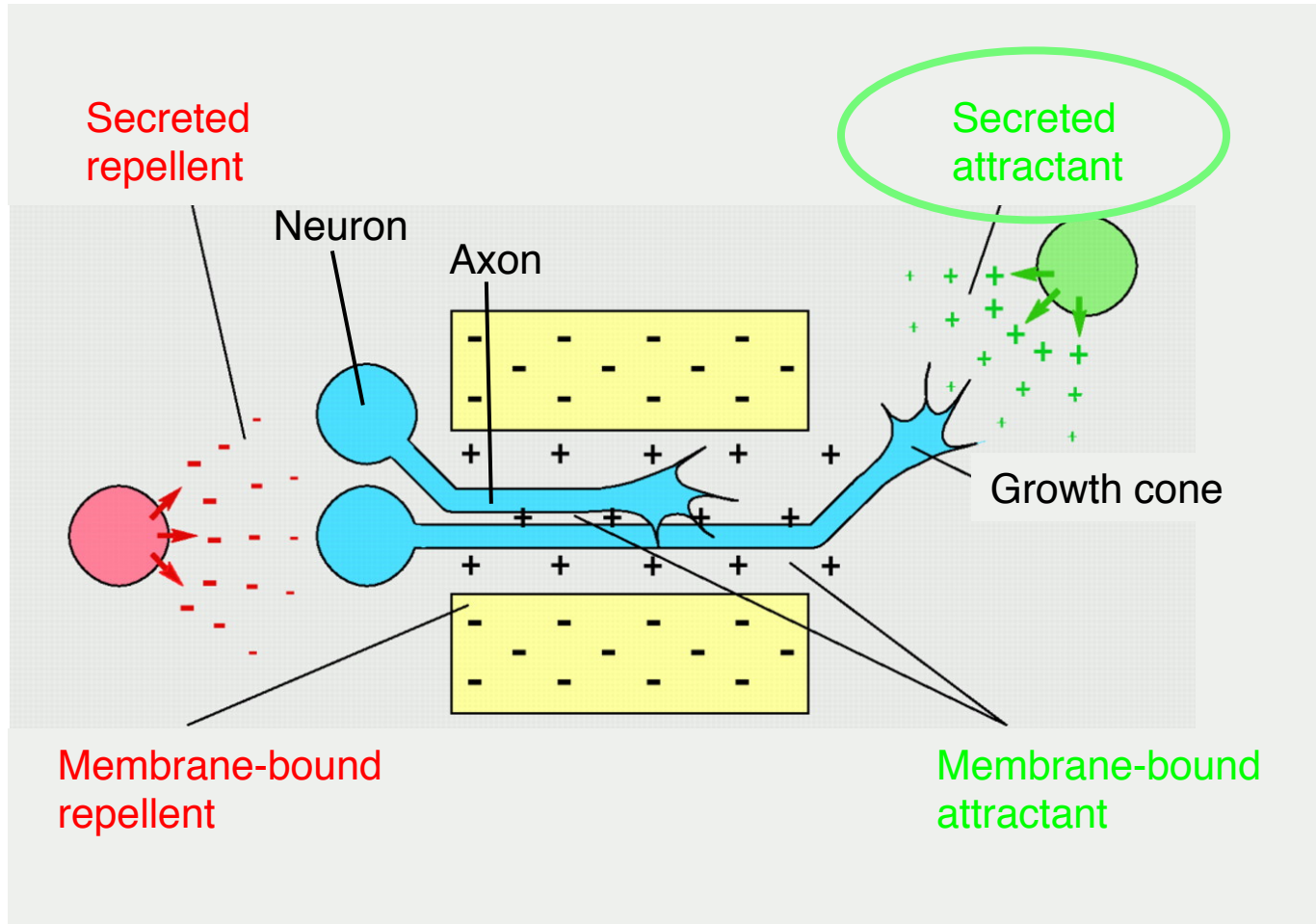


Light sheet imaging



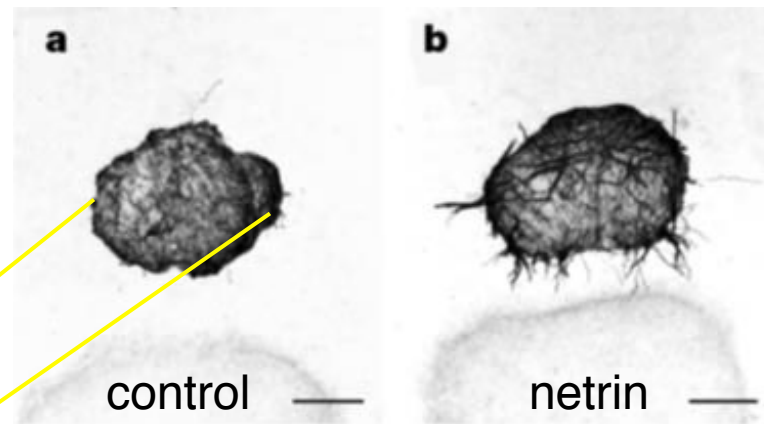
Whole embryonic mouse spinal cord, commissural axons, 3DISCO cleared (A. Chedotal)

Principles of axon guidance

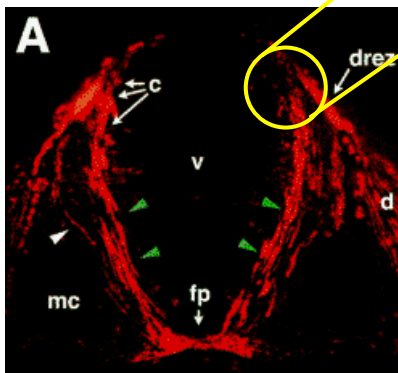


Chemoattraction

In vitro
(collagen matrix assay)



Corset et al., 2000



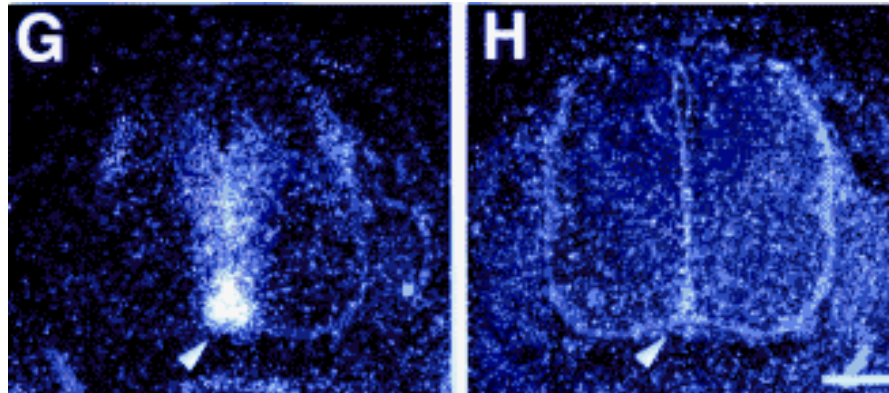
Commissural neurons

Chemoattraction

In vivo
(mouse knockout)

Netrin

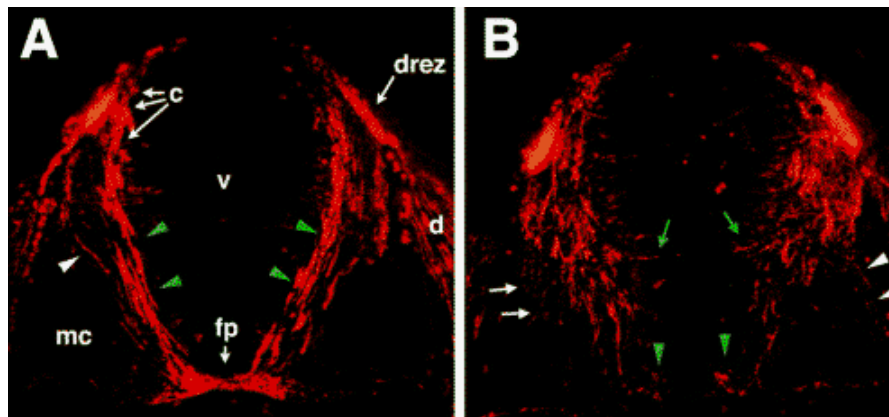
No Netrin



Netrin expression

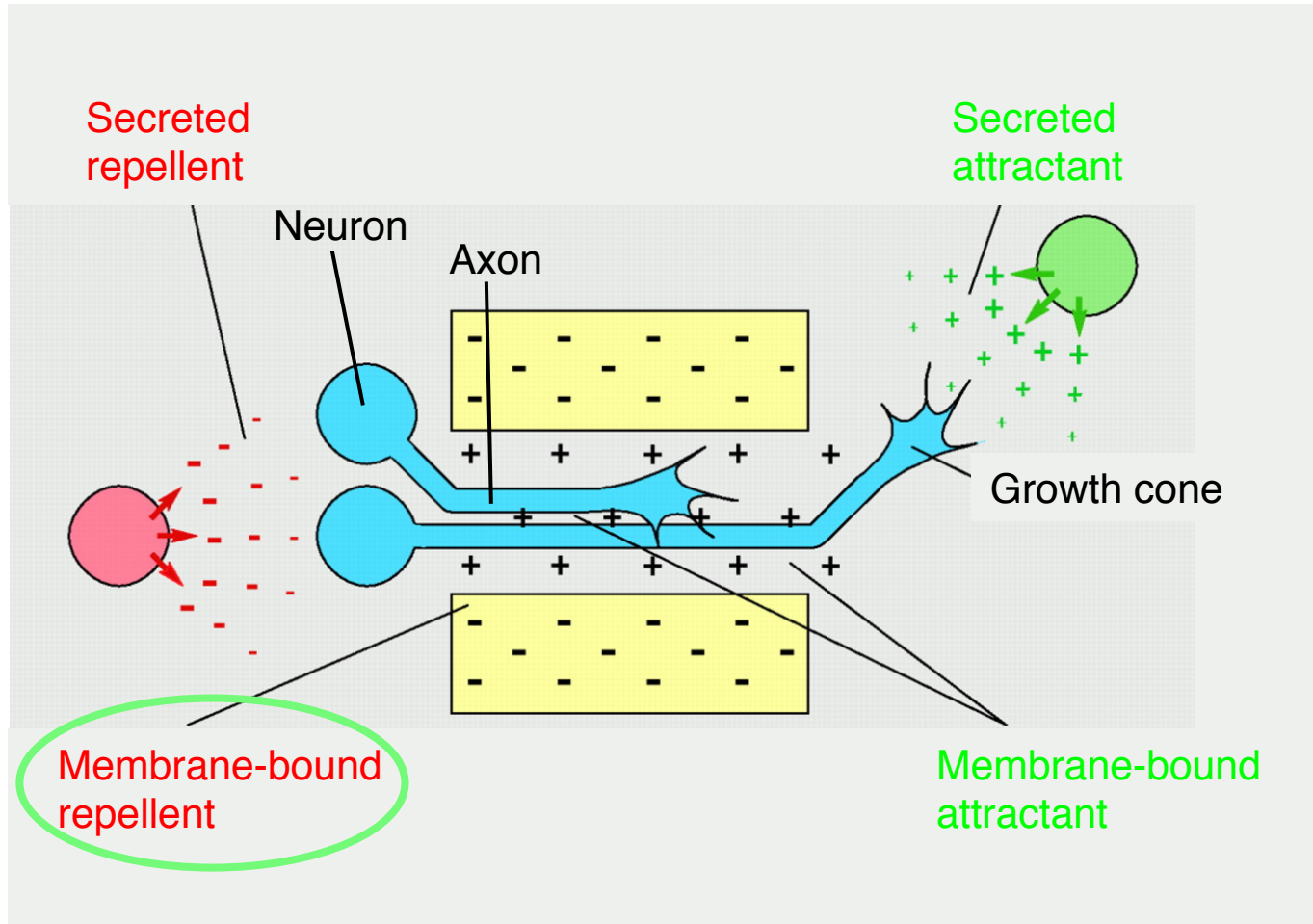
Control

No Netrin

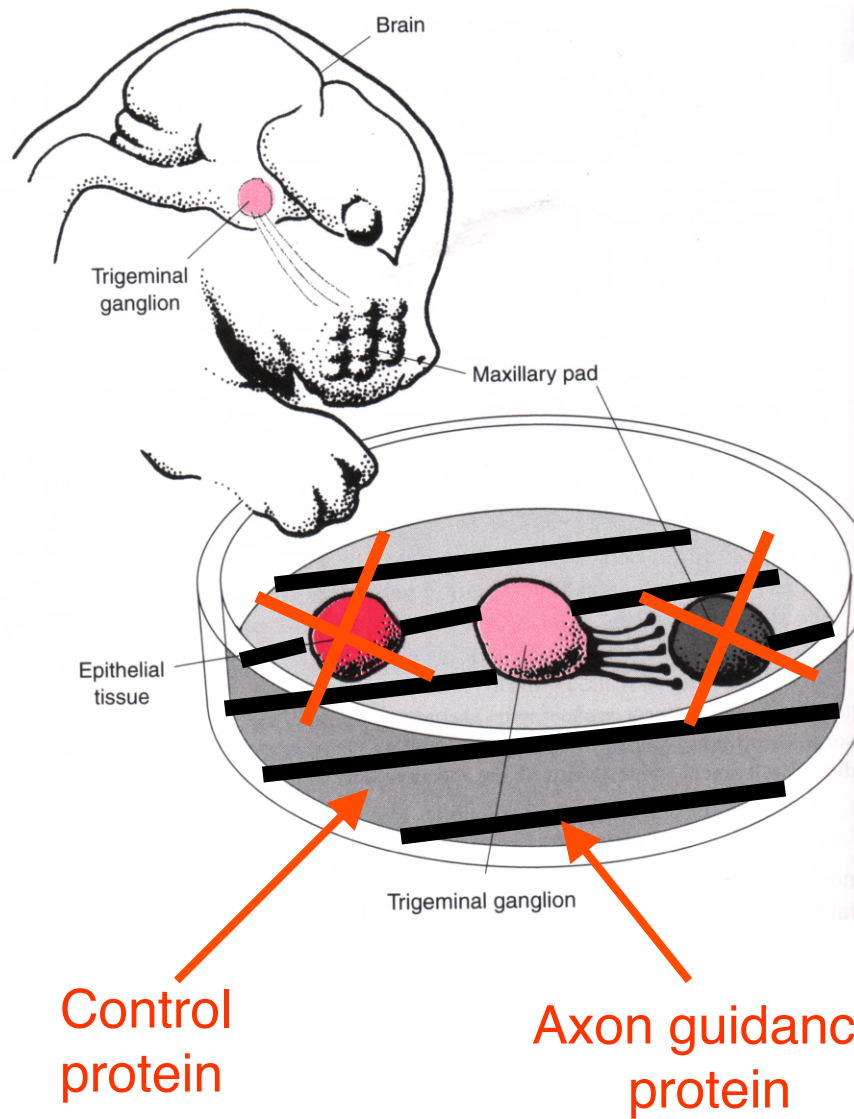


Netrin knockout
mouse

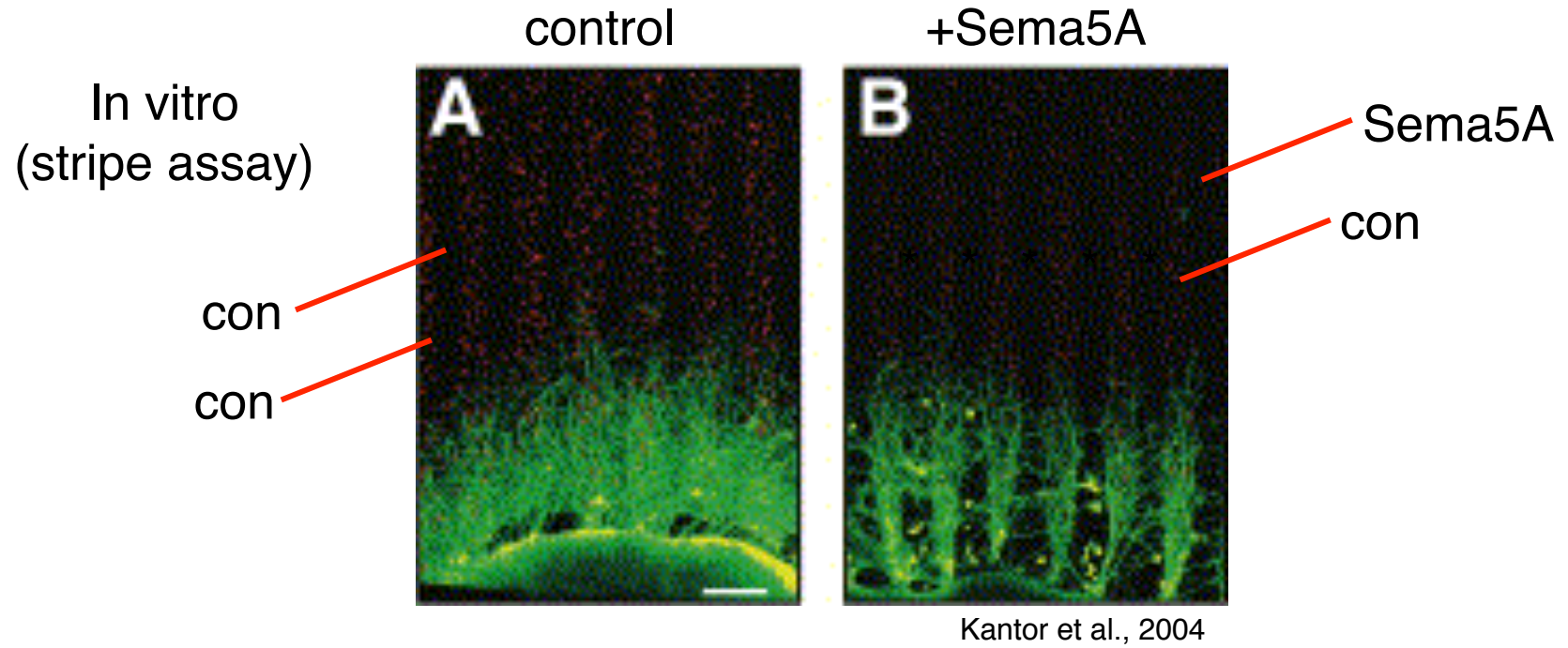
Principles of axon guidance



Stripe assay



Contact repulsion



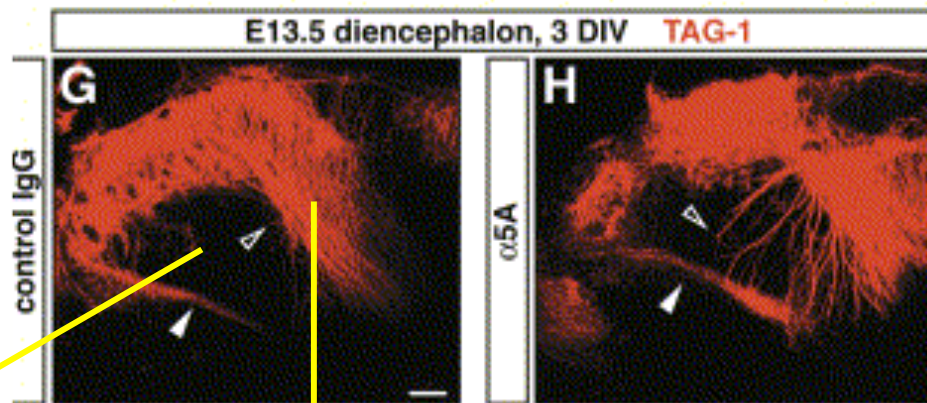
Contact repulsion

Control
antibody

Sema5A
antibody

Ex vivo

Sema5A
expression

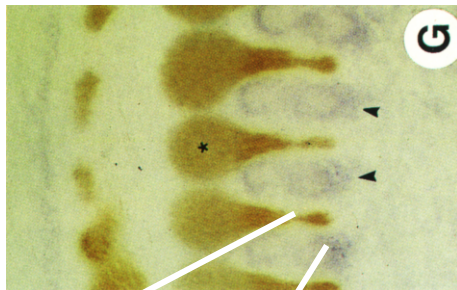


Kantor et al., 2004

Fasciculus retroflexus

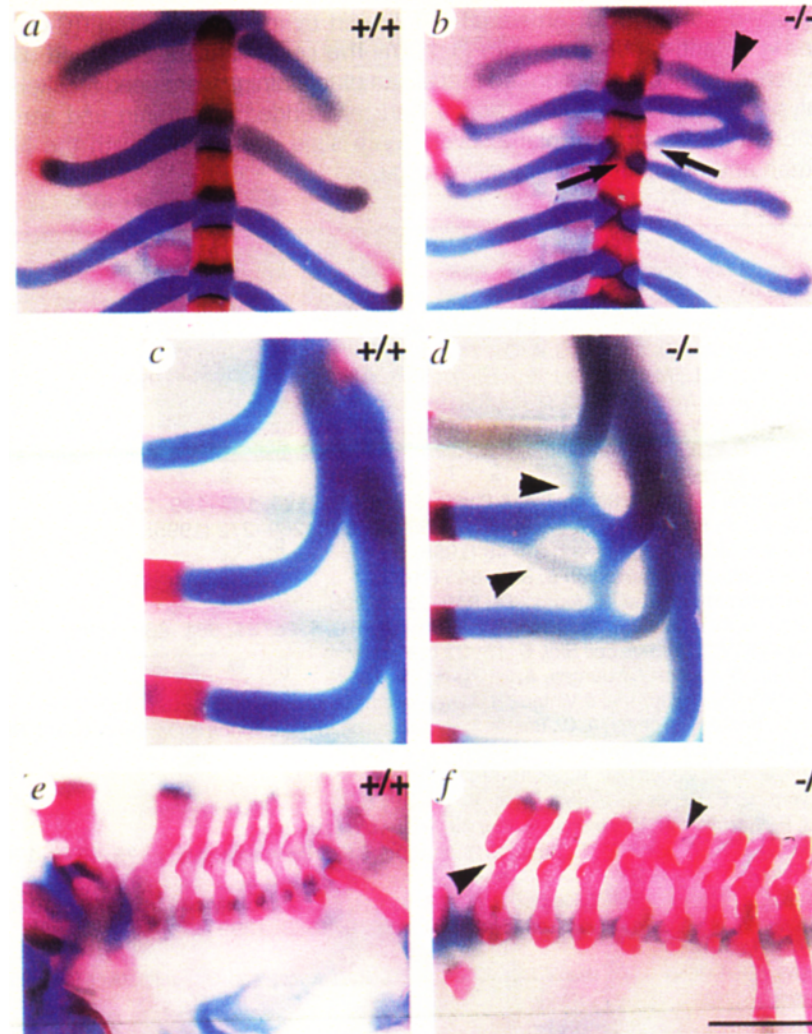
Axon guidance proteins have many functions outside the nervous system

Sensory neurons



Axon

Sema3A (rib)



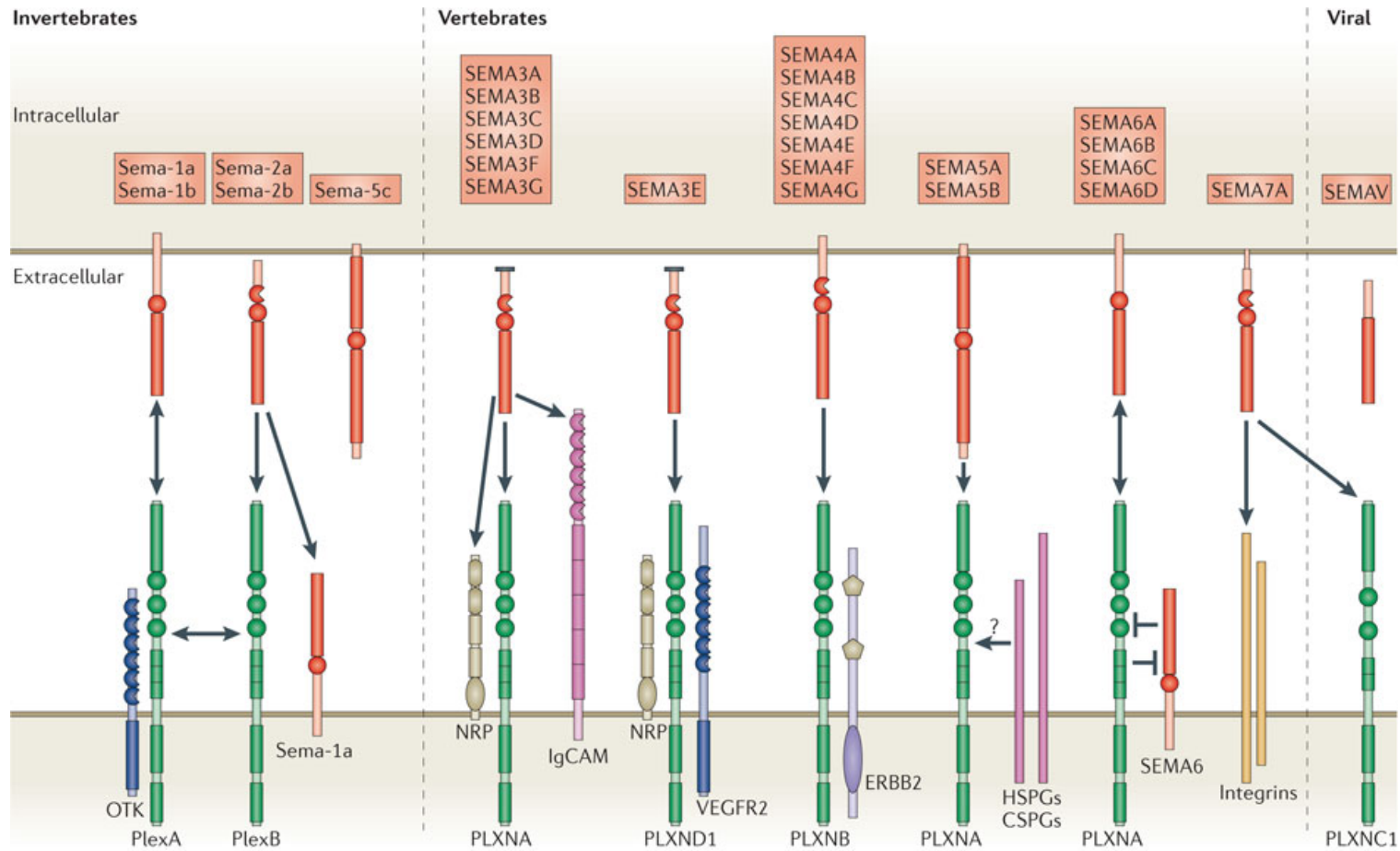
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Axon guidance receptors

- Expression of receptor determines growth cone sensitivity
- Composition of receptor complex determines response
- Response can change in time

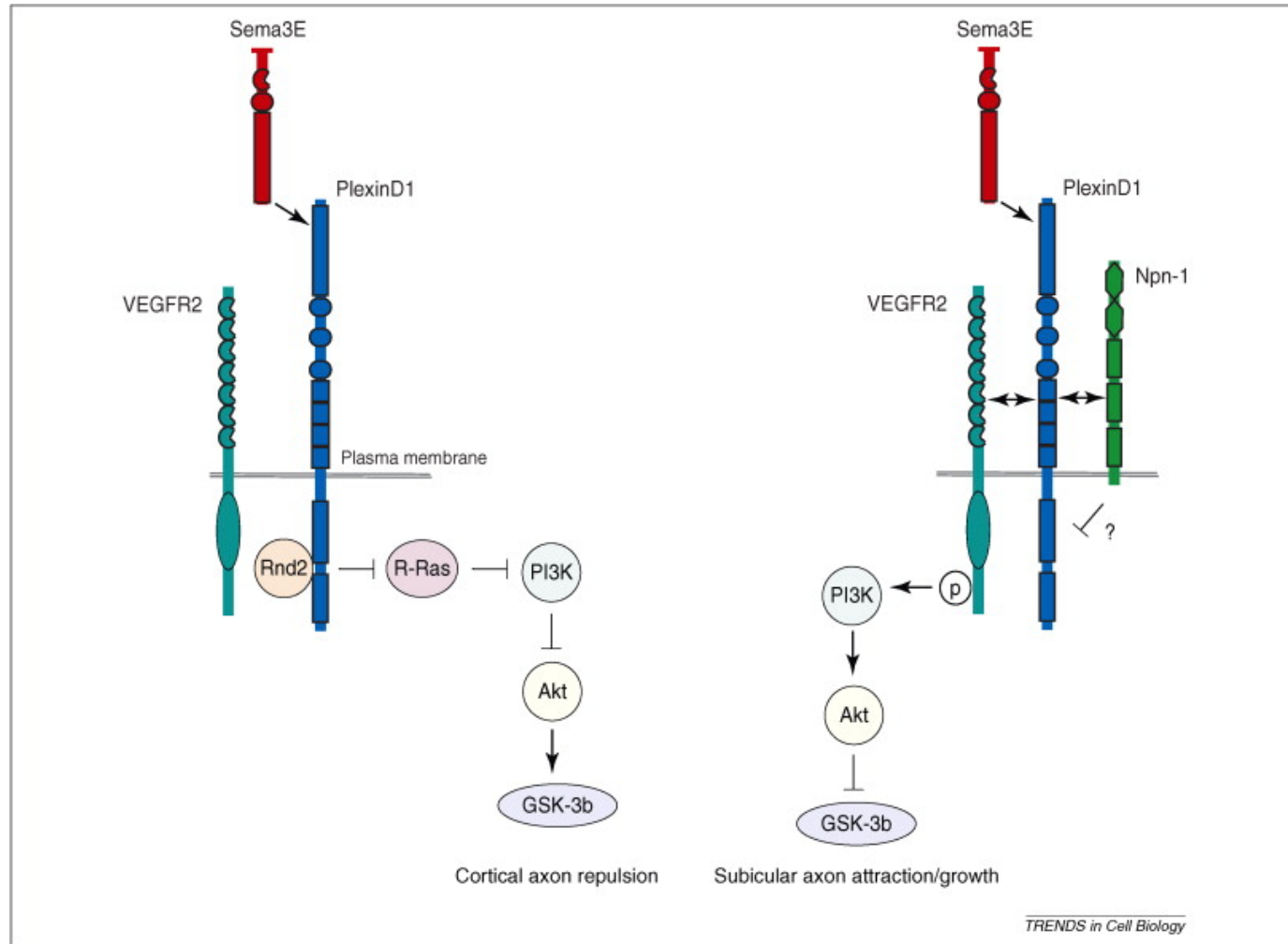
Composition of receptor complex determines response



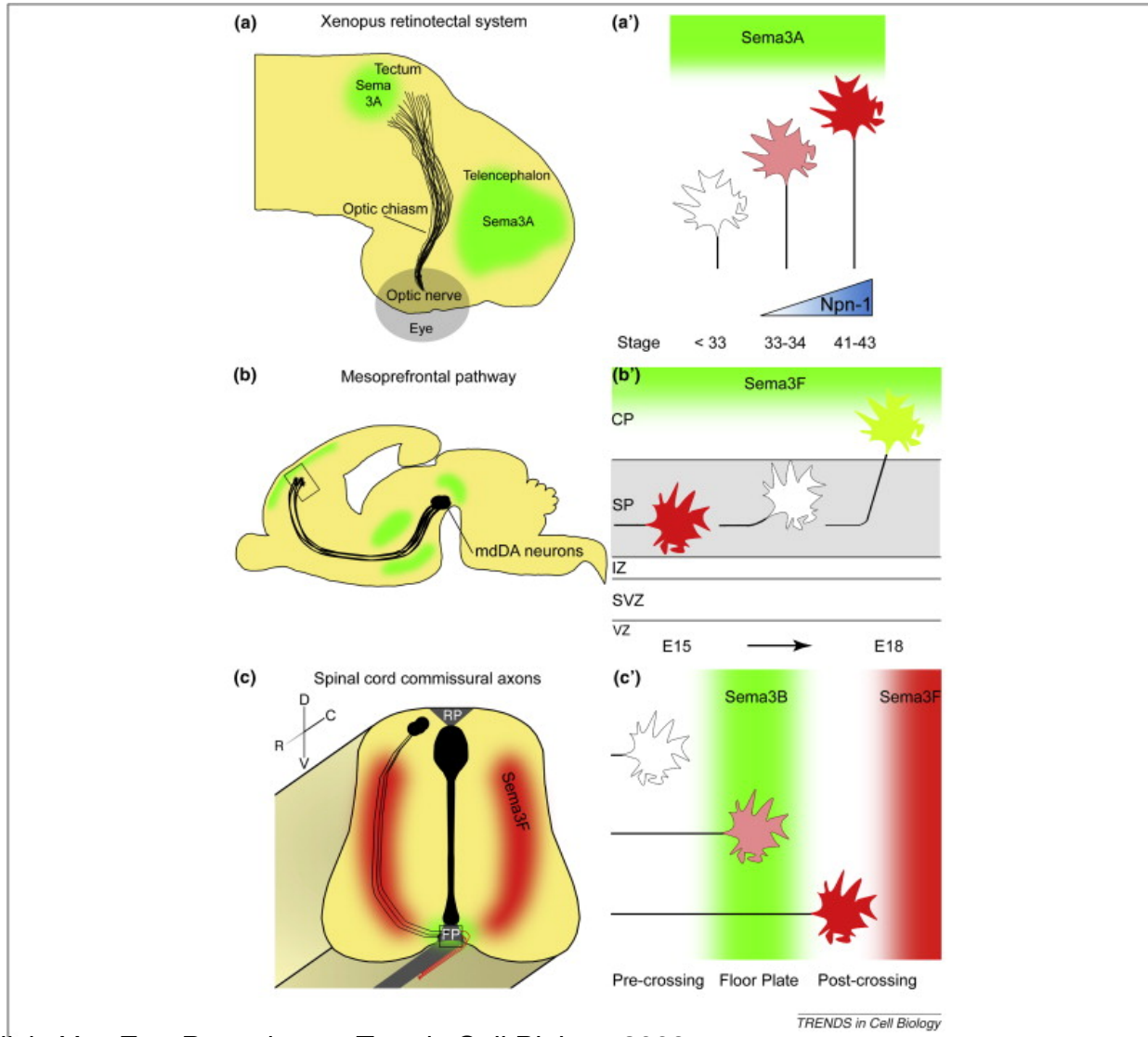
Nature Reviews | Neuroscience

Pasterkamp, 2012

Composition of receptor complex determines response



Response changes in place and time



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Regulation of Neogenin ectodomain shedding

NEOGENIN

Ligand/binding proteins

Repulsive guidance molecules (RGM)

Netrin-1

Bone morphogenetic proteins (BMP)

Uncoordinated 5 (Unc5)

....

Cellular function

Axon repulsion

Cell migration and adhesion

Apoptosis

....

Organ systems

Nervous system

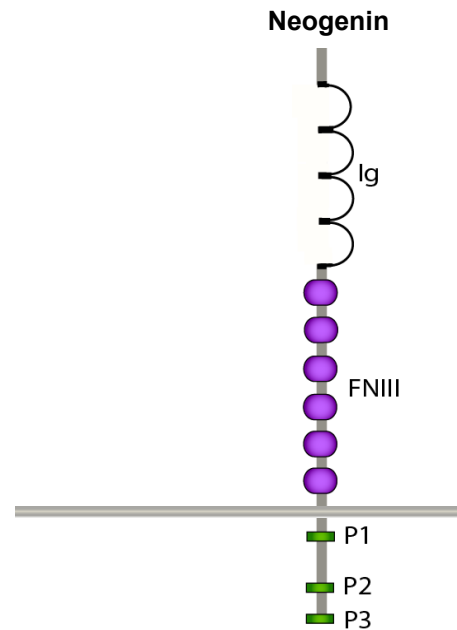
Immune system

Bone

Muscle

Blood

....



Disease

Inhibition of axon regeneration

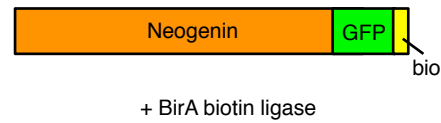
Autoimmune disease

Cancer

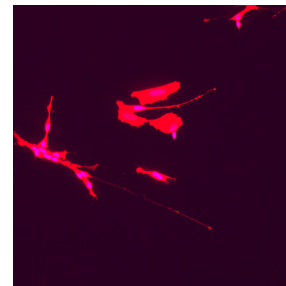
....

NEOGENIN INTERACTORS

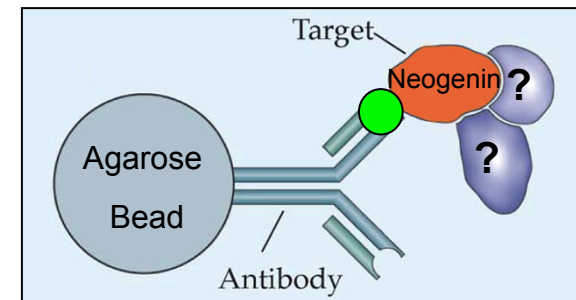
1. In vitro



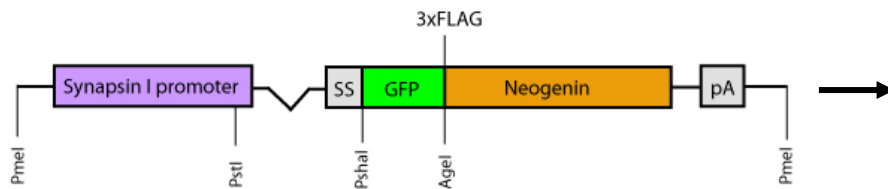
(Neuronal) cells



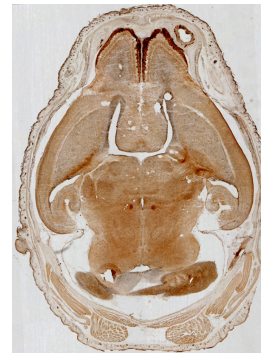
Immunoprecipitation



2. In vivo

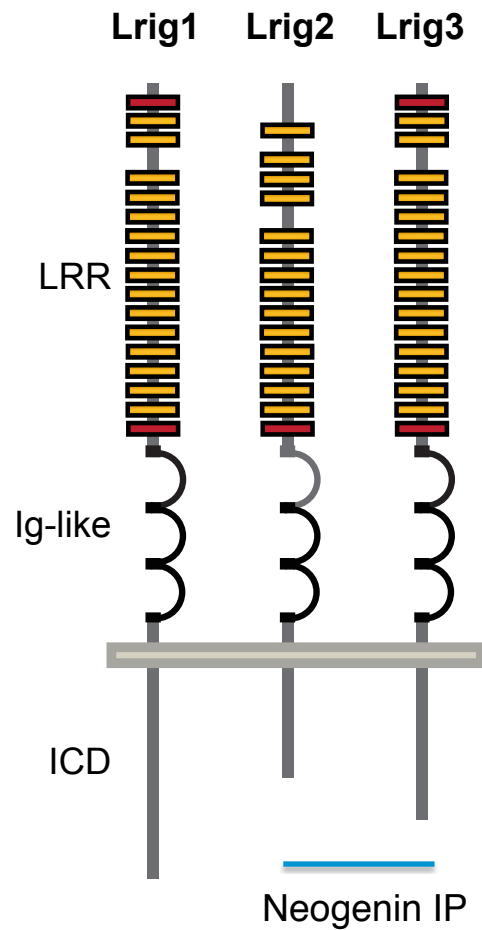


Transgenic mice



+ MS analysis

LRIGs

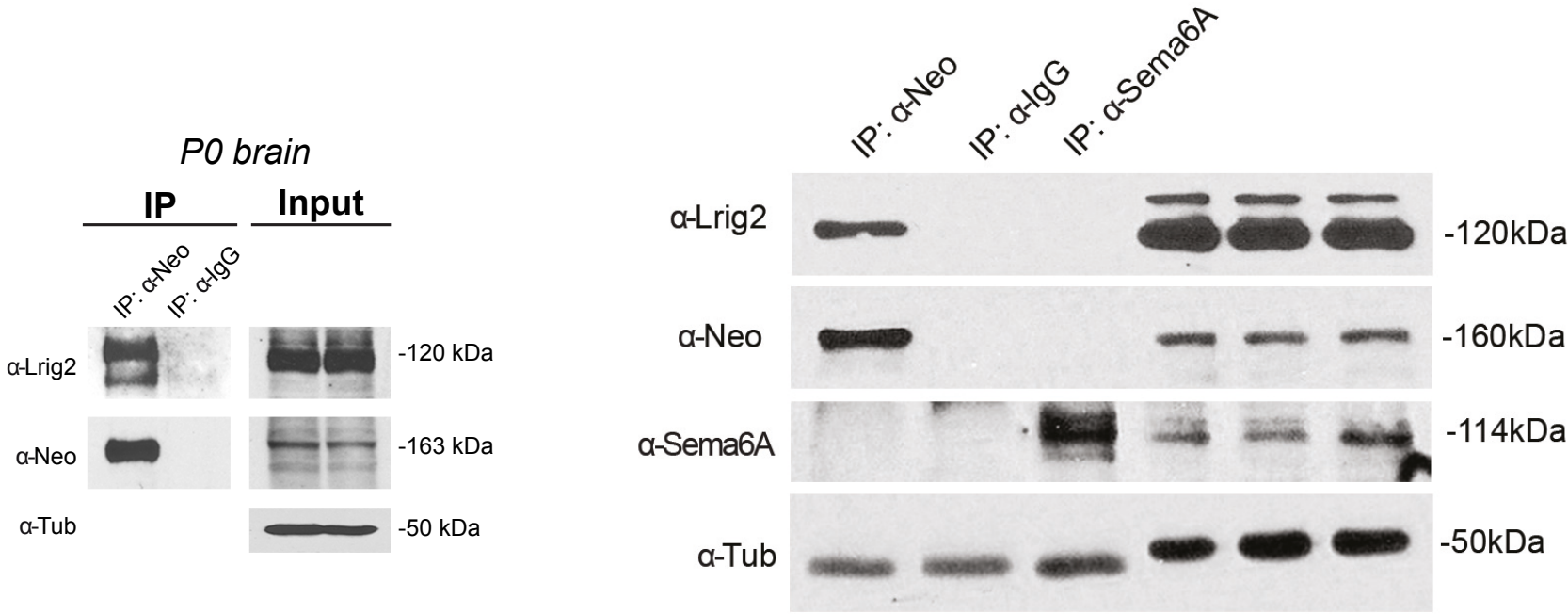


LRIG family

- Stem cell quiescence
- Cancer
- Negative regulator growth factor signaling
- Nervous system?

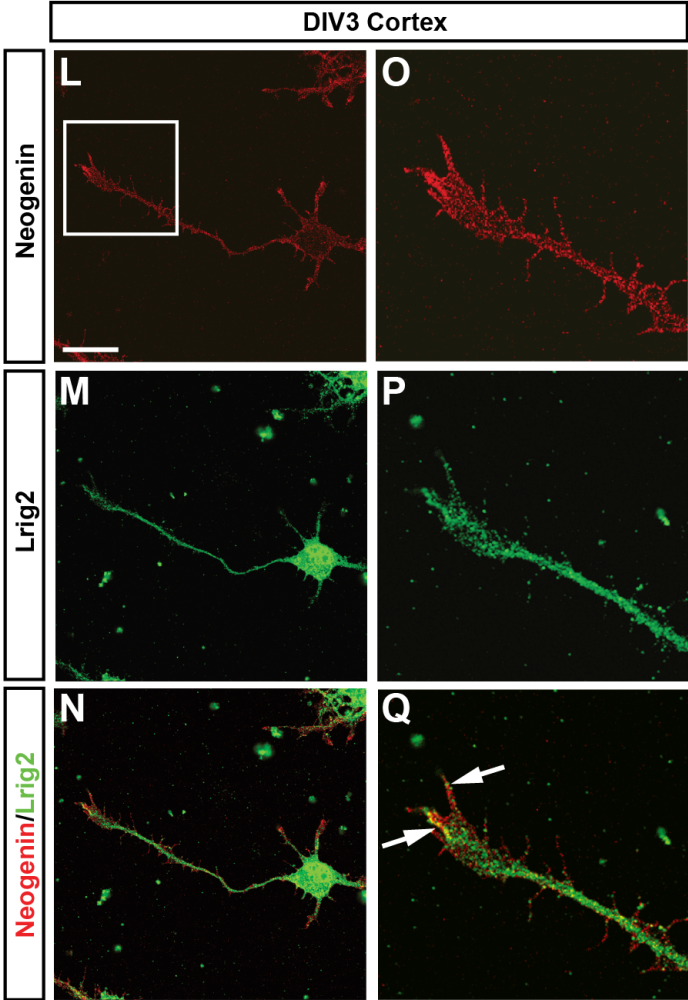
Leucine-rich repeats and immunoglobulin-like domains (Lrig) – Guo et al., Genomics 2004
(member extracellular leucine-rich repeat superfamily)

LRIG2 AND NEOGENIN INTERACT AT THE MEMBRANE



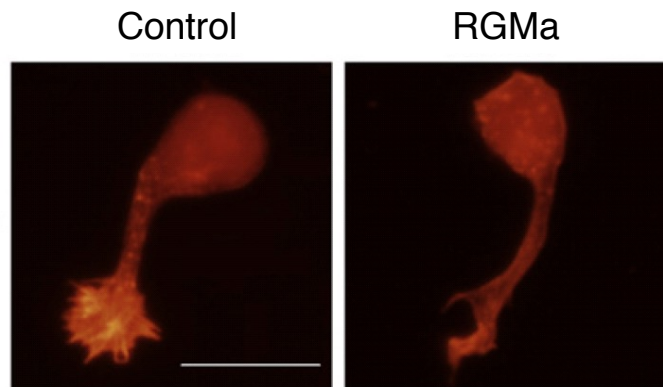
IP on neuronal membranes

LRIG2 AND NEOGENIN CO-LOCALIZE IN NEURONS



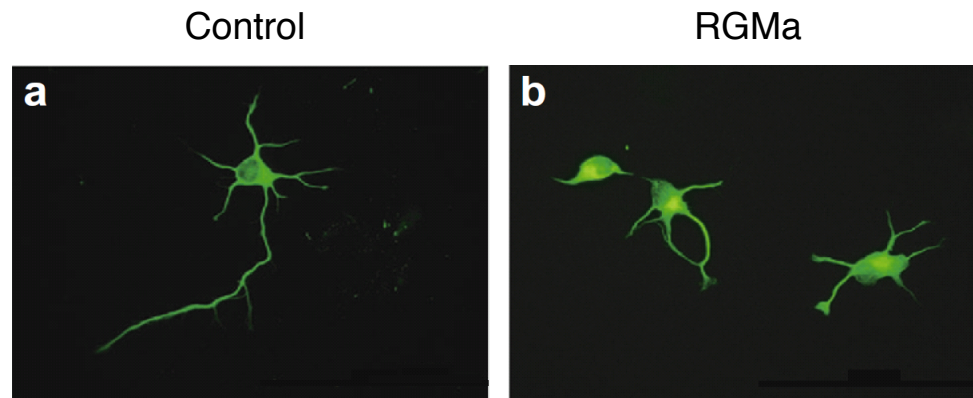
Cortex, ICC,
E14.5, DIV3

RGMa CAUSES GROWTH CONE COLLAPSE AND INHIBITS NEURITE GROWTH



Suda *et al.*, 2008

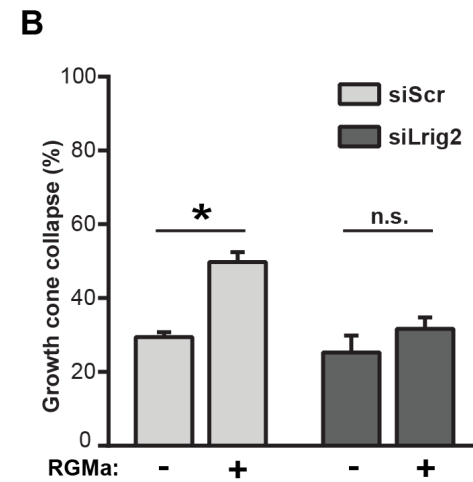
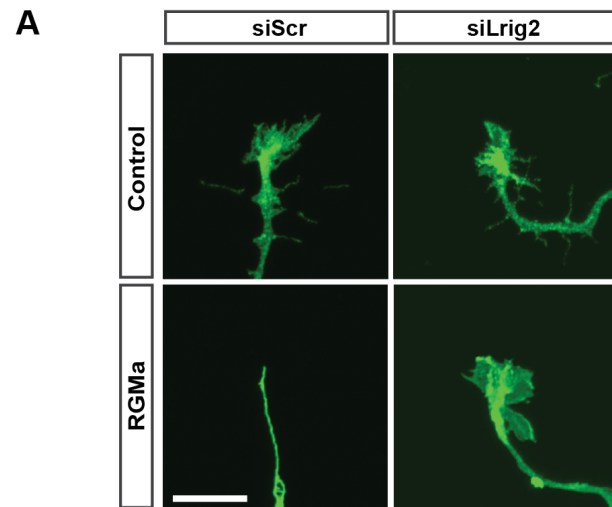
Growth cone collapse



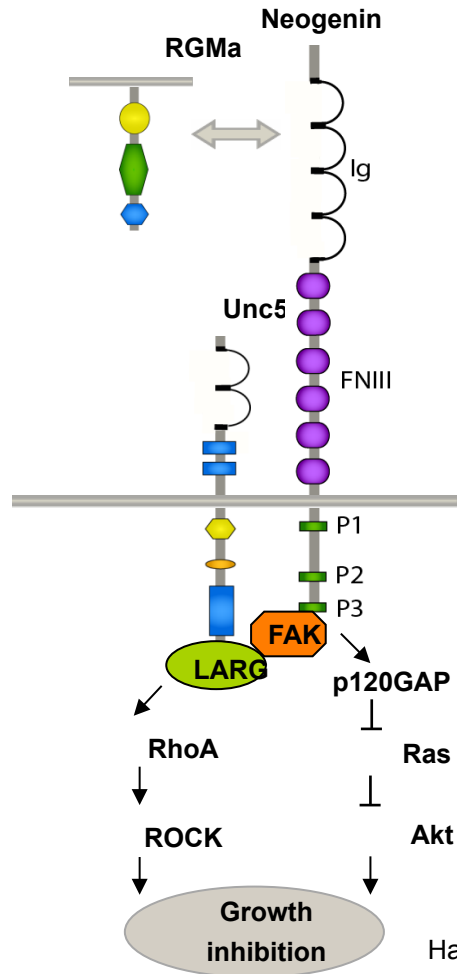
Yoshika *et al.*, 2008

Neurite growth inhibition

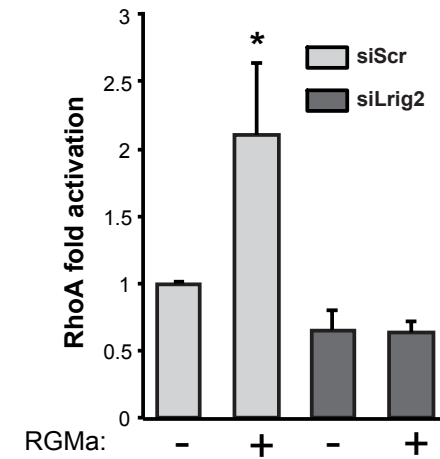
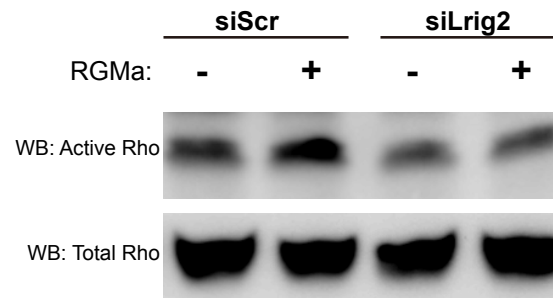
GROWTH CONE COLLAPSE BY RGMa REQUIRES LRIG2



NEOGENIN-RGMa SIGNALING REQUIRES LRIG2



Hata et al., 2006; Conrad et al., 2007; Endo and Yamashita, 2009



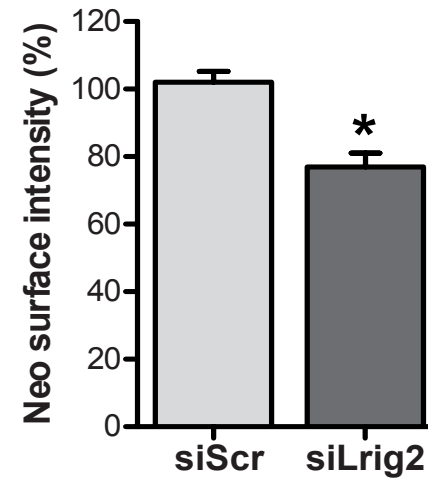
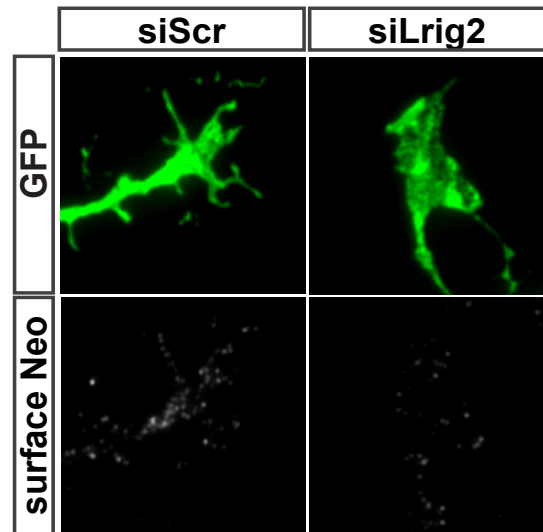
HOW DOES LRIG2 REGULATE RGMa FUNCTION?

HOW DOES LRIG2 REGULATE RGM α FUNCTION?

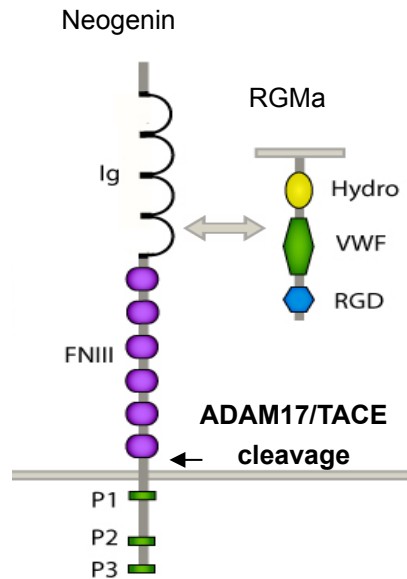
Lrig2 knockdown did not affect:

- Neogenin endocytosis
- Neogenin exocytosis
- Lipid raft localization
- Neogenin degradation

HOW DOES LRIG2 REGULATE RGMa FUNCTION?



AXON GUIDANCE RECEPTOR SHEDDING BY ADAMS



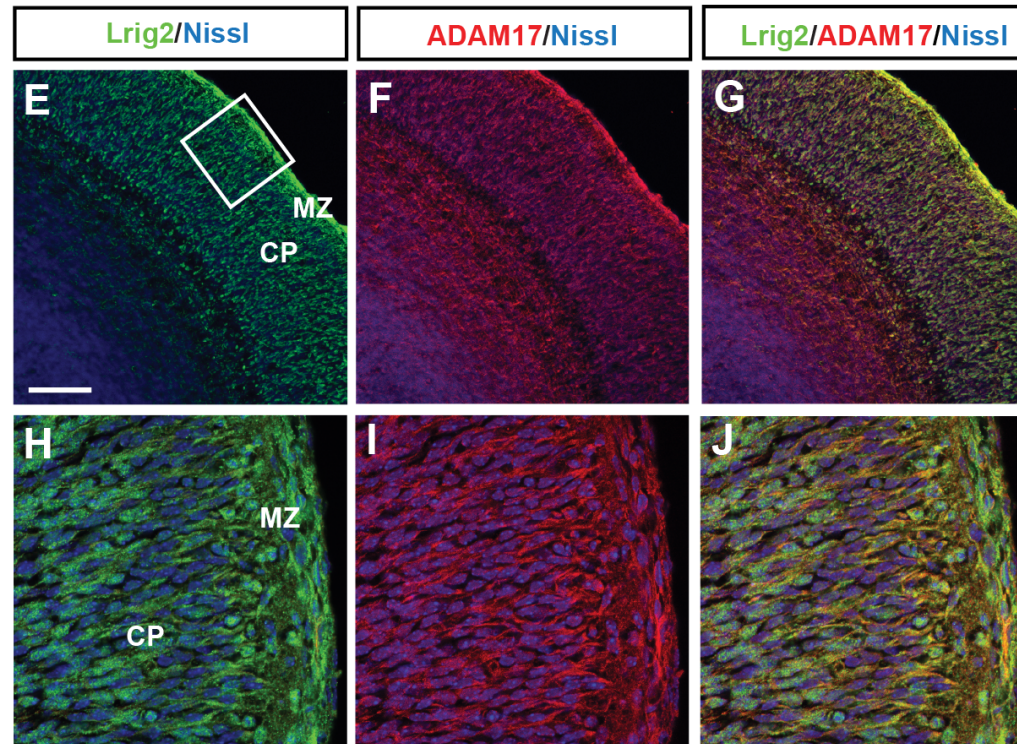
Axon guidance receptor shedding by ADAMs:

- Control of receptor cell surface levels
- Activation of downstream signaling
- Disassembly of ligand-receptor complexes
- Signal termination and duration
- ...

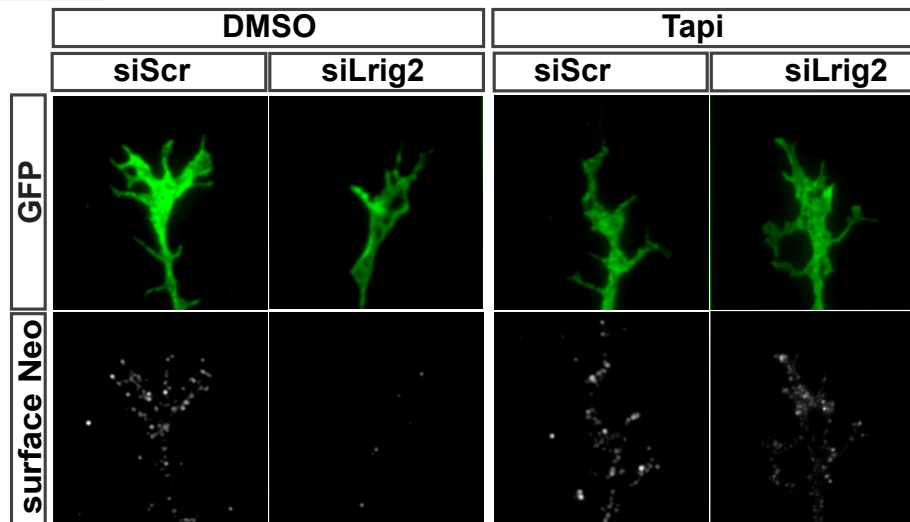
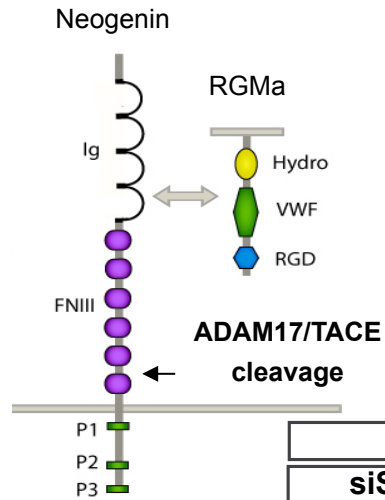
e.g. Chen et al., 2007; Coleman et al., 2010; Fambrough et al., 1996; Gatto et al., 2014; Hattori et al., 2000; Janes et al., 2005; Okamura et al., 2011; Romi et al., 2014

ADAM17= A Disintegrin and Metalloprotease 17
TACE= tumor necrosis factor- α converting enzyme

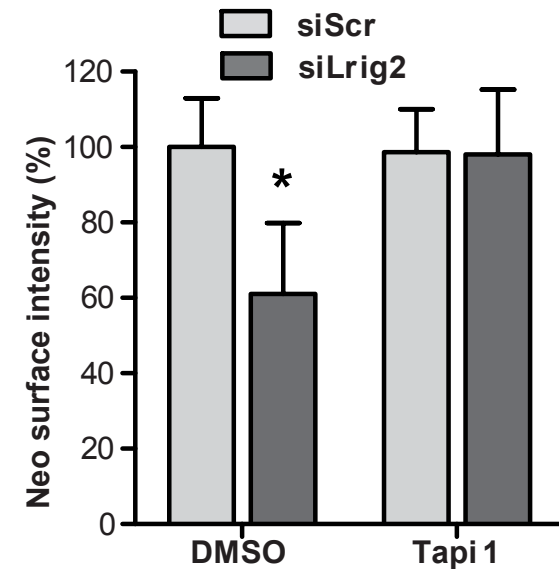
LRIG2 AND ADAM17 CO-LOCALIZE IN NEURONS



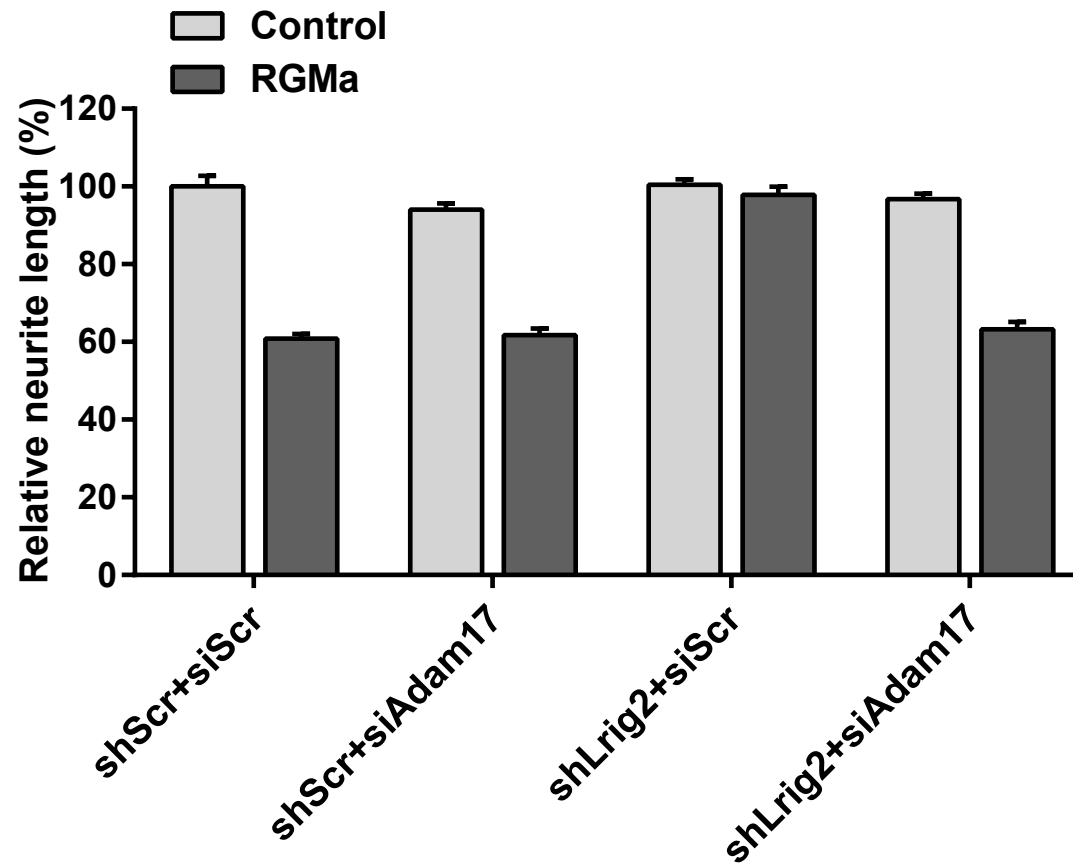
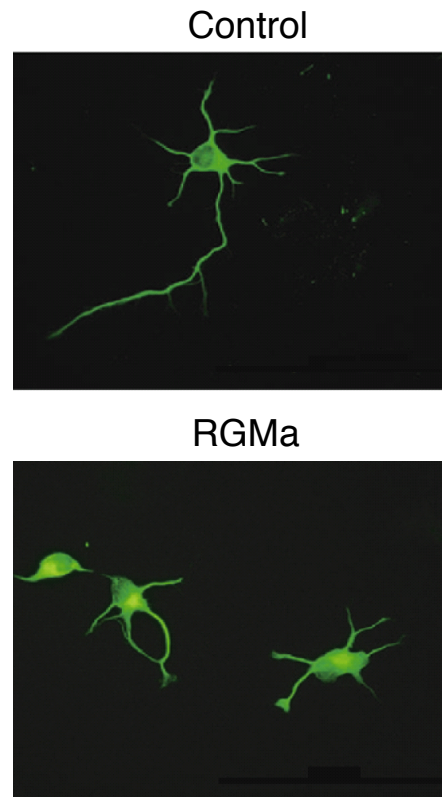
LRIG2 ANTAGONIZES NEOGENIN CLEAVAGE BY ADAM17



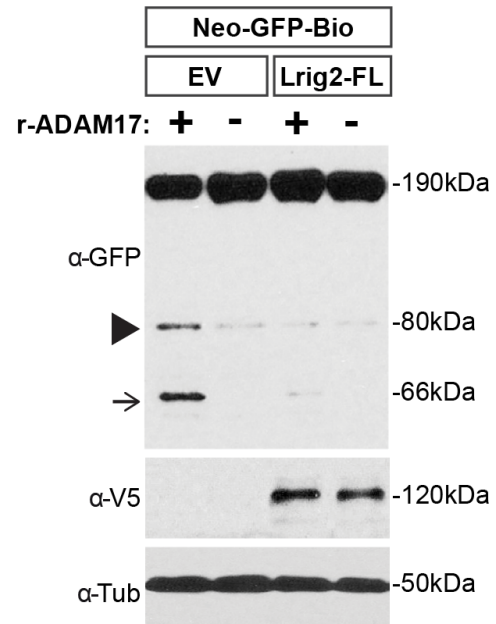
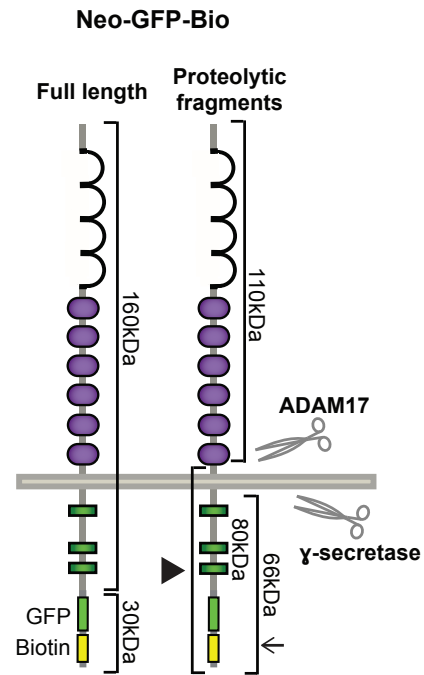
ADAM17 knockdown reduces Neogenin cell surface levels (not shown)



LRIG2 AND ADAM17 COOPERATE DURING RGMa-MEDIATED NEURITE GROWTH INHIBITION

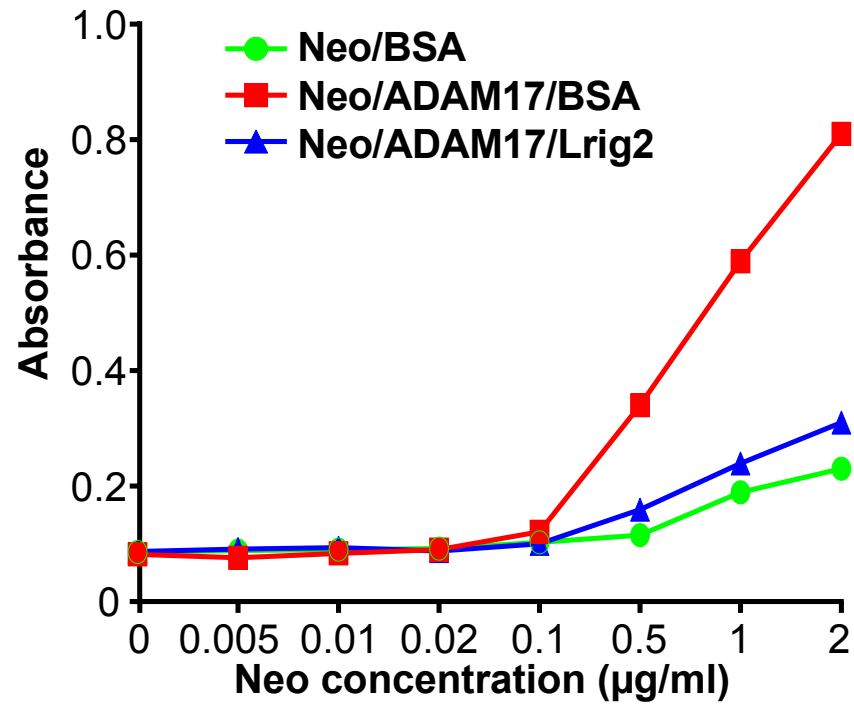


LRIG2 ANTAGONIZES NEOGENIN CLEAVAGE BY ADAM17



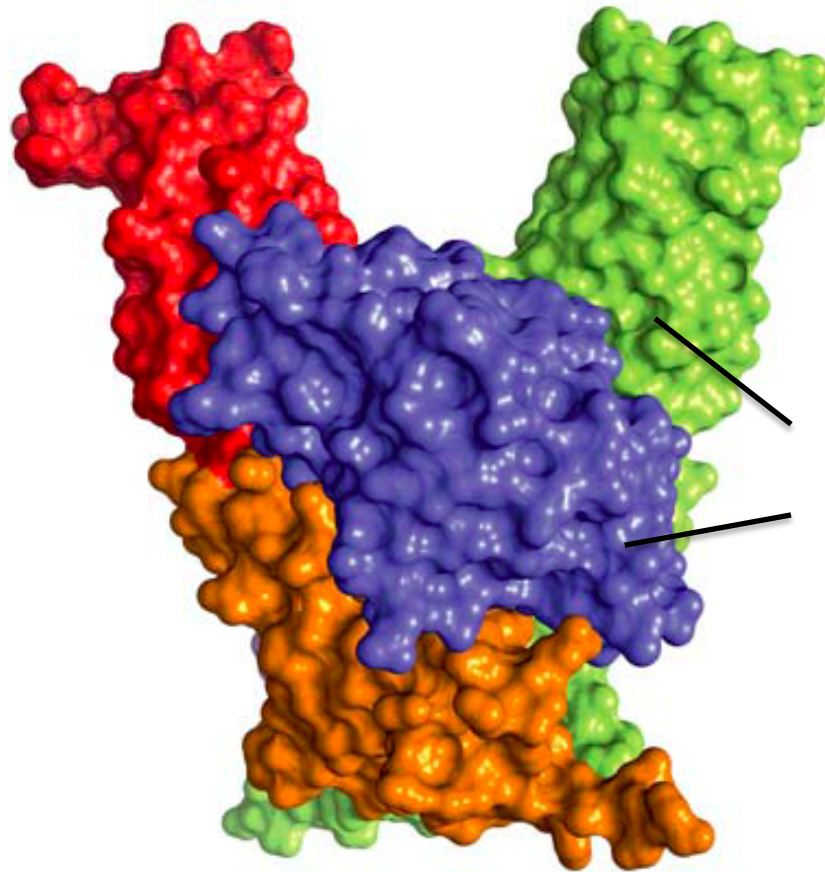
No cleavage by other ADAMs
e.g. ADAM9, ADAM10

LRIG2 INTERFERES WITH ADAM17-NEOGENIN BINDING



Okamura et al., 2011

RGMa AND LRIG2 BIND NEOGENIN FN5/6



- co-IP Δ Neogenin and Δ Lrig2 constructs from cells

- Surface plasmon resonance (SPR) analysis purified Neogenin and Lrig2 proteins (fragments)

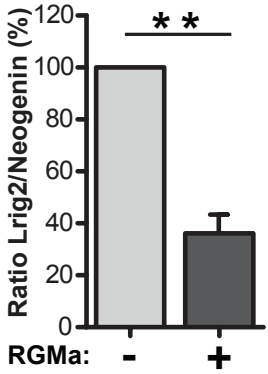
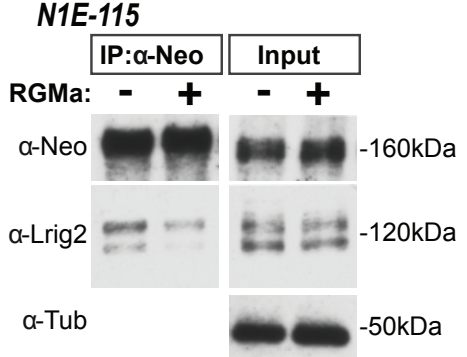
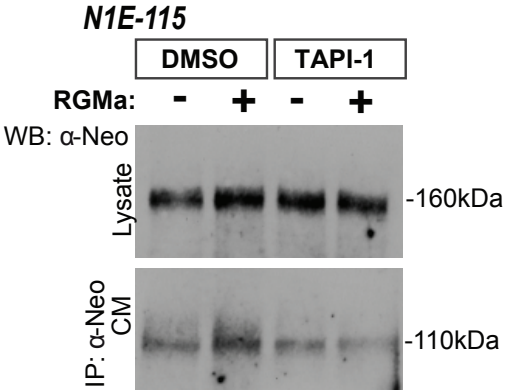
Neogenin

RGMa

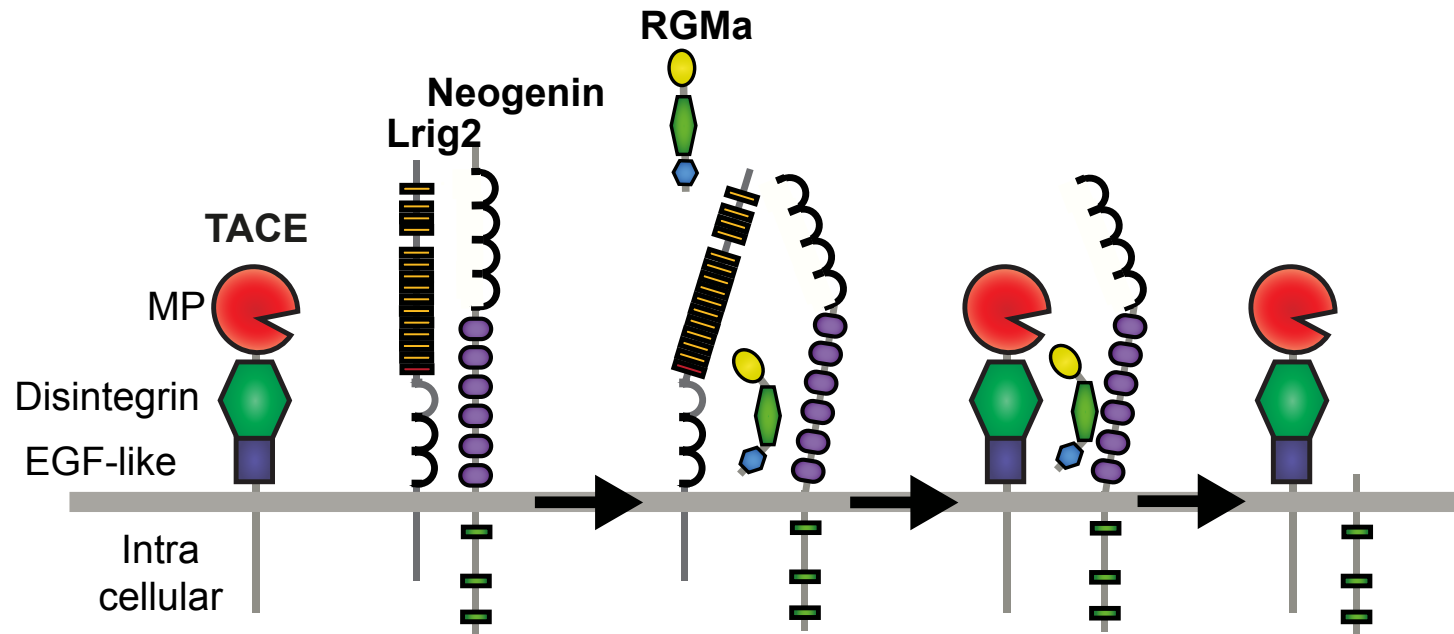
RGMa and Lrig2 bind FN5/6 in Neogenin

Bell et al., Science 2013

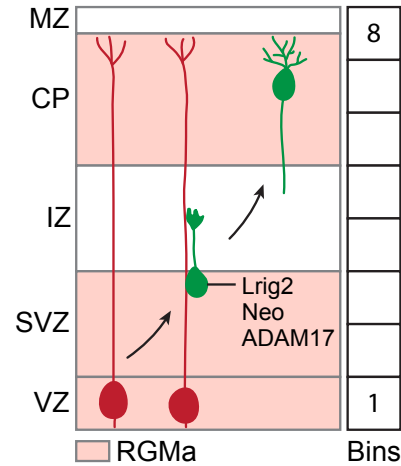
RGMa REGULATES NEOGENIN SHEDDING AND LRIG2-NEOGENIN BINDING



ROLE FOR LRIG2 IN RGMa-NEOGENIN SIGNALLING

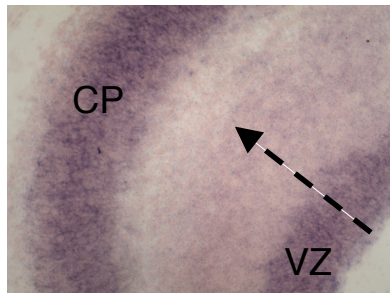


LRIG2 CONTROLS ADAM17-MEDIATED NEOGENIN SHEDDING DURING CORTICAL NEURON MIGRATION

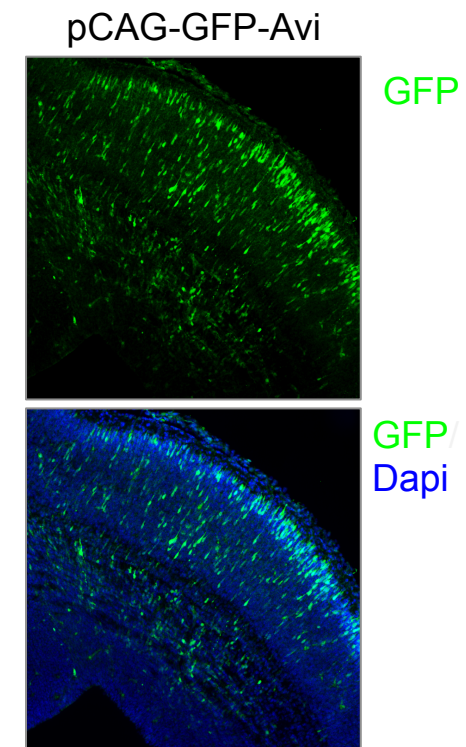
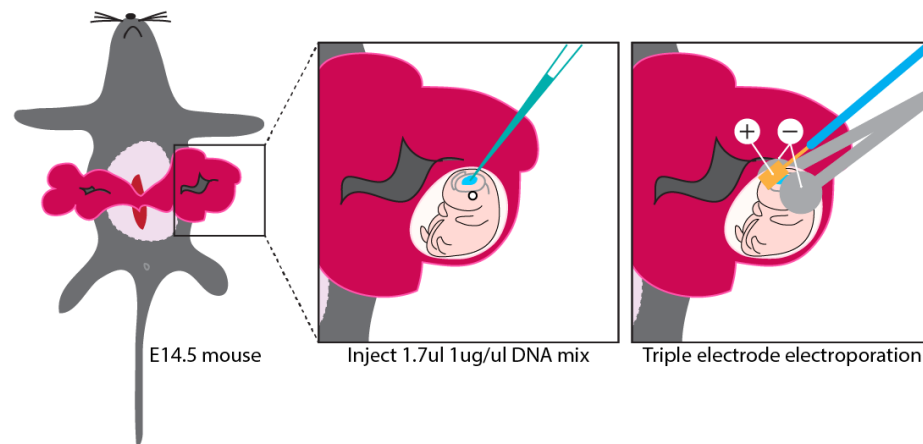


e.g. Bradford et al., 2010; Van den Heuvel et al., 2013;
O'Leary et al., 2013

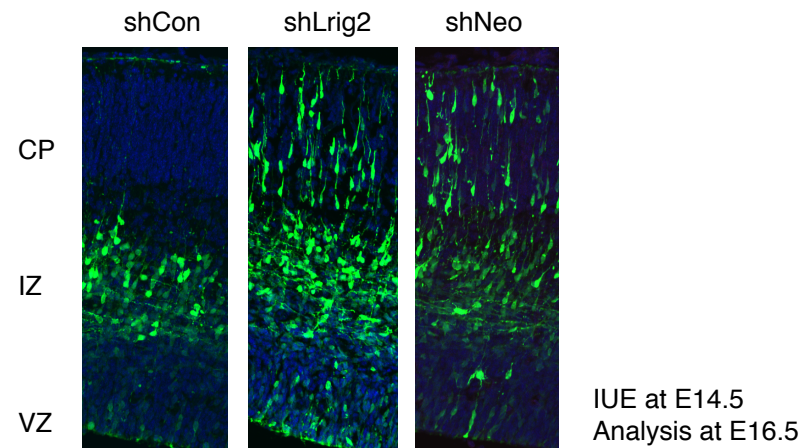
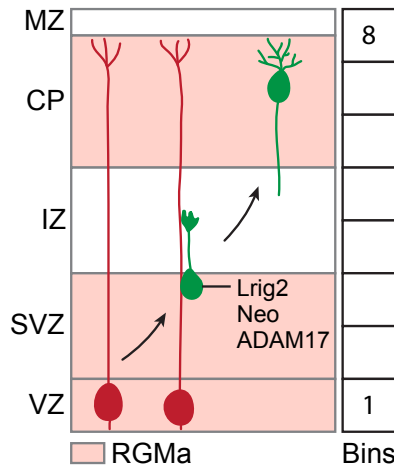
RGMa



ROLE FOR LRIG2 IN RGMa-NEOGENIN SIGNALLING

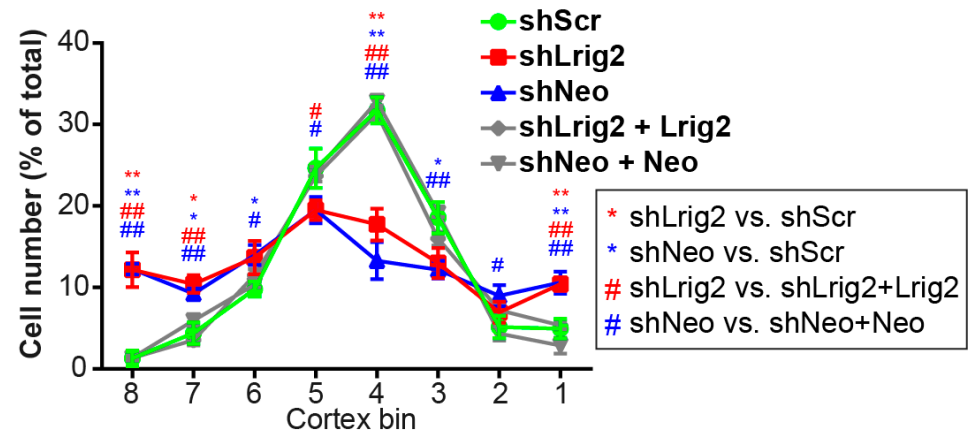
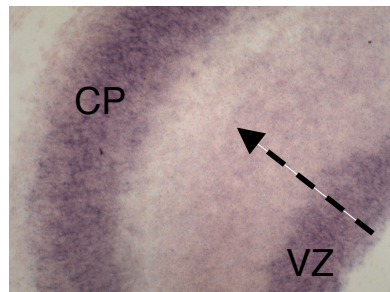


LRIG2 CONTROLS ADAM17-MEDIATED NEOGENIN SHEDDING DURING CORTICAL NEURON MIGRATION

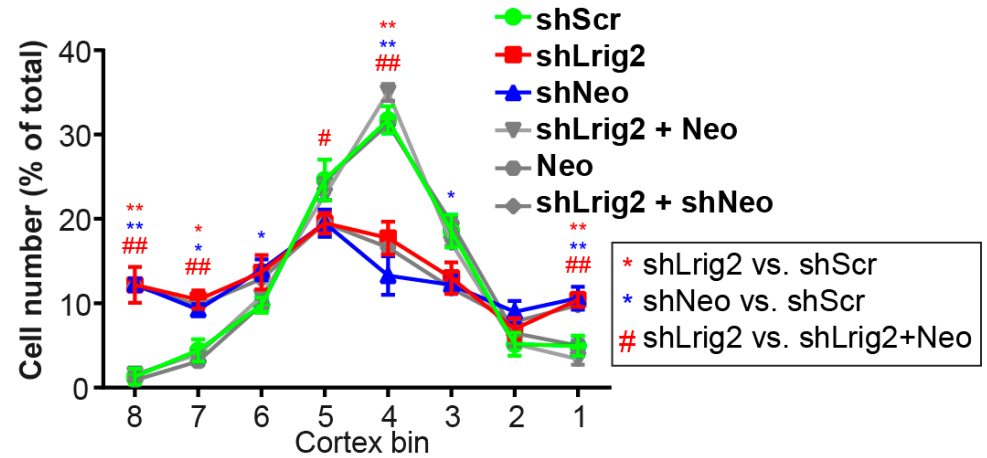
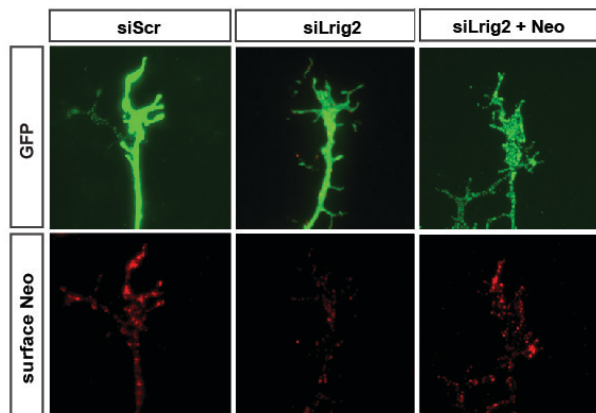
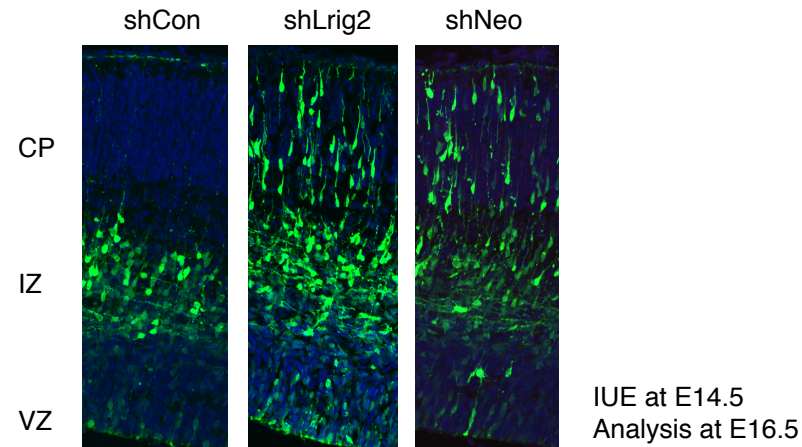
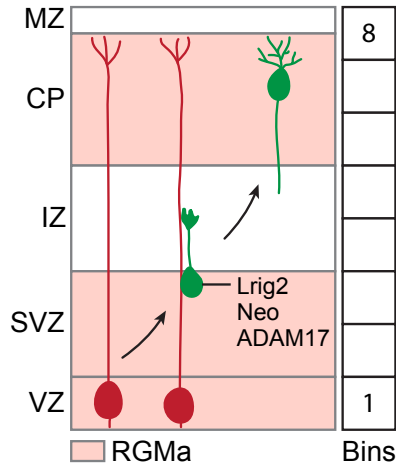


e.g. Bradford et al., 2010; Van den Heuvel et al., 2013;
O'Leary et al., 2013

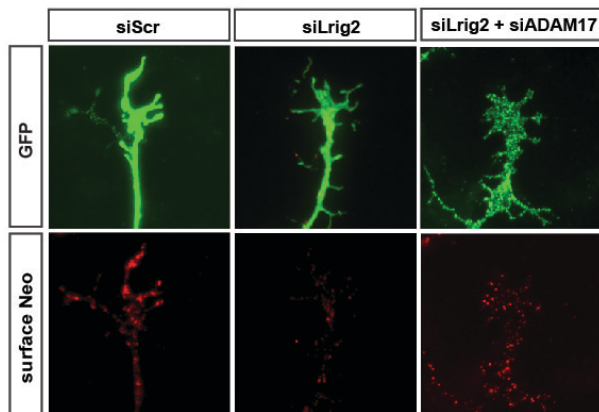
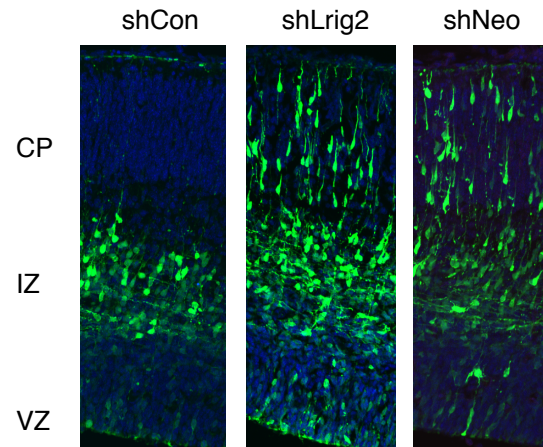
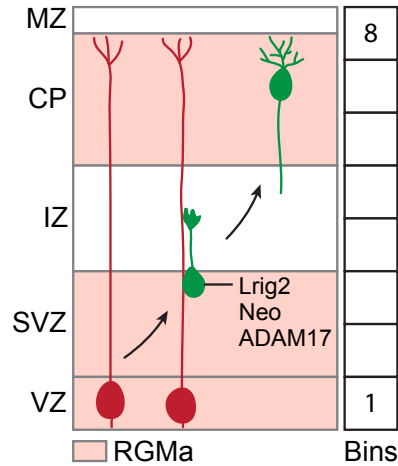
RGMa



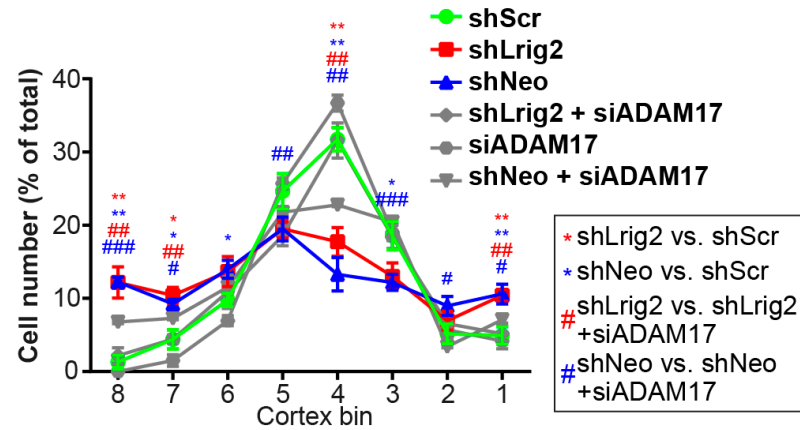
LRIG2 CONTROLS ADAM17-MEDIATED NEOGENIN SHEDDING DURING CORTICAL NEURON MIGRATION



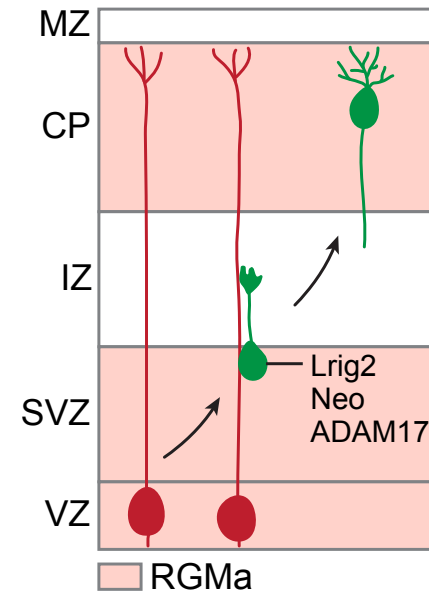
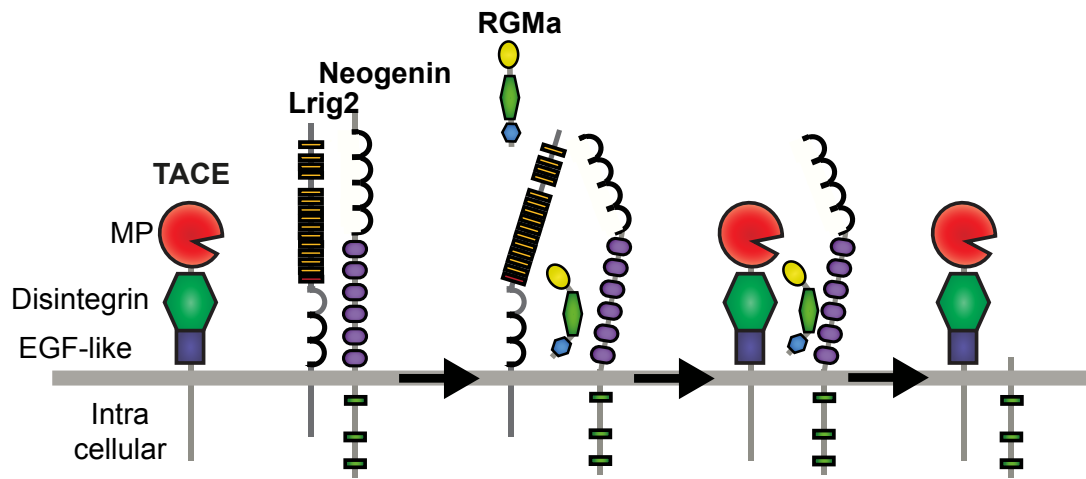
LRIG2 CONTROLS ADAM17-MEDIATED NEOGENIN SHEDDING DURING CORTICAL NEURON MIGRATION



ADAM17 rescue



CONCLUSIONS II



CONCLUSIONS

- Biochemical and genetic screening led to the identification of ~100 axon guidance proteins
- Axon guidance cues act as attractants and repellents, can be membrane-bound or secreted
- Receptors on growth cones detect axon guidance cues and can diversify the effects of these cues
- **Take home:** Knowledge of axon guidance cues can provide insight or treatment possibilities for disease