AXON GUIDANCE

Monday 02/27/2023

Reading:

Kandel et al., Principles of Neuroscience, 6th edition. Chapter 47, *Growth and Guidance of Axons.*

Gorla and Bashaw (2020) *Molecular mechanisms regulating axon responsiveness at the midline*. Developmental Biology 466, 12-21

References:

Colak et al., (2013) *Regulation of Axon Guidance by Compartmentalized Nonsense-Mediated mRNA Decay*. Cell 153, 1252-1265.

Zelina et al., (2014) *Signaling Switch of the Axon Guidance Receptor Robo3 during Vertebrate Evolution*. Neuron 84, 1258-1272.

Zhuang et al., (2019) *The m6A reader YTHDF1 regulates axon guidance through translational control of Robo3.1 expression*. Nucleic Acids Research 79, 4765-4777.

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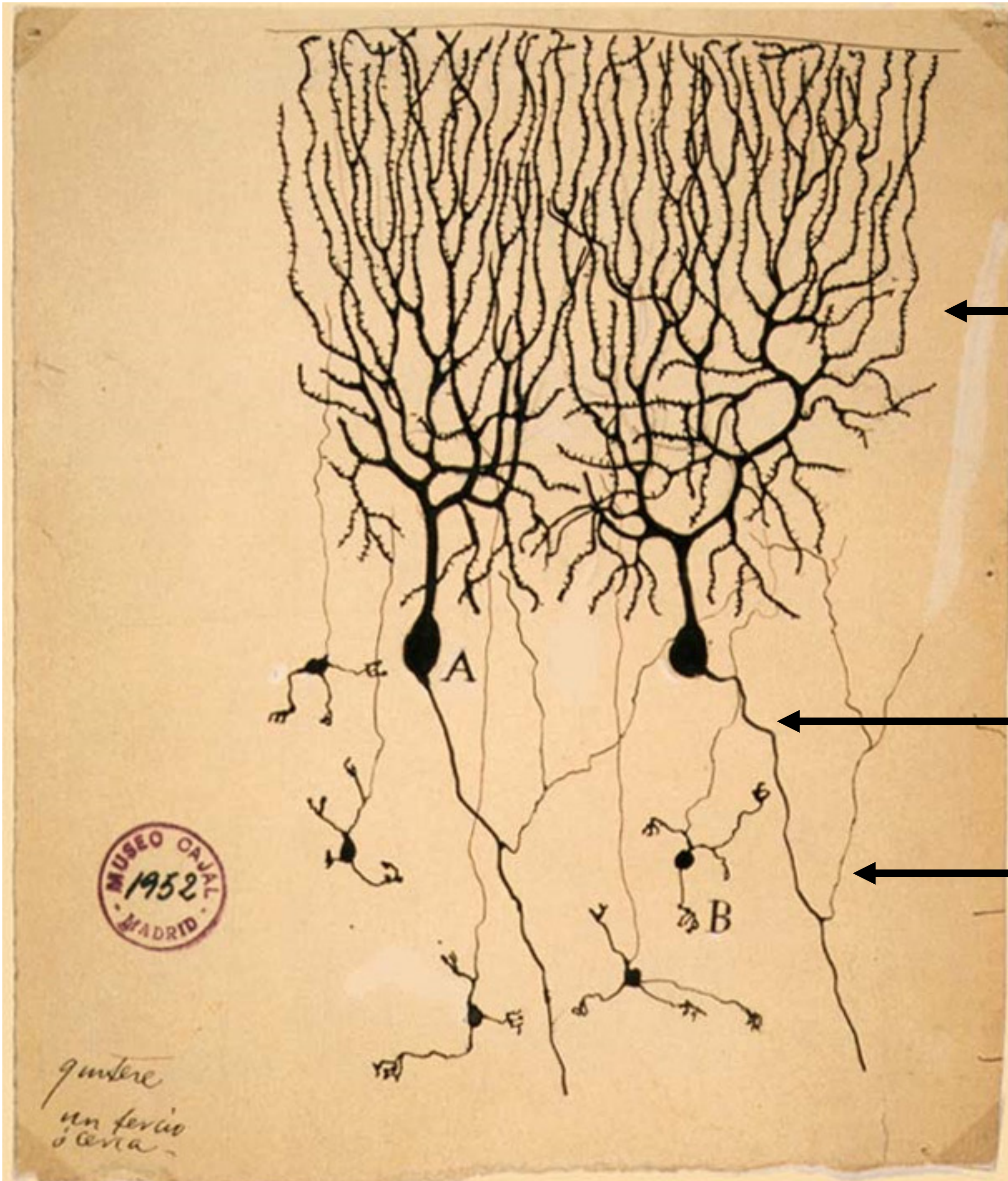
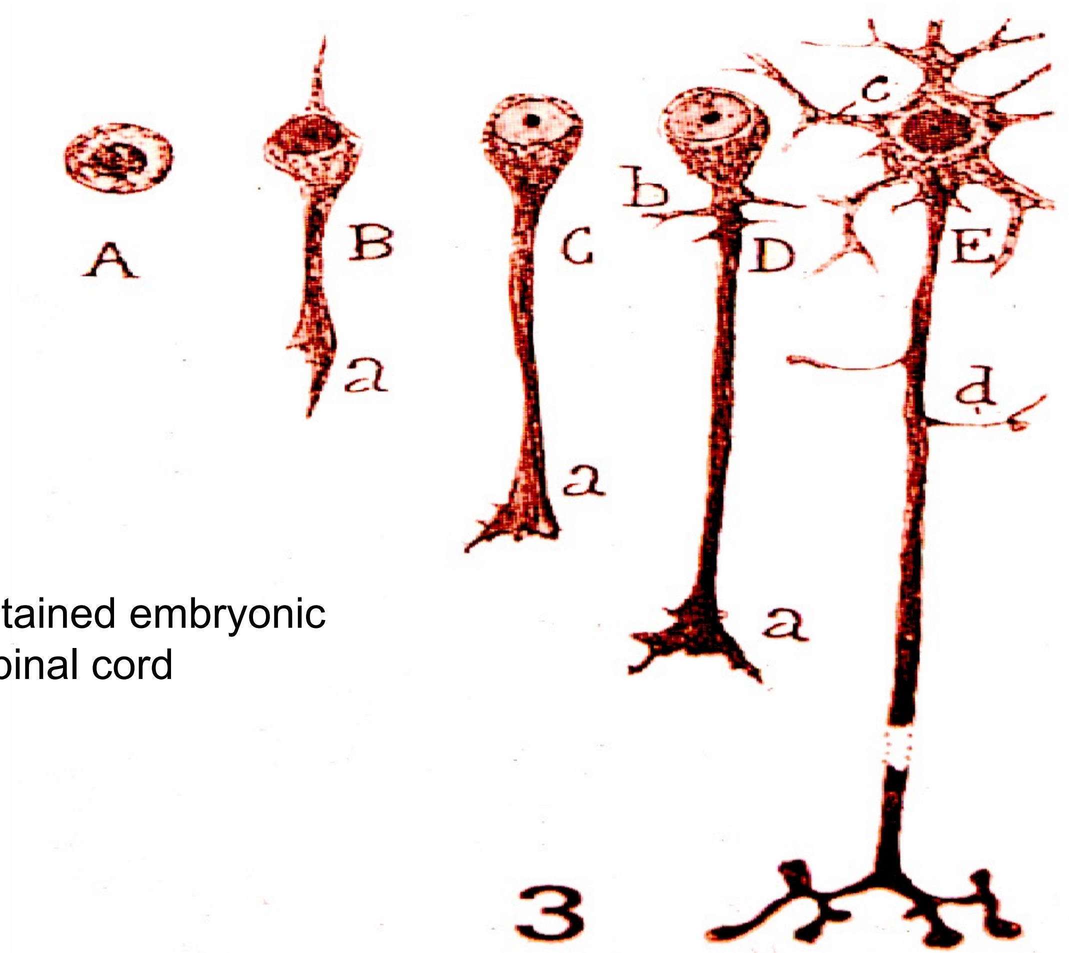
NURO8345

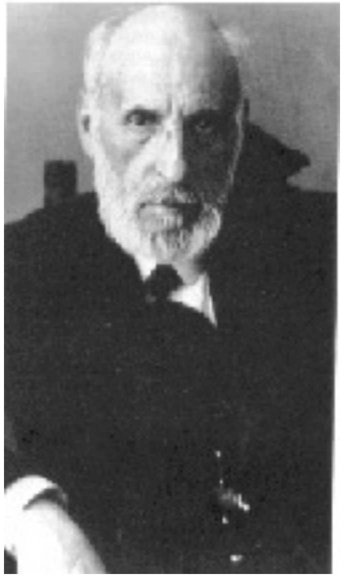
Camera Lucida of Purkinje neurons by S. Ramon y Cajal



S. Ramon y Cajal describes the growth cone



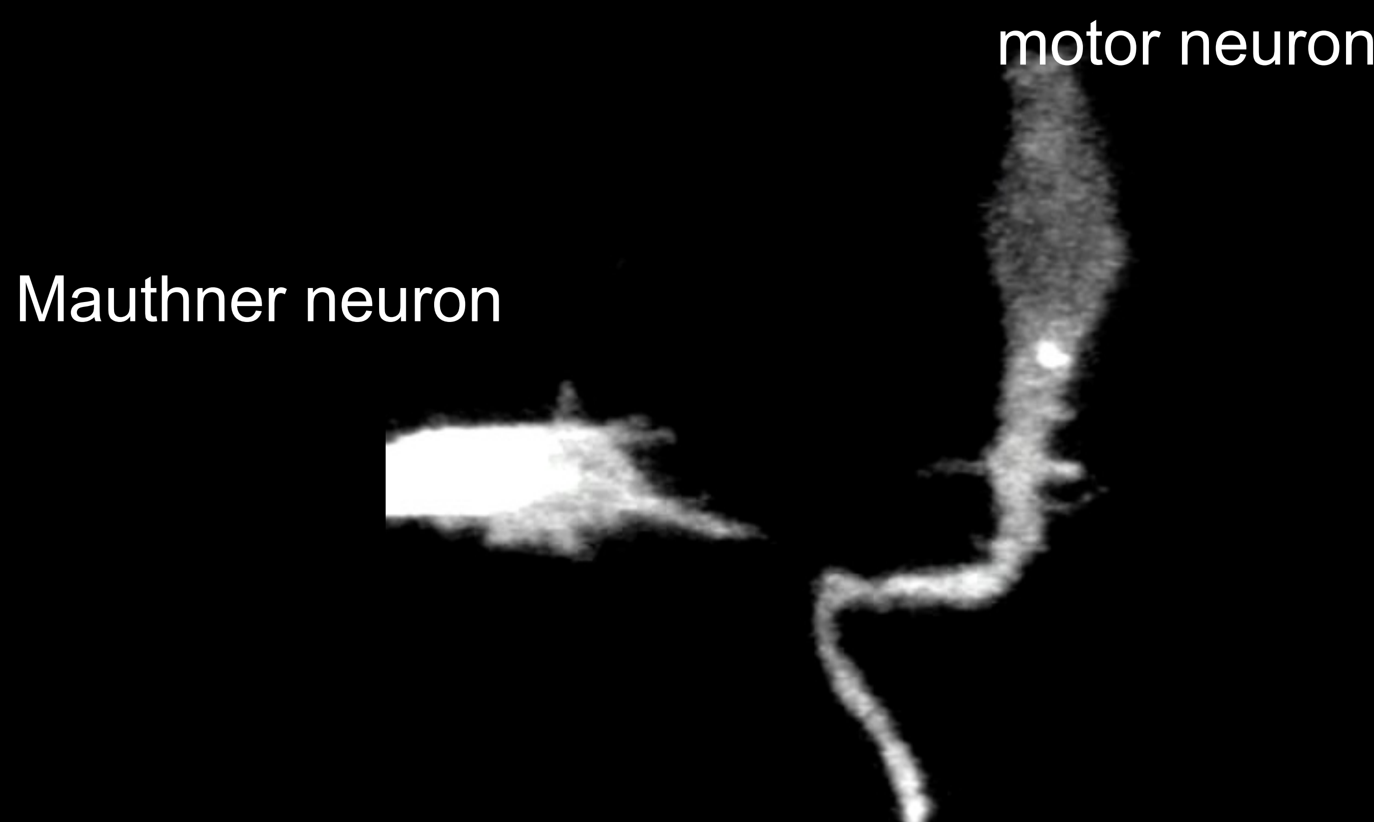
Silver-stained embryonic
chick spinal cord



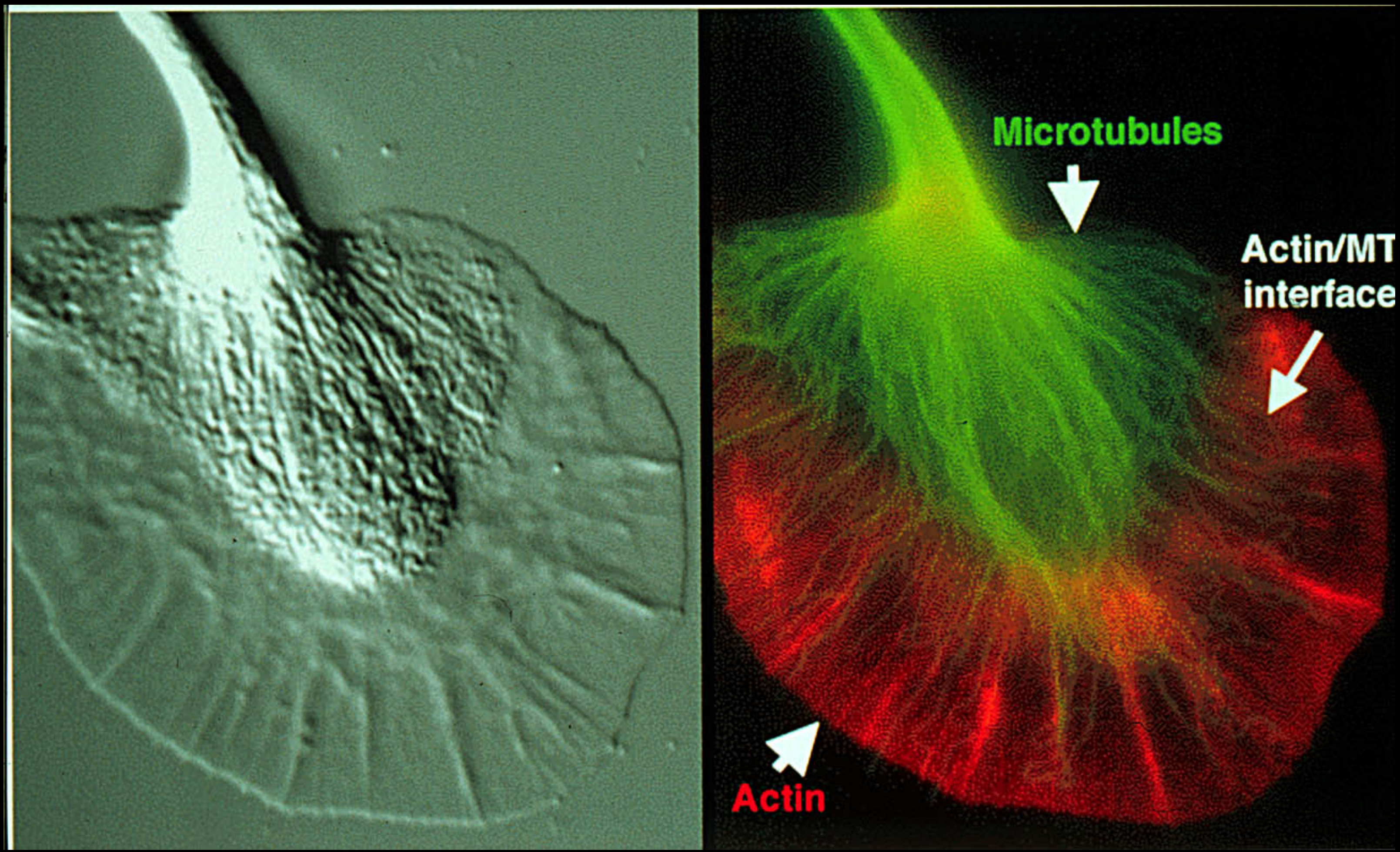
"…endowed with exquisite chemical sensitivity, with rapid ameboid movements, and with certain impulsive force to…overcome obstacles…, forcing cellular interstices until it arrives at its destination"

http://8e.devbio.com/article.php?id=145

Mauthner neuron axonal growth cone navigates the zebra fish spinal cord

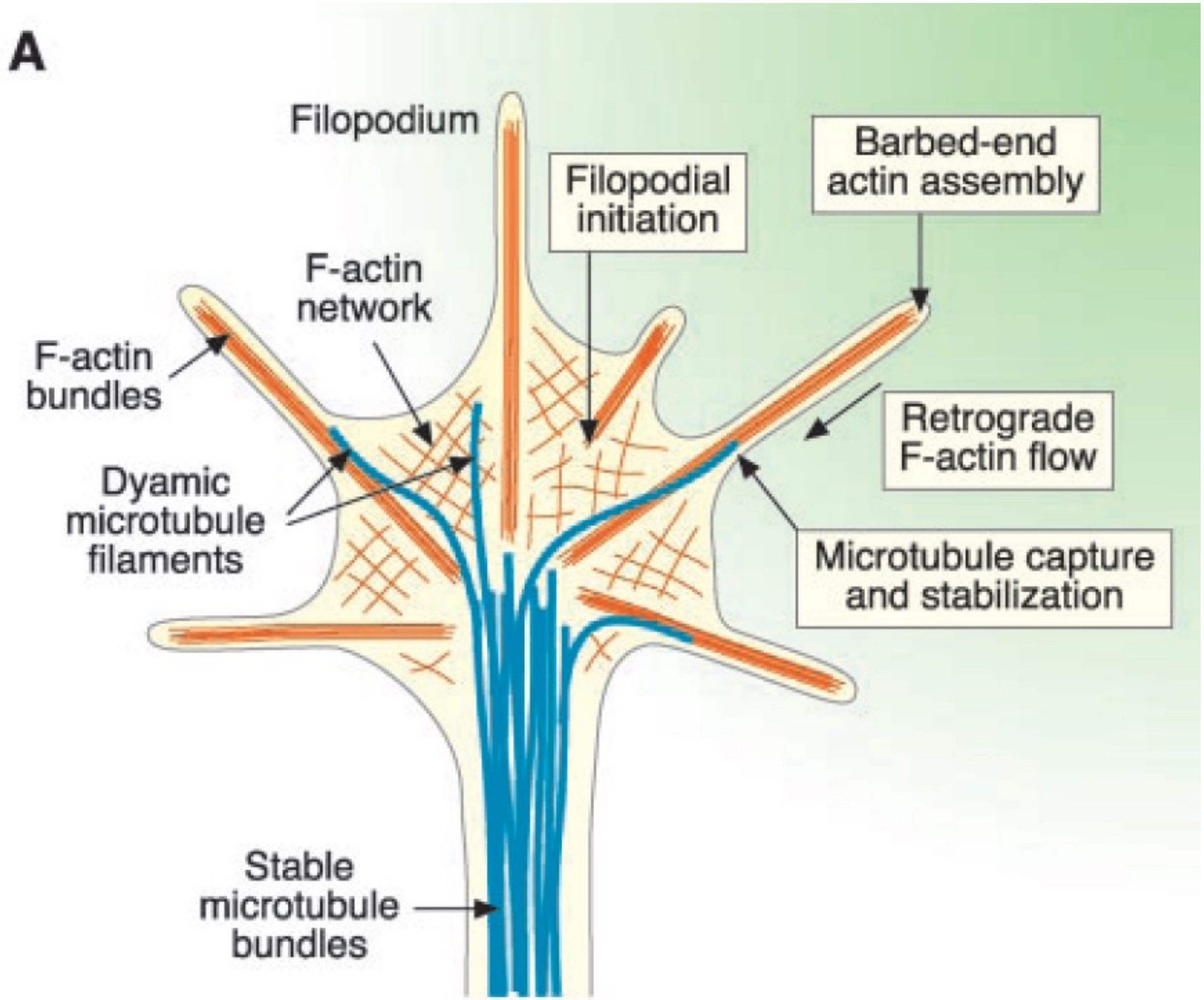


The growth cone cytoskeleton directs axon guidance

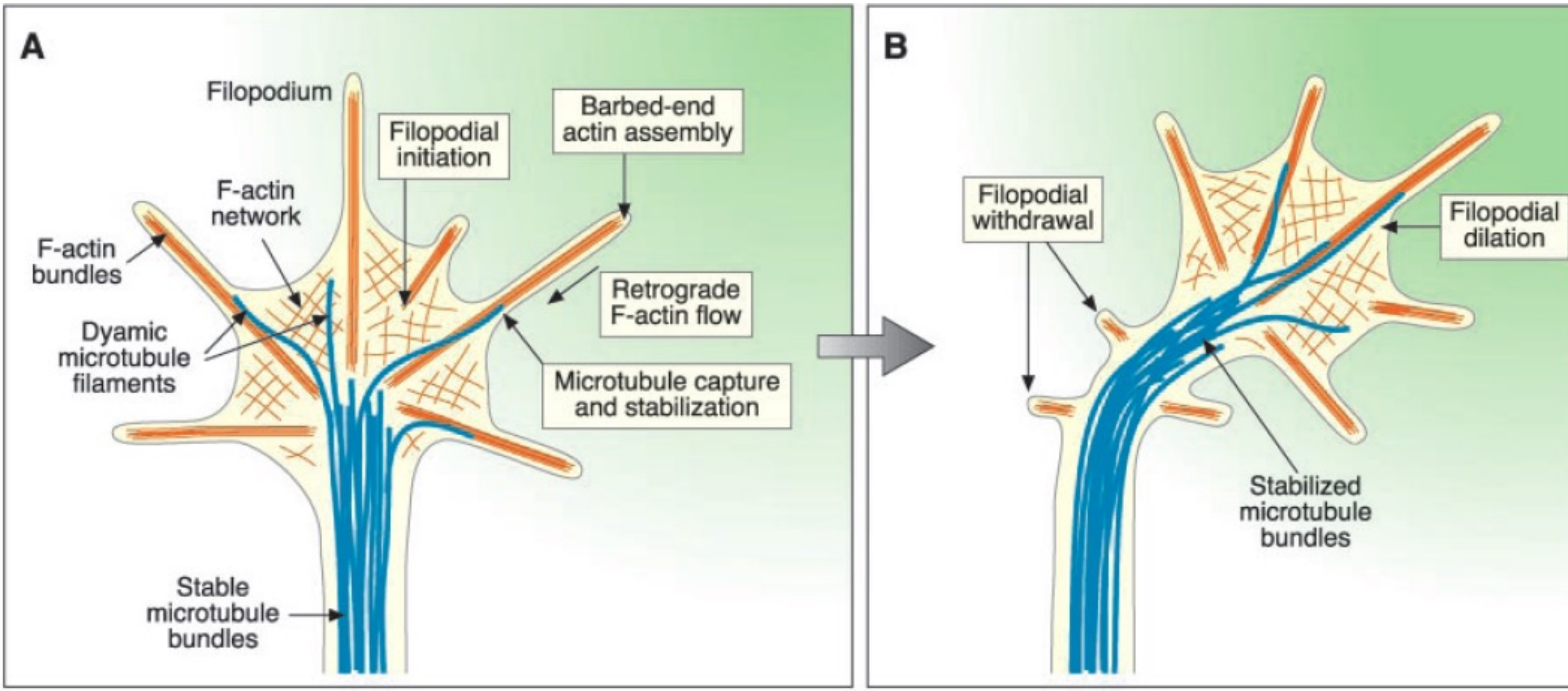


Aplysia neuron growth cone

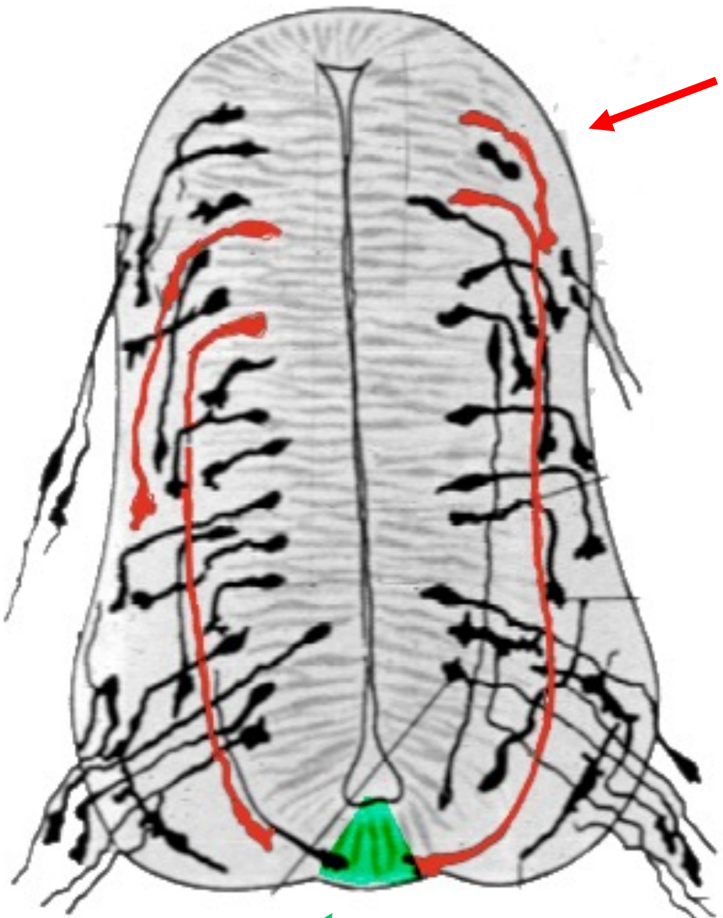
Image by Paul Forscher


A
Filopodium
Filopodial initiation
Barbed-end actin assembly
F-actin network
F-actin bundles
Retrograde F-actin flow
Dyamic microtubule filaments
Microtubule capture and stabilization
Stable microtubule bundles

Model of cytoskeletal rearrangement in turning Growth Cone



Axon guidance along D/V axis of vertebrate spinal cord



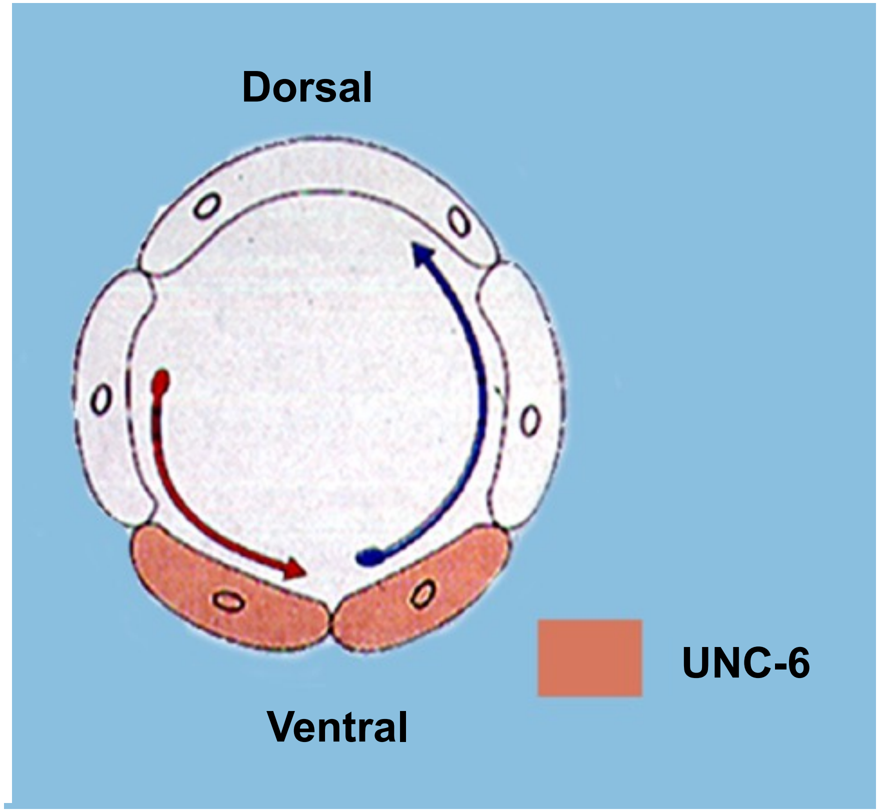
Commissural interneurons
Project ventrally around circumference then dive toward midline

Floorplate

Ramon y Cajal (1890s)
proposed that floorplate attracts commissural axons with diffusible cue

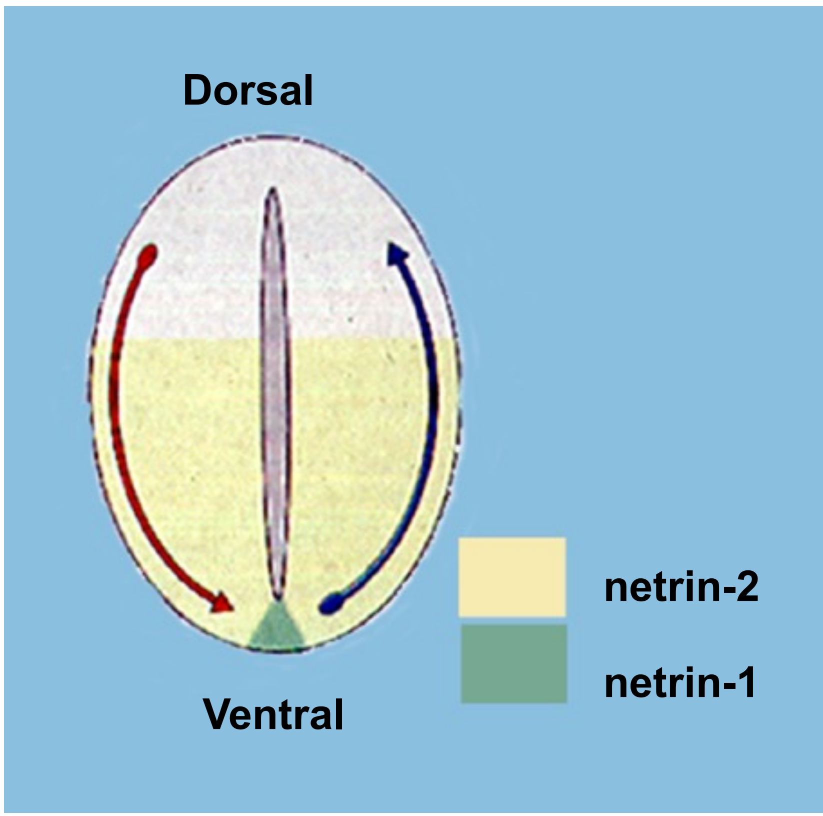
Netrins/UNC-6 are ventral-derived axon guidance cues in nematodes and in the vertebrate spinal cord (1990s)

C. elegans (round worm)



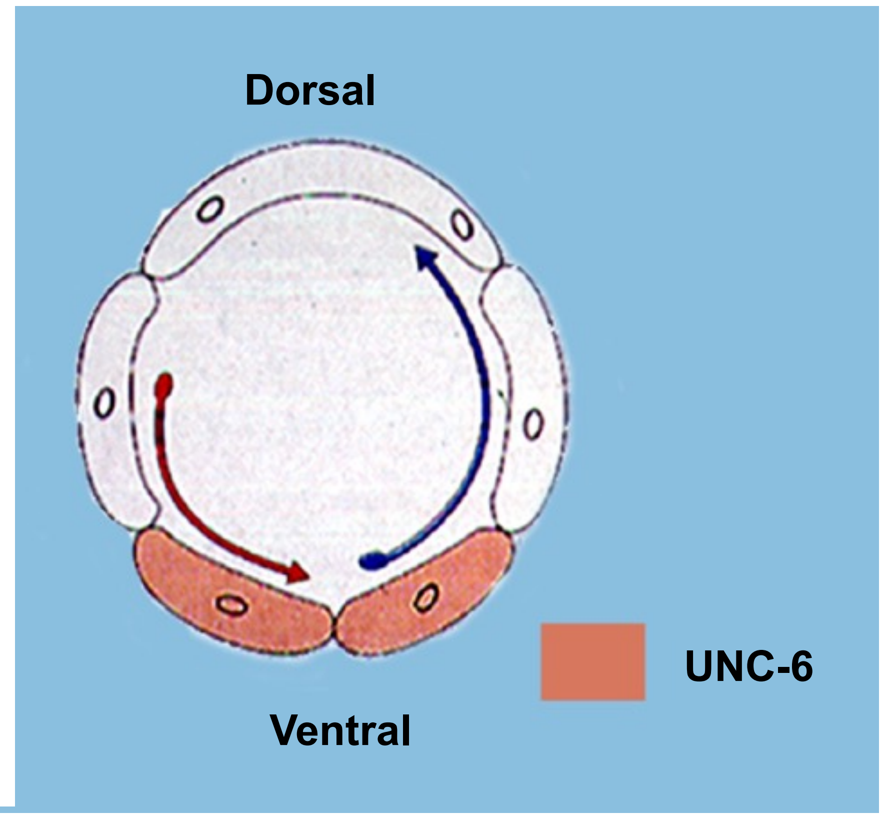
Netrins/UNC-6 are ventral-derived axon guidance cues in nematodes and in the vertebrate spinal cord (1990s)

Vertebrate spinal cord



Chick

***C. elegans* (round worm)**



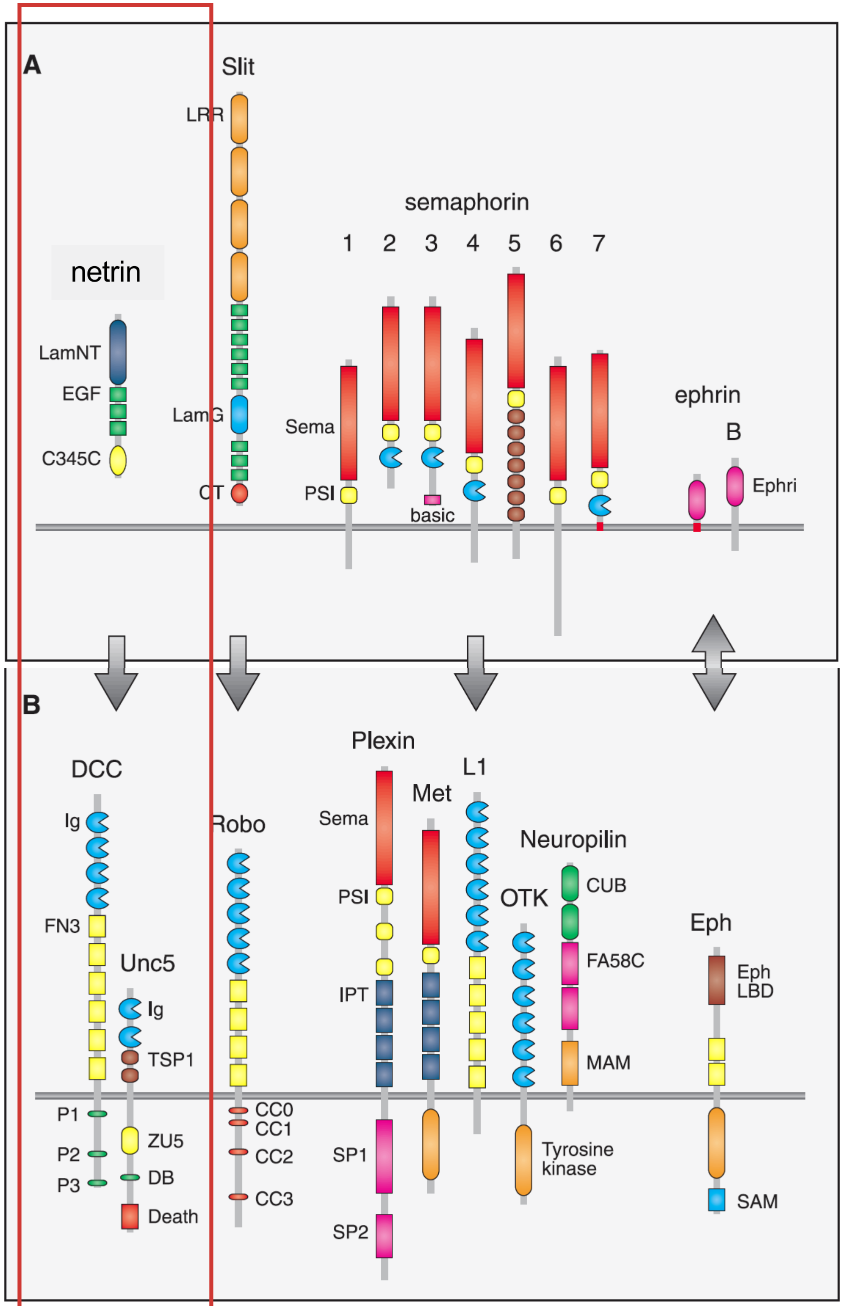
Major Guidance Cue/Receptor Families

Cue	Receptor
Netrin/UNC-6	DCC/UNC-40, UNC-5
Slit	Robo
Semaphorin	Plexin, Neuropilin
Ephrin	Eph

Four Major Guidance Cue/Receptor Families

Cues

Receptors



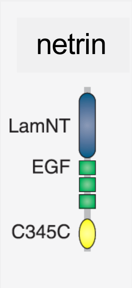
Dickson, B. (2002) *Molecular Mechanisms of Axon Guidance* **Science 298**, 1959-1964

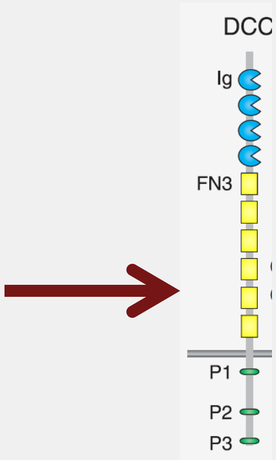
Netrin is secreted from the floorplate to direct axon outgrowth along the dorsal/ventral axis

Attraction

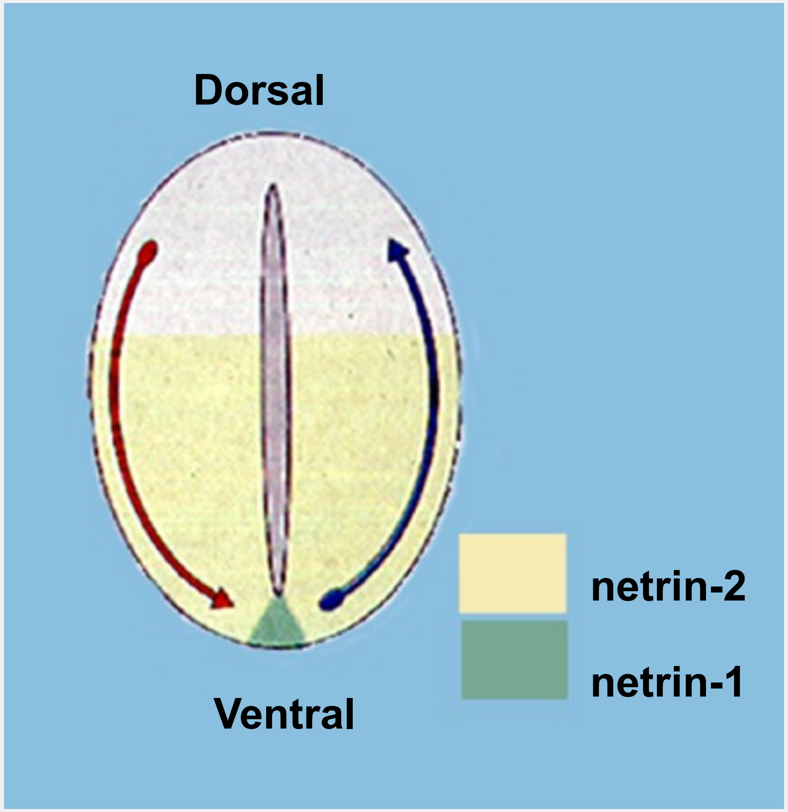
Cue

Receptor

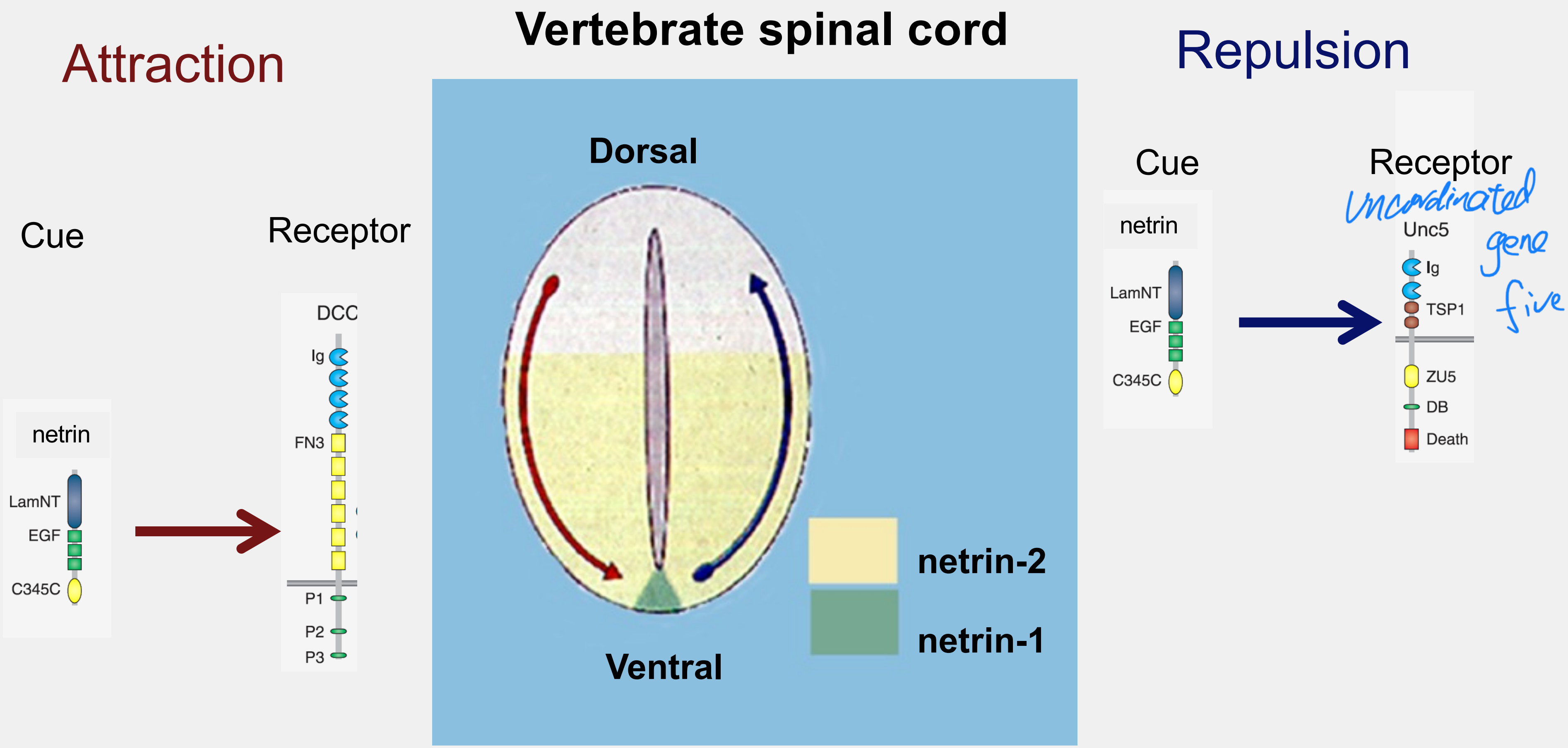




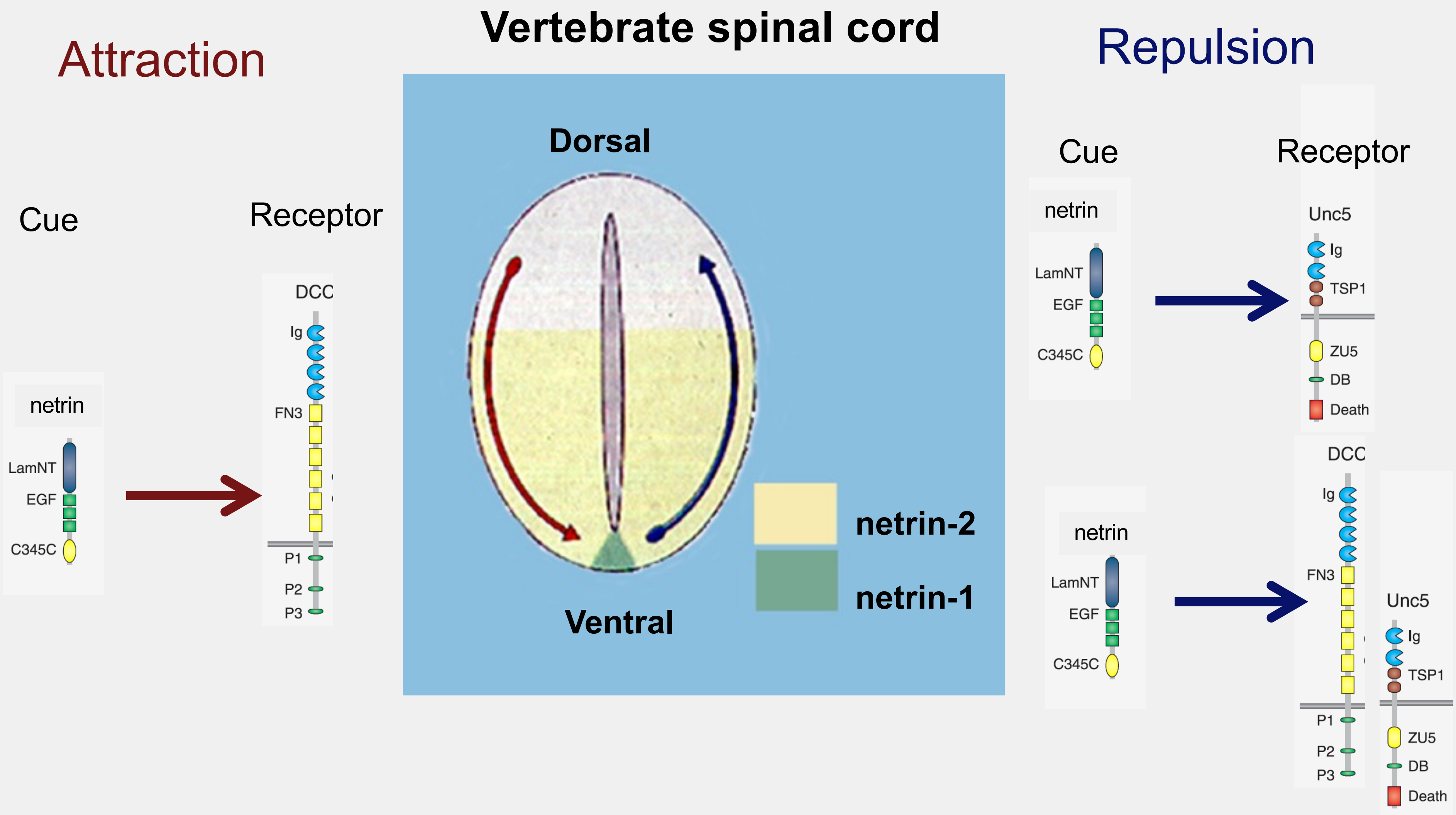
Vertebrate spinal cord



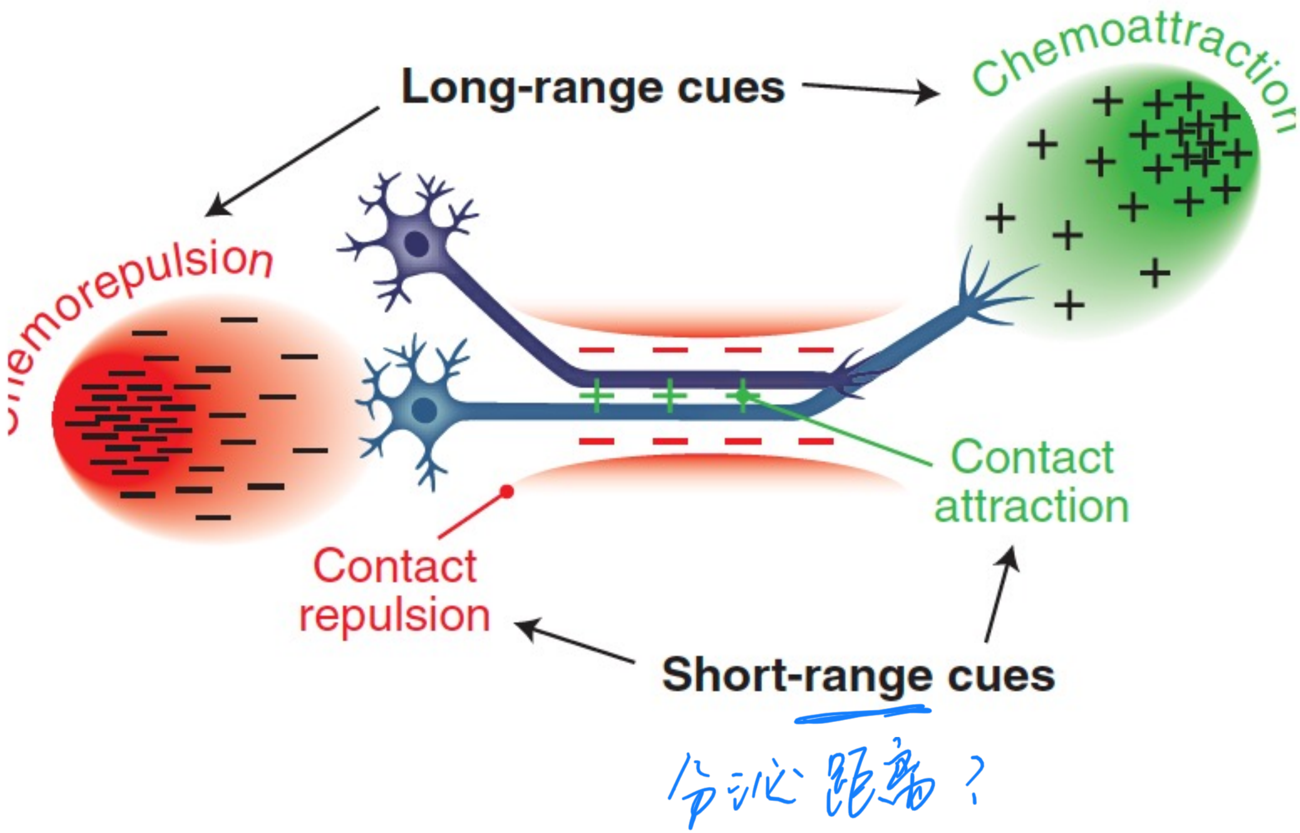
Netrin is secreted from the floorplate to direct axon outgrowth along the dorsal/ventral axis



Netrin is secreted from the floorplate to direct axon outgrowth along the dorsal/ventral axis

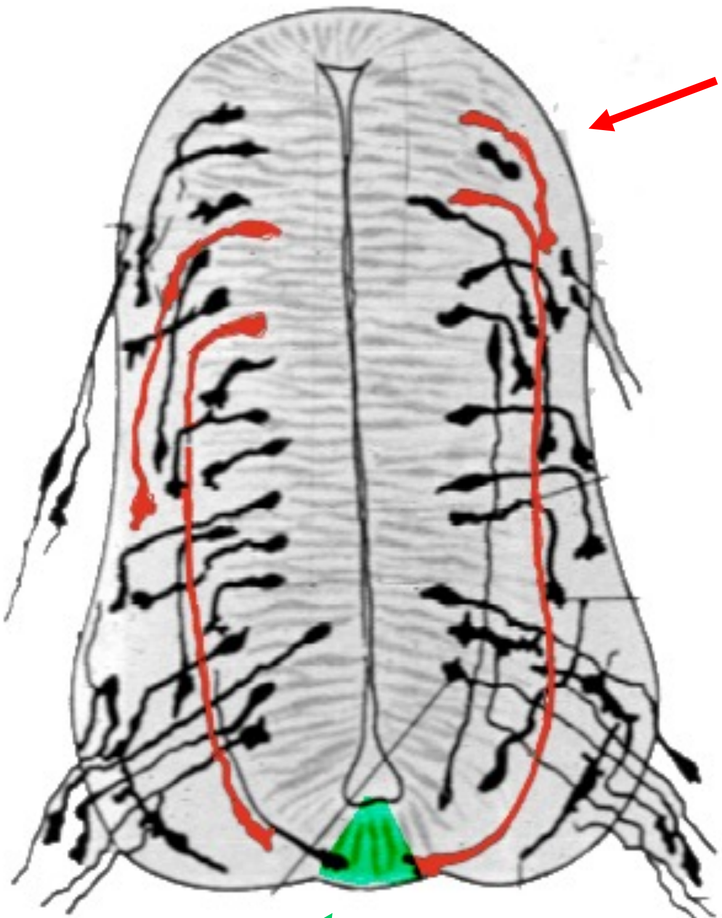


Guidance cues regulate axon outgrowth



AL Kolodkin & M Tessier-Lavigne (2011). *Mechanisms and molecules of neuronal wiring: A primer*. **Cold Spring Harbor Perspectives in Biology 3**:a001727

Axon guidance along D/V axis of vertebrate spinal cord



Commissural interneurons
Project ventrally around circumference then dive toward midline, turn along A/P axis

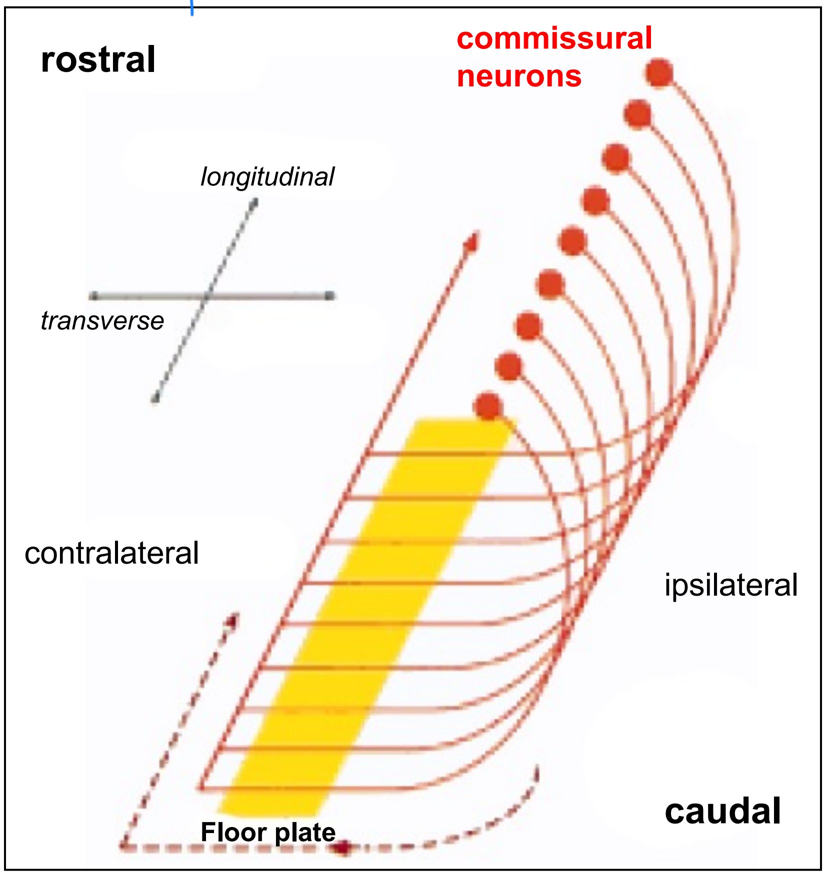
Ramon y Cajal (1890s)
proposed that floorplate attracts commissural axons with diffusible cue

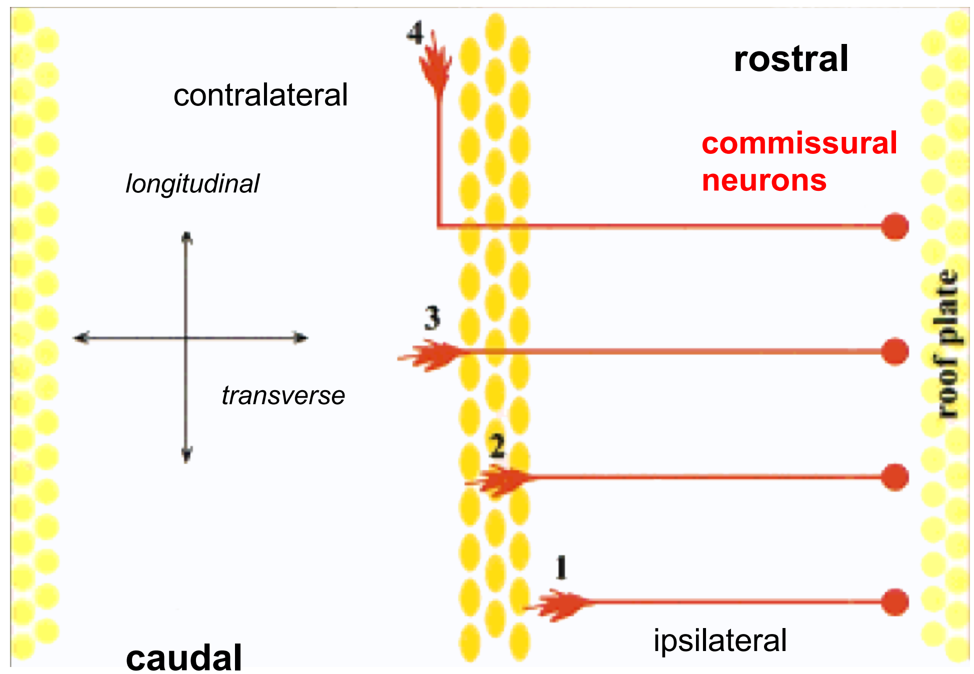
Netrin/UNC-6

Floorplate

Commissural neurons cross the midline and turn along the body axis

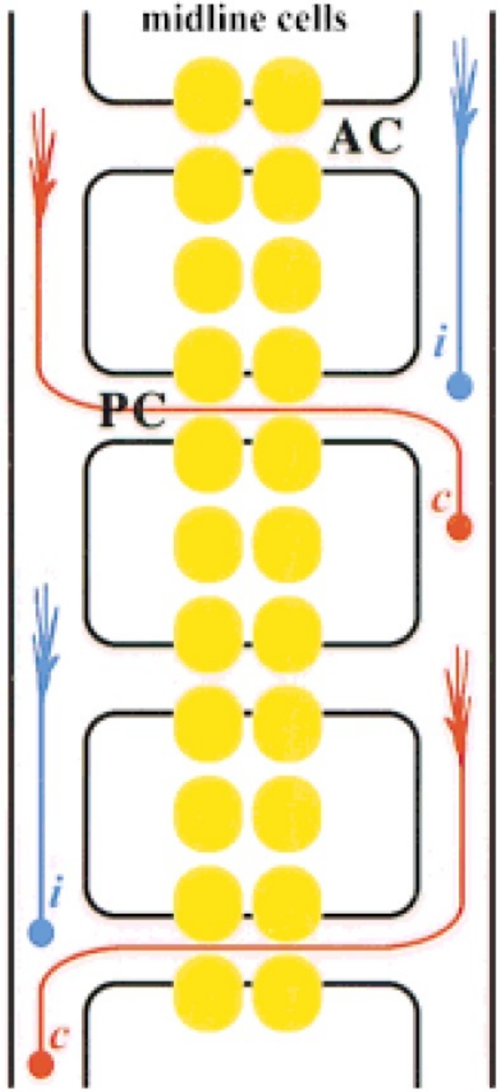
" open book look"

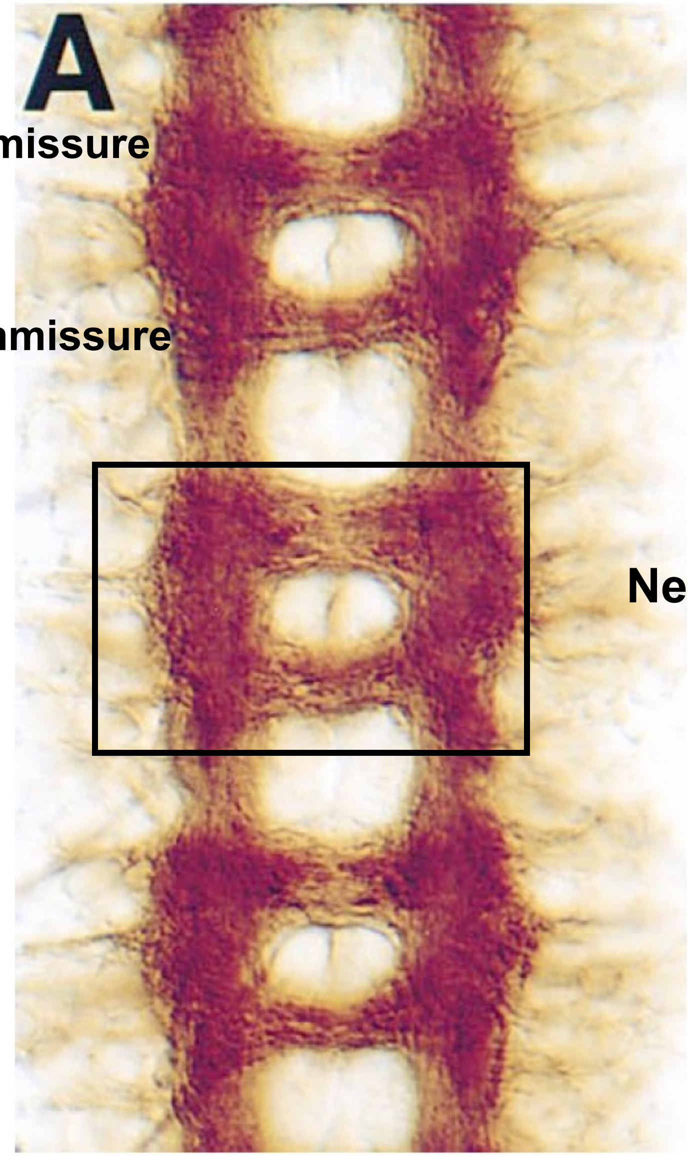




"open book" view

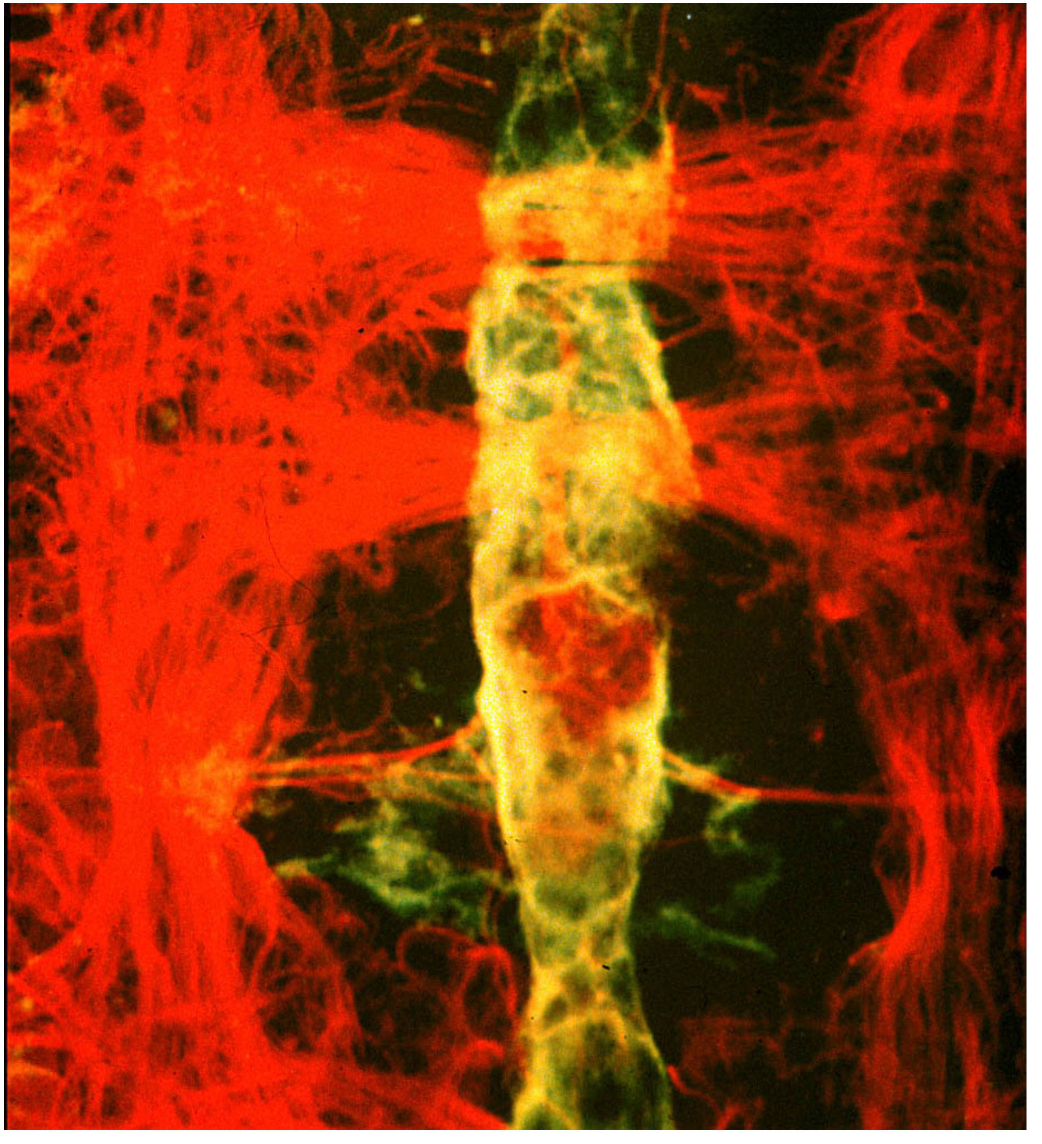
Commissures cross the midline from bilateral axon tracts





fly nerve cord

Midline organizes CNS



insect nerve cord

Major Guidance Cue/Receptor Families

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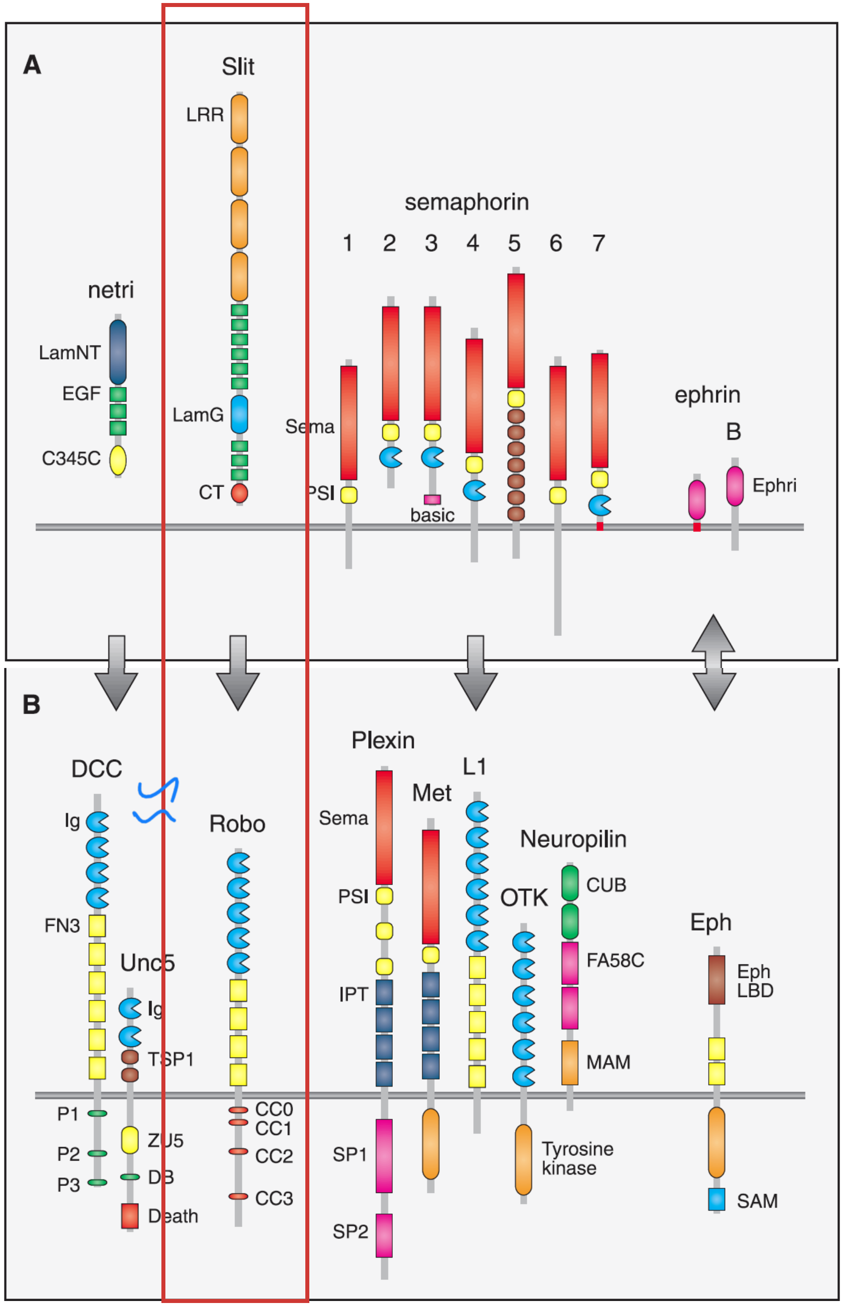
Ephrin	Eph

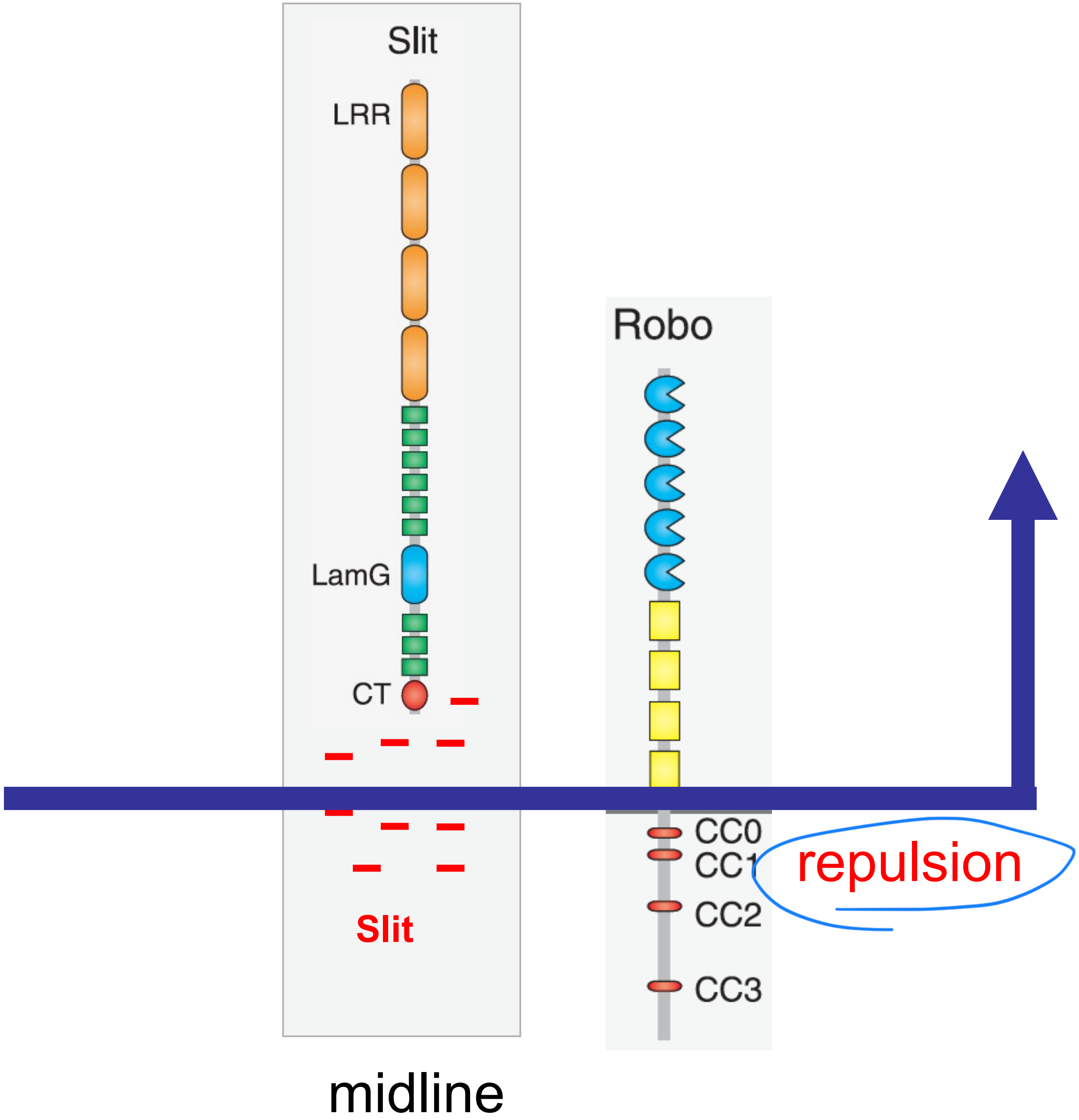
Four Major Guidance Cue/Receptor Families

Cues

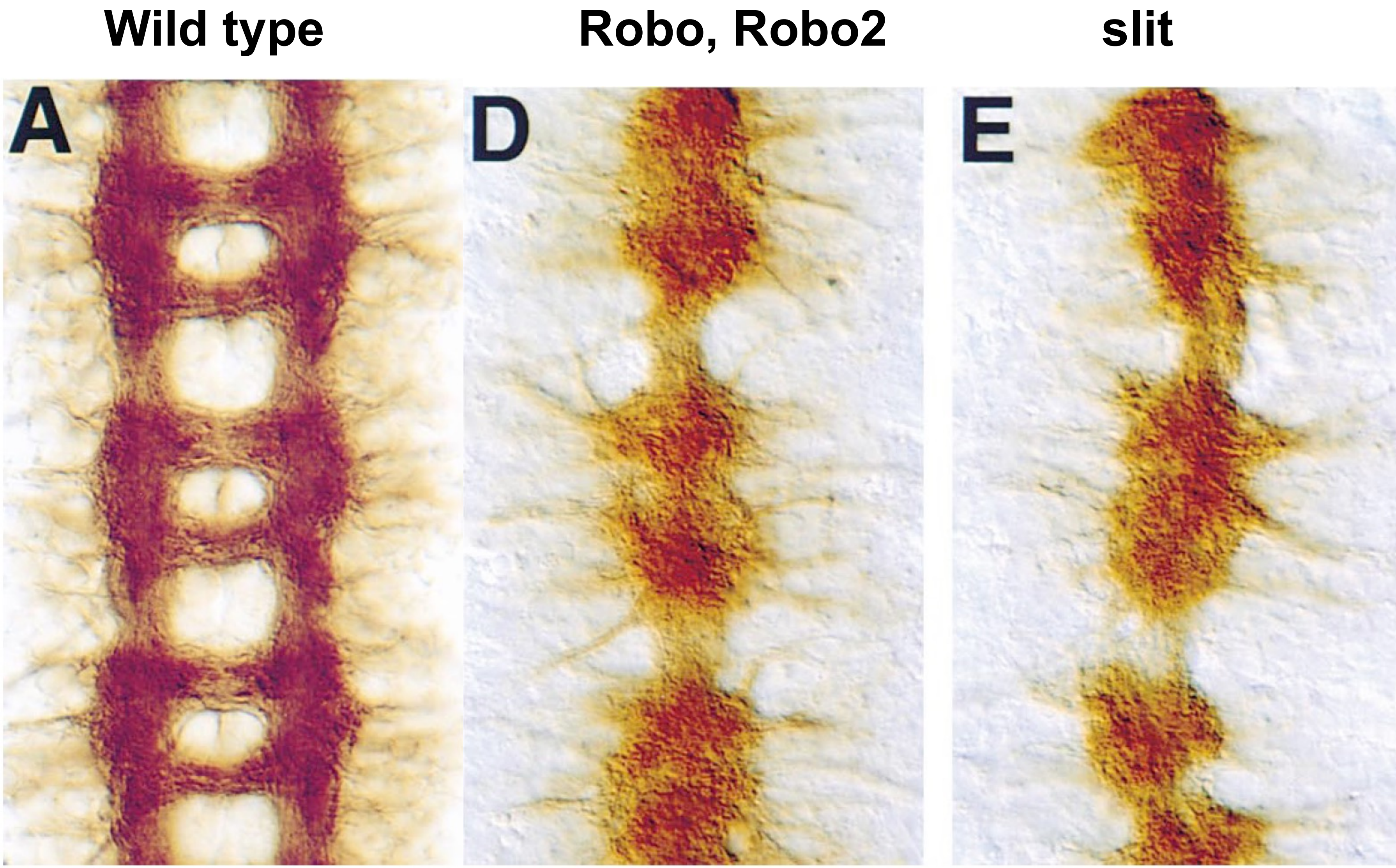
Receptors



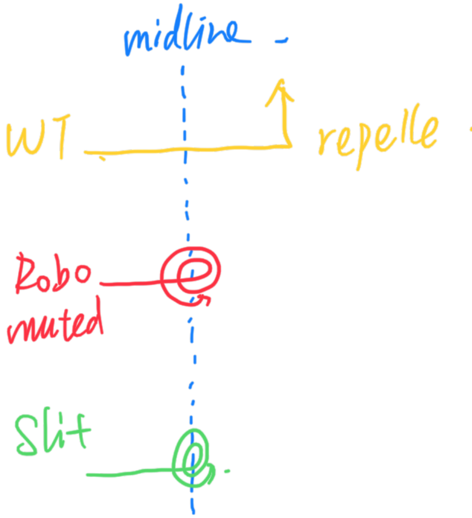
Robo receptor responds to repulsive slit cue



Excessive midline crossing in robo and slit mutants



Drosophila embryos



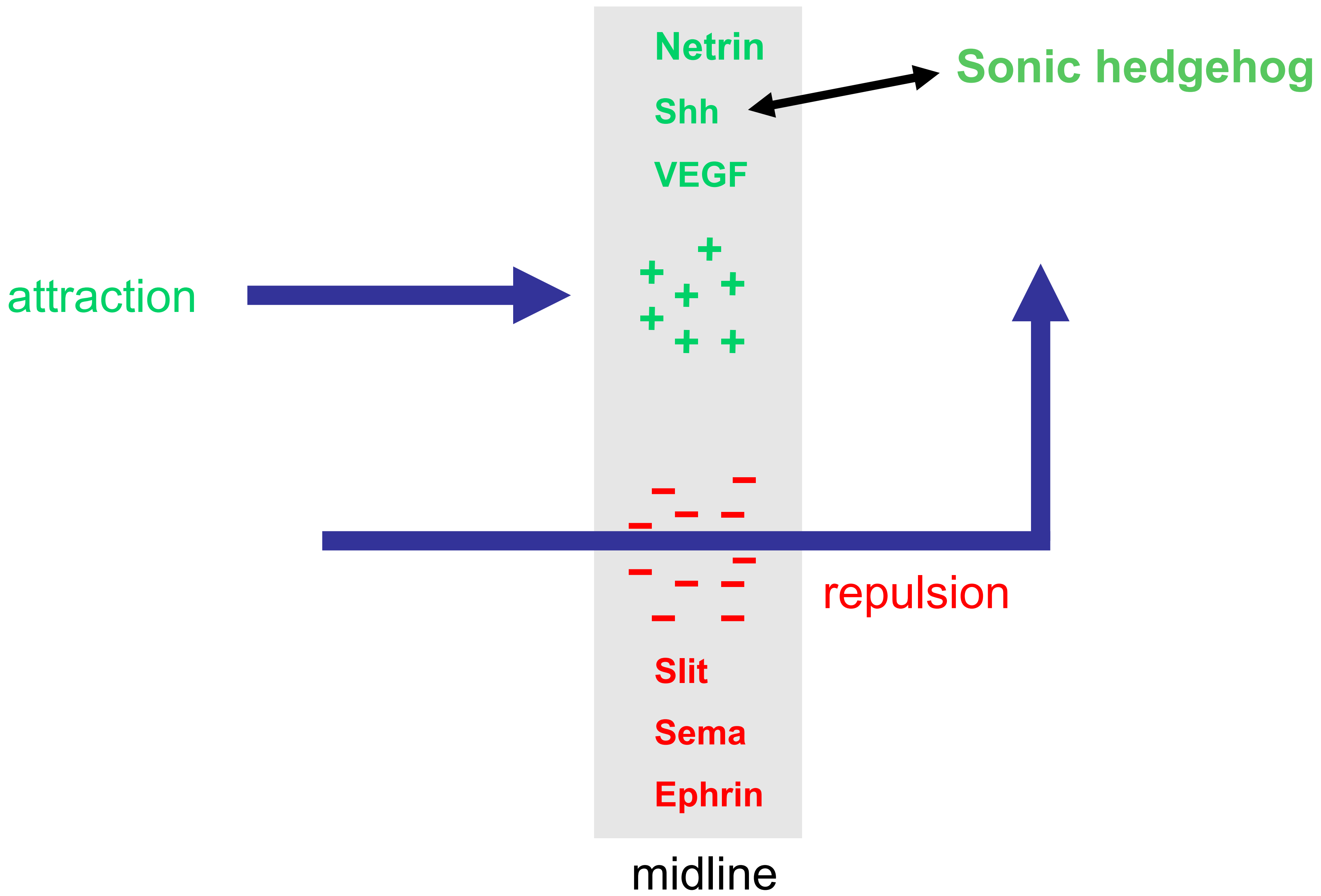
Midline :

Robo : "Roundabout"

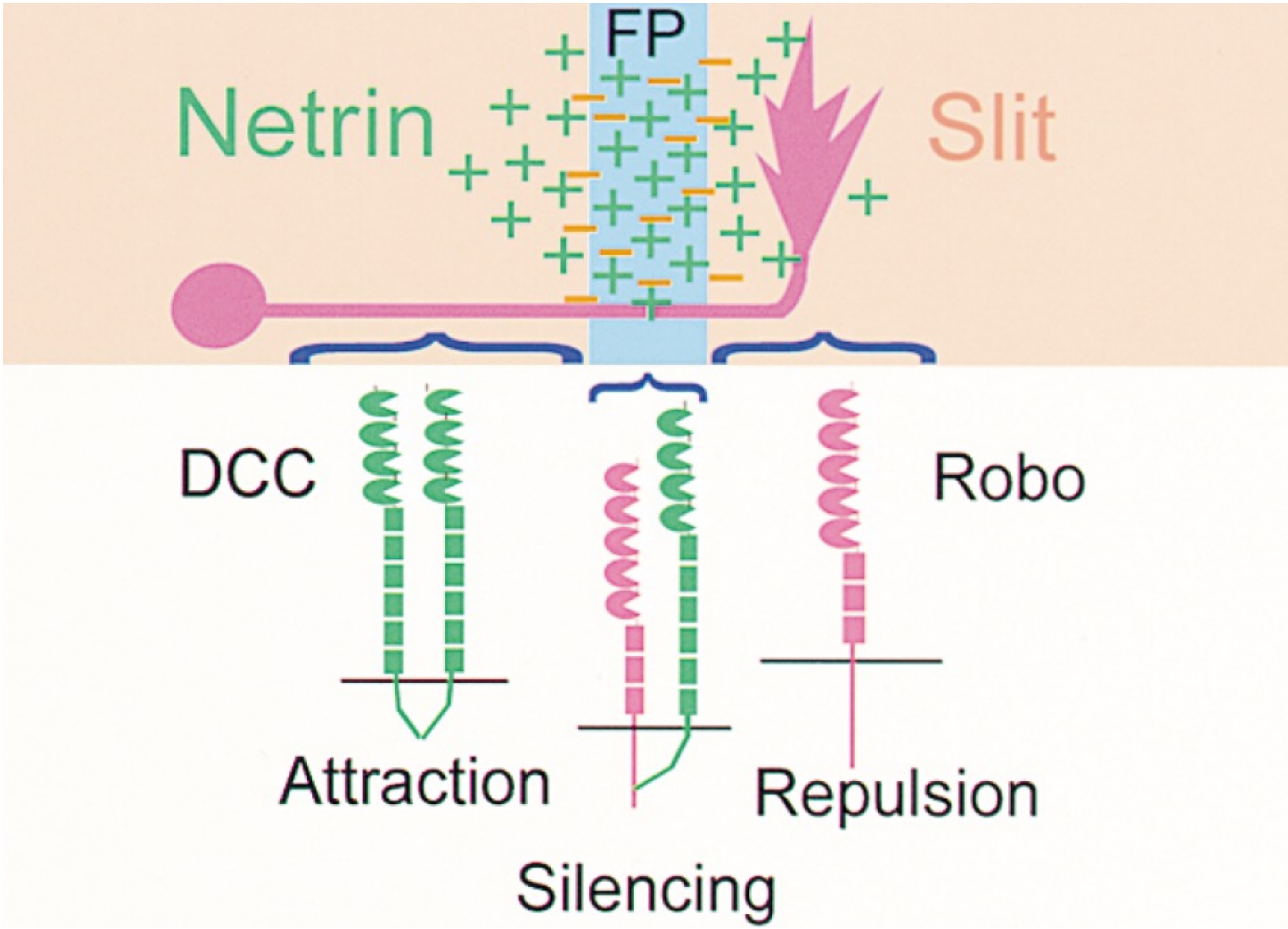
Slit $\longrightarrow$ Robo $\longrightarrow$ Repulsion.

ligand receptor.

Positive and negative signals regulate midline crossing

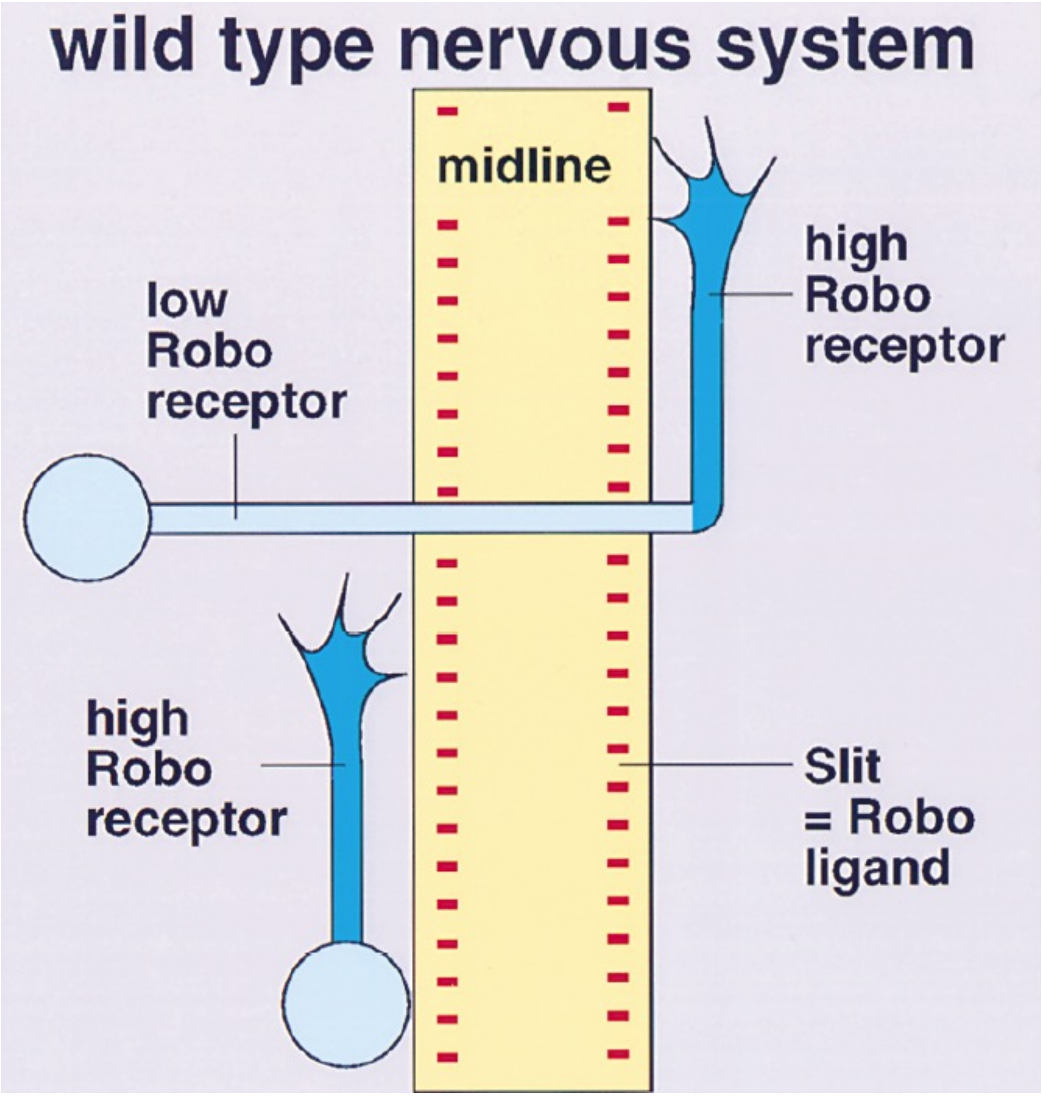


Specific axonal receptors respond to midline signals

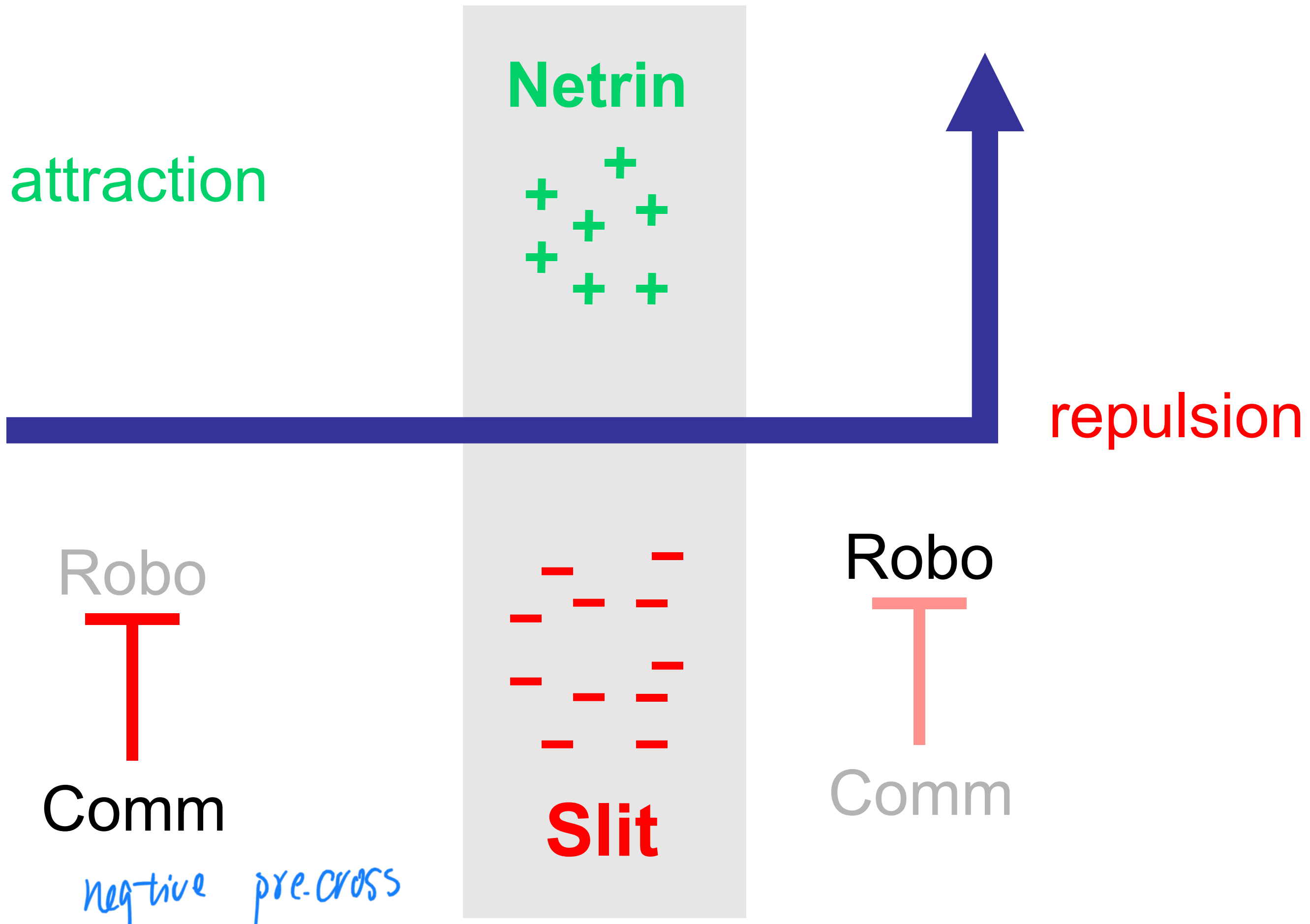


Stein & Tessier-Lavigne (2001) Science 291, 1928 – 1938.

Repulsive response to slit is determined by Robo expression



Choreographing the midline switch in flies



Commissureless (Comm) antagonizes Robo expression

Mid line.

Robo muted

Comm muted

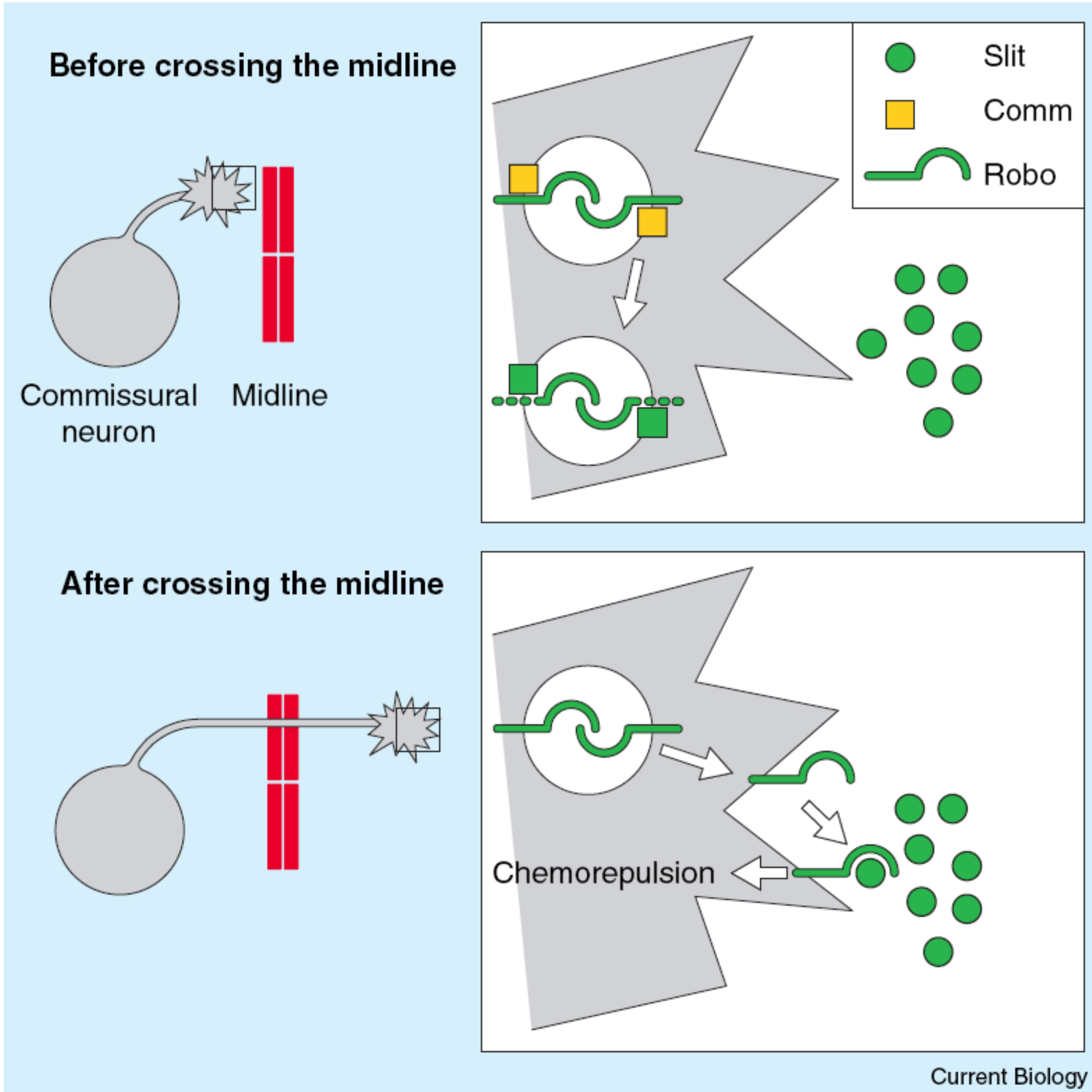
No crossing. (Robo works hard!).

	Comm	$\dashv$ Robo	(repulsion)
robo(-)	on	off	no repulsion
comm(-)	OFF	ON (pre)	no crossing.
both(-)	OFF	OFF	no repulsion

why this model? change to Robo $\dashv$ comm

not same result.

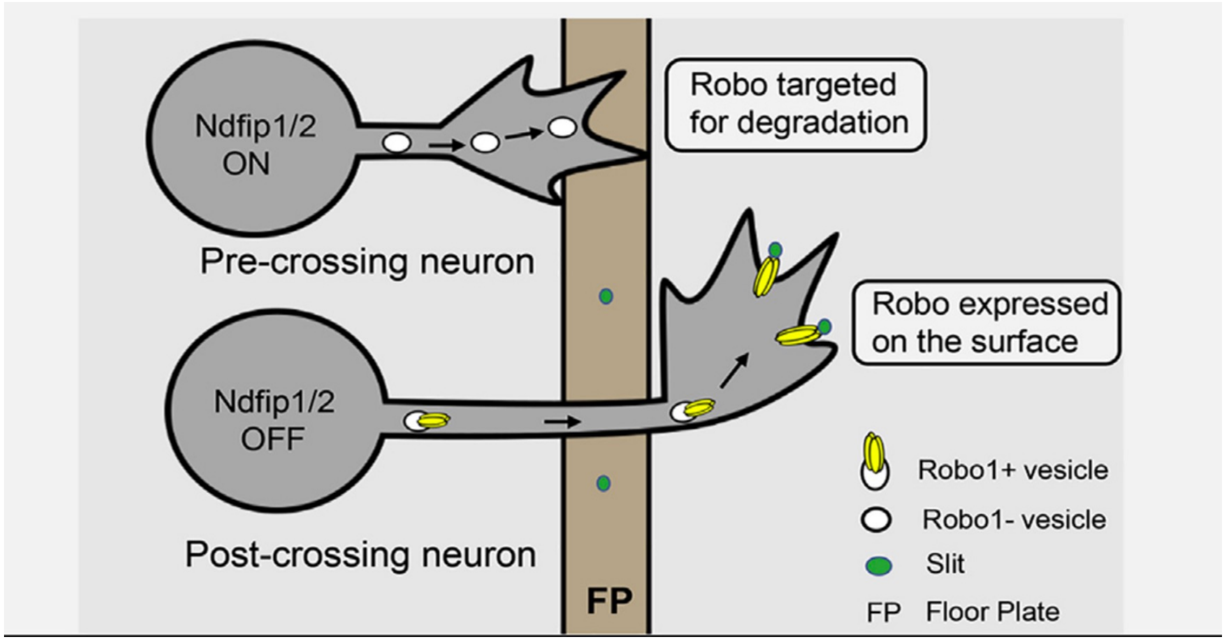
Comm directs Robo to endosomes for degradation



Couch & Condron, 2002

Ndfip Proteins Target Robo Receptors for Degradation and Allow Commissural Axons to Cross the Midline in the Developing Spinal Cord

Madhavi Gorla,[1] Celine Santiago,[1] Karina Chaudhari,[1] Awo Akosua Kesewa Layman,[2,3] Paula M. Oliver,[2,3] and Greg J. Bashaw[1,4,*]
[1]Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

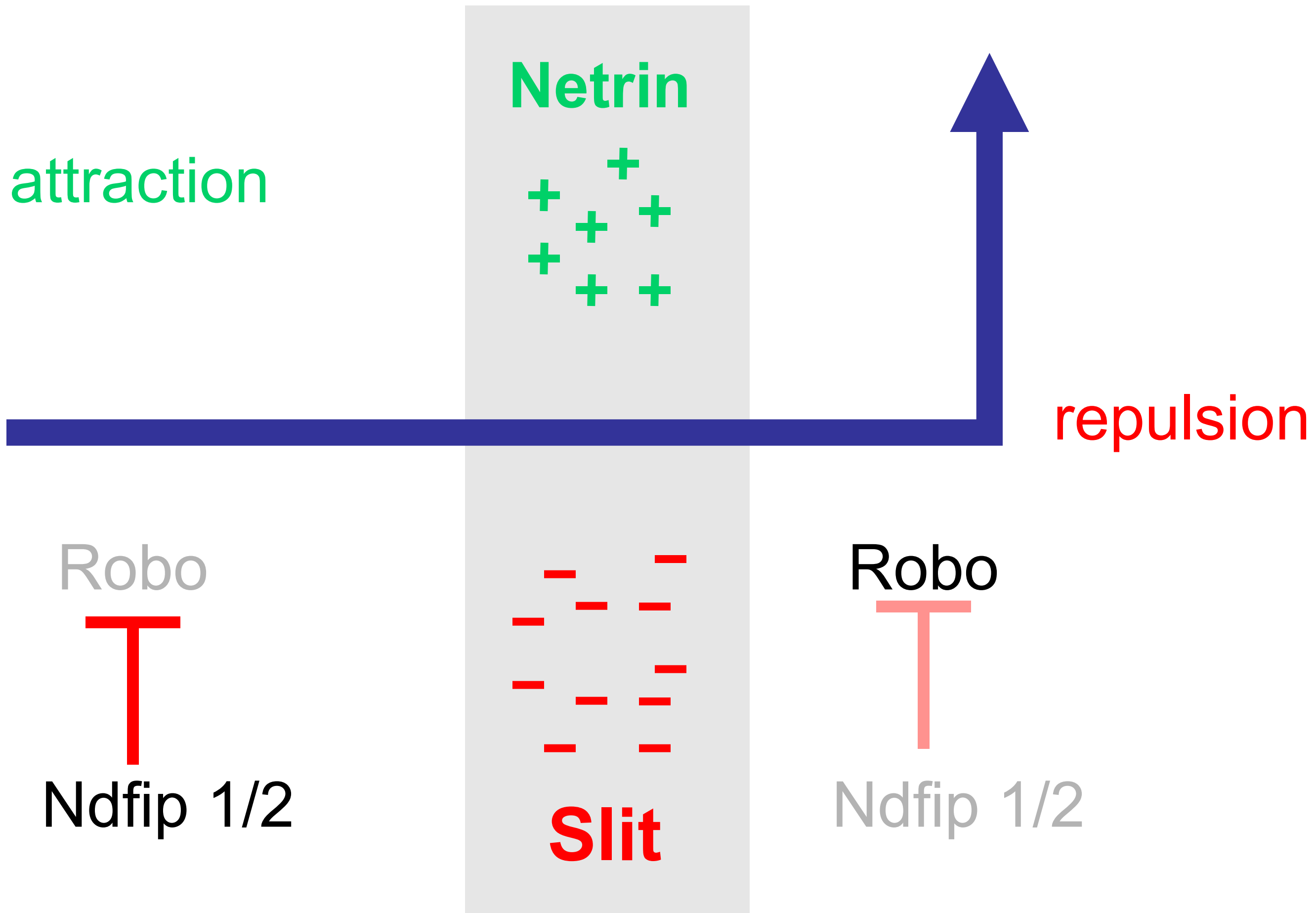


Mammals

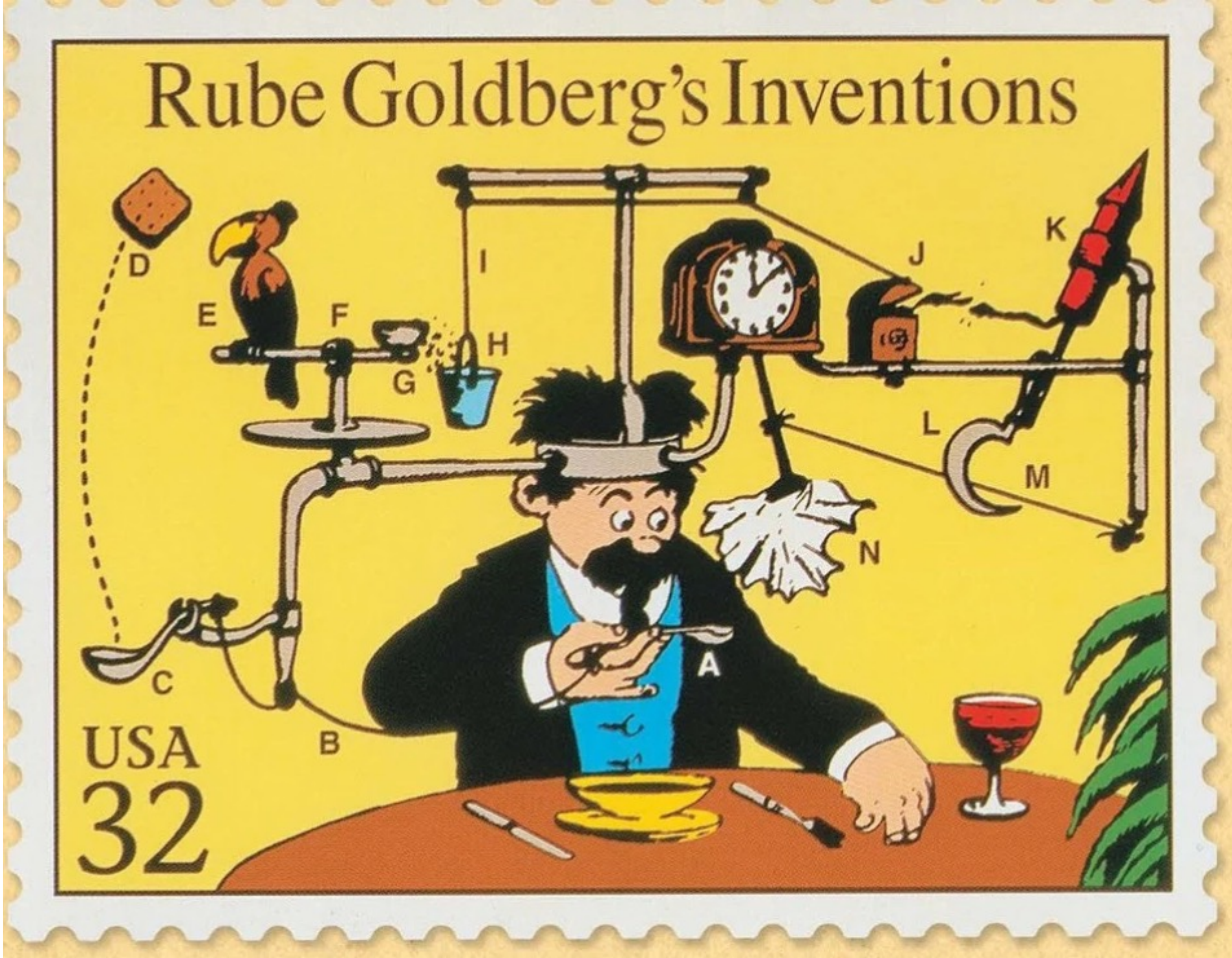
- Ndfip 1/2 (Nedd-4 interacting proteins) comm in mammals.
- Recruit Nedd4 E3 ubiquitin ligases → degradation
- Functional homologs of Comm in mammals

Gorla et al., 2019, Cell Reports *26*, 3298–3312
March 19, 2019 © 2019 The Authors.
https://doi.org/10.1016/j.celrep.2019.02.080

Choreographing the midline switch in mice



Ndfip 1/2 antagonizes Robo expression


Rube Goldberg's Inventions
D
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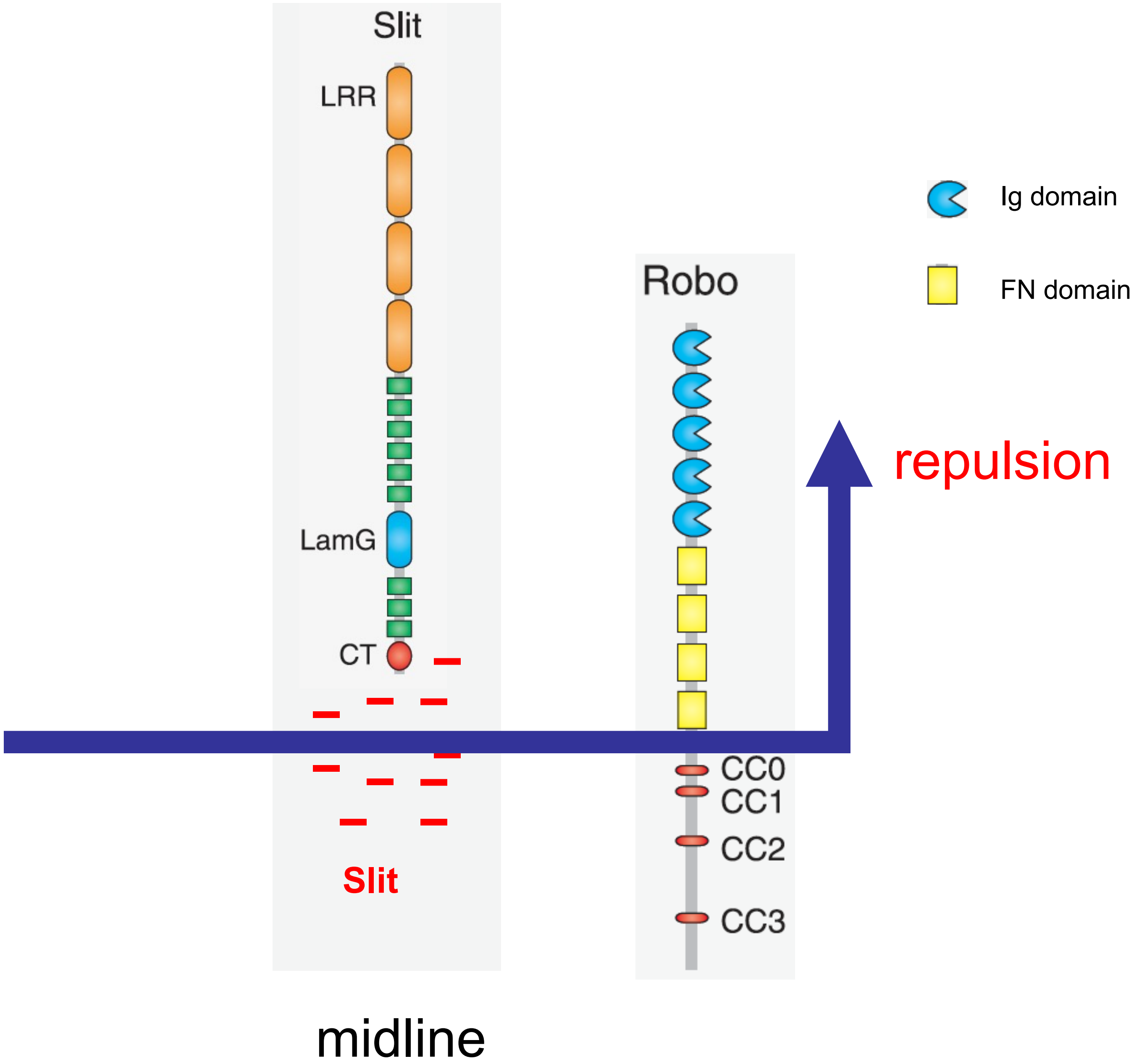
USA
32

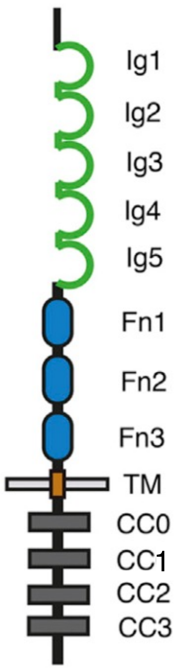

JIWI'S
MACHINES

Robo receptor responds to repulsive slit cue from midline cells

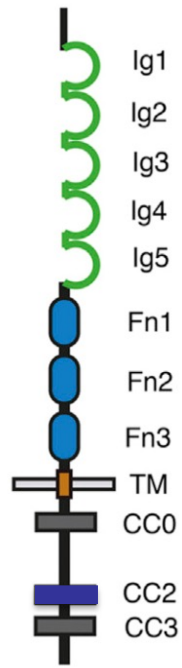


Four Mammalian Robo genes

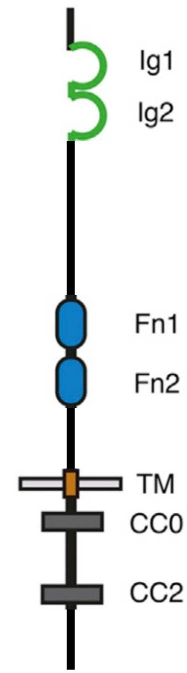
Robo1/2
Repulsion



Robo3
Attraction & Repulsion

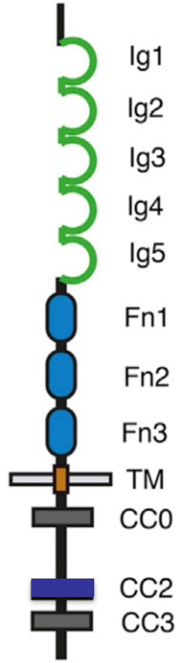


Robo 4
Angiogenesis



Robo3 mediates repulsion and attraction?

Robo3
Attraction & Repulsion

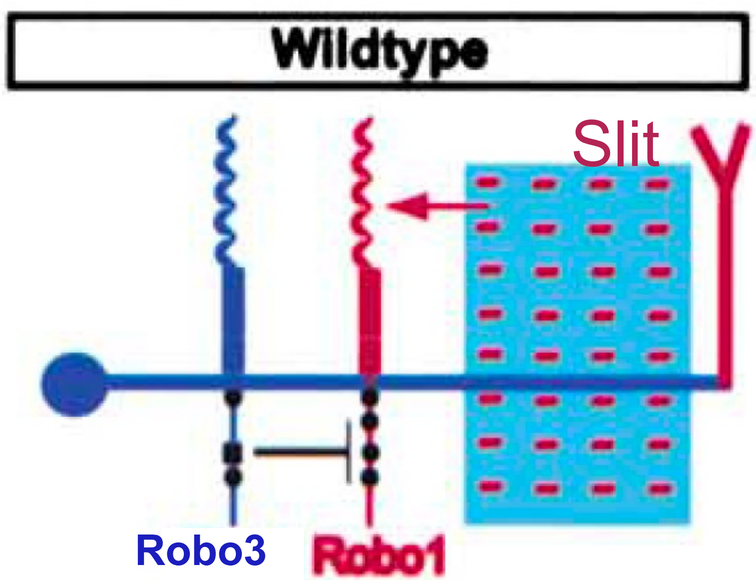


Cell, Vol. 117, 157–169, April 16, 2004, Copyright ©2004 by Cell Press

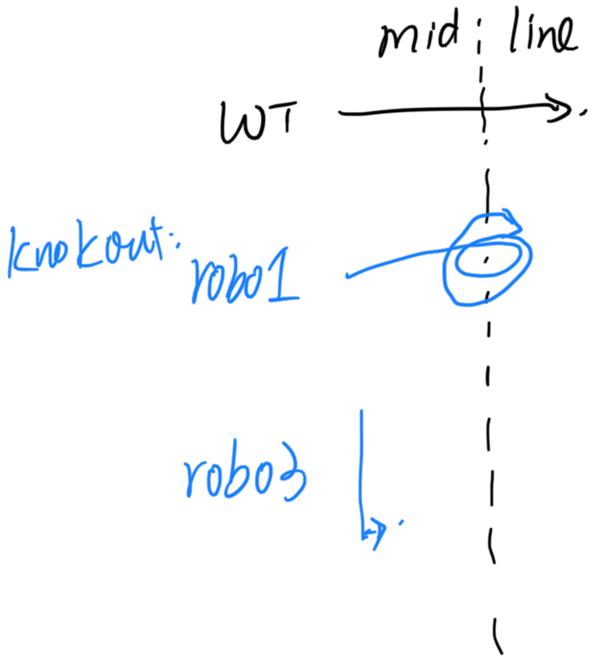
The Divergent Robo Family Protein Rig-1/Robo3 Is a Negative Regulator of Slit Responsiveness Required for Midline Crossing by Commissural Axons

Christelle Sabatier,[1,2] Andrew S. Plump,[2,6] Le Ma,[1,2]
Katja Brose,[2,7] Atsushi Tamada,[3]
Fujio Murakami,[3] Eva Y.-H. P. Lee,[4]
and Marc Tessier-Lavigne[1,2,5,*]

How it regulate attraction.



Robo3 = Attraction (by blocking repulsive response to slit)



Robo3 ⊣ Robo1. → repulsion.

robo1(-)	+	-	stall
robo3(-)	-	+	no crossing
robo3(-)	-	-	stall

robo 1 is epistatic to robo 3

"

robo 1 is required to robo 3 muted phenotype

Robo3 is required for midline crossing

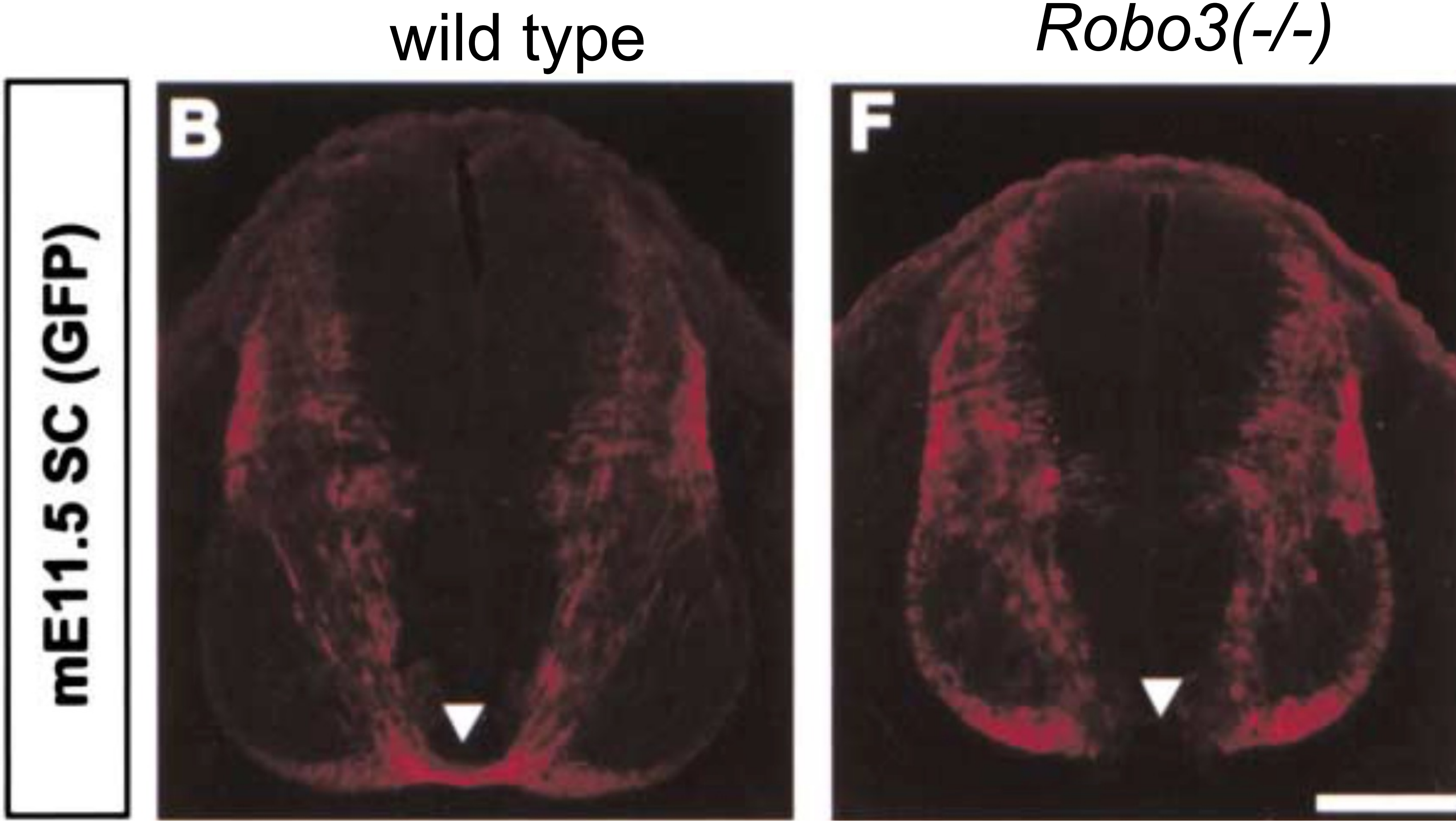


Figure 3

Sabatier et al., 2004

Mutations in a Human *ROBO* Gene Disrupt Hindbrain Axon Pathway Crossing and Morphogenesis

Joanna C. Jen,[1*] Wai-Man Chan,[7] Thomas M. Bosley,[10] Jijun Wan,[1] Janai R. Carr,[1] Udo Rüb,[11] David Shattuck,[1] Georges Salamon,[2] Lili C. Kudo,[3] Jing Ou,[1] Doris D. M. Lin,[12] Mustafa A. M. Salih,[13] Tülay Kansu,[14] Hesham al Dhalaan,[15] Zayed al Zayed,[16] David B. MacDonald,[15] Bent Stigsby,[15] Andreas Plaitakis,[17] Emmanuel K. Dretakis,[18] Irene Gottlob,[19] Christina Pieh,[20] Elias I. Traboulsi,[21] Qing Wang,[21] Lejin Wang,[21†] Caroline Andrews,[7,9] Koki Yamada,[7,9] Joseph L. Demer,[1,4] Shaheen Karim,[4] Jeffry R. Alger,[2] Daniel H. Geschwind,[1] Thomas Deller,[11] Nancy L. Sicotte,[1] Stanley F. Nelson,[5] Robert W. Baloh,[1,6] Elizabeth C. Engle[7,8,9*]

The mechanisms controlling axon guidance are of fundamental importance in understanding brain development. Growing corticospinal and somatosensory axons cross the midline in the medulla to reach their targets and thus form the basis of contralateral motor control and sensory input. The motor and sensory projections appeared uncrossed in patients with horizontal gaze palsy with progressive scoliosis (HGPPS). In patients affected with HGPPS, we identified mutations in the *ROBO3* gene, which shares homology with *roundabout* genes important in axon guidance in developing *Drosophila*, zebrafish, and mouse. Like its murine homolog Rig1/Robo3, but unlike other Robo proteins, ROBO3 is required for hindbrain axon midline crossing.

Science 304, 1509-1513 (2004)

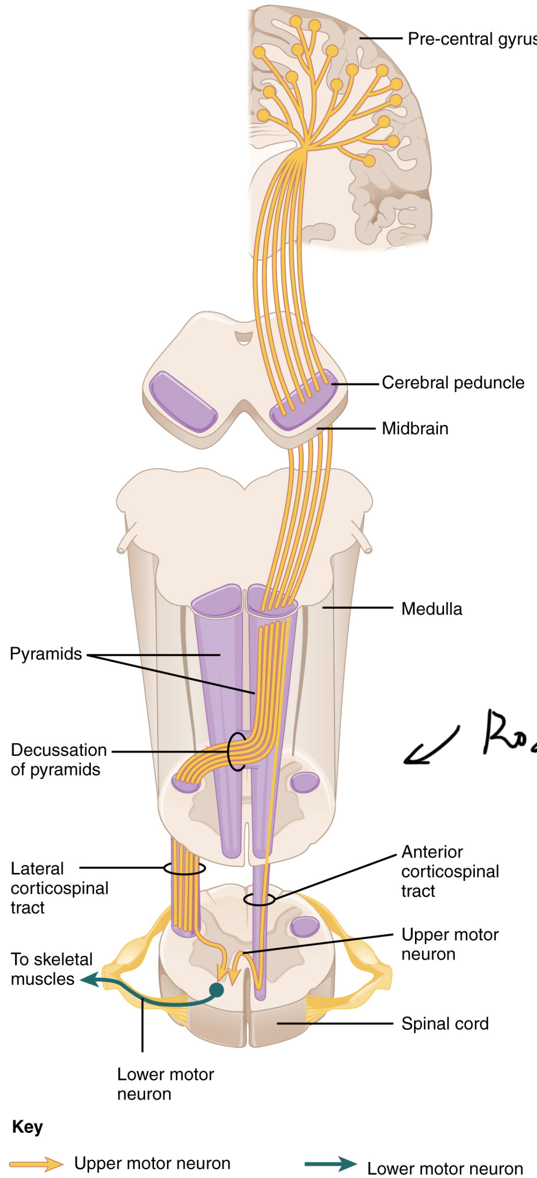
HGPPS (Horizontal Gaze Palsy with Progressive Scoliosis)

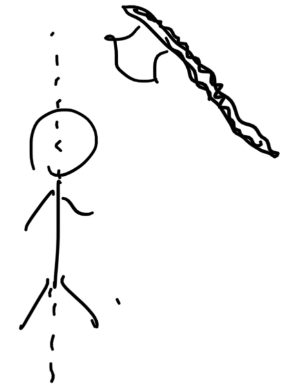
Absent horizontal eye movement

Scoliosis

Robo3 mutation disrupts midline crossing (decussation) of corticospinal axons

Corticospinal neurons from motor cortex normally decussate in medulla to innervate contralateral motor neurons in spinal cord.





Robo 3

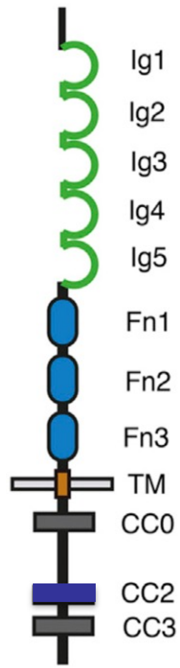
https://en.wikipedia.org/wiki/Corticospinal_tract

Robo3 mediates repulsion and attraction?

Robo3

Repulsion & Attraction

?

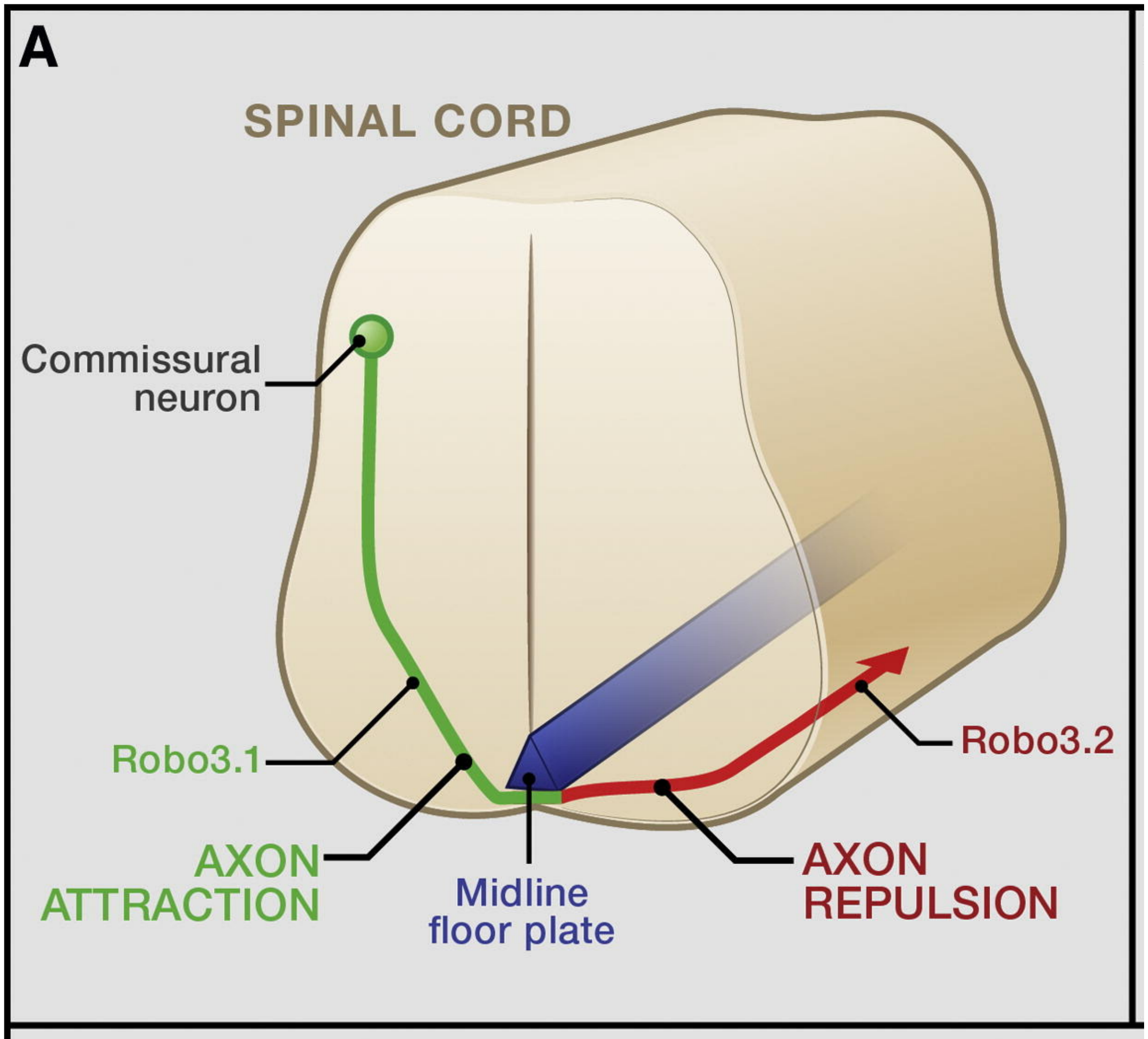


Neuron 58, 325–332, May 8, 2008

Alternative Splicing of the Robo3 Axon Guidance Receptor Governs the Midline Switch from Attraction to Repulsion

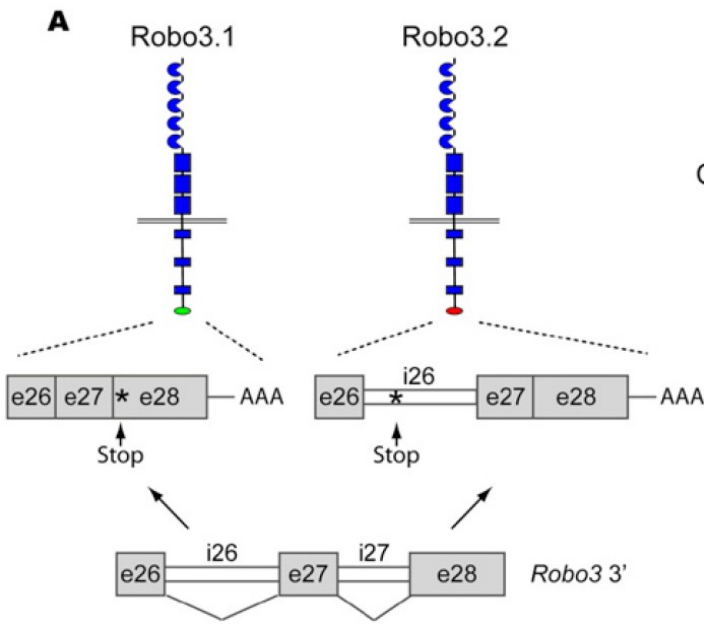
Zhe Chen,[1,3] Bryan B. Gore,[1,2] Hua Long,[1,2,3] Le Ma,[2,4] and Marc Tessier-Lavigne[1,*]

Robo3.1



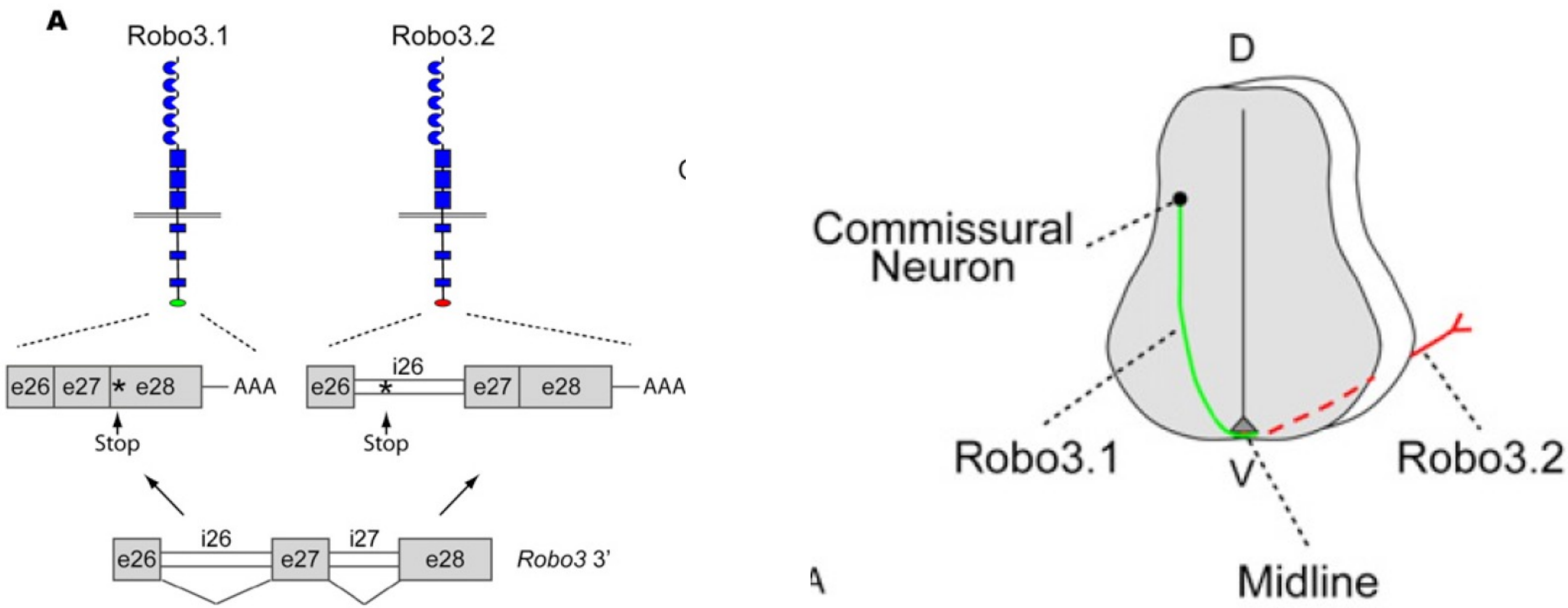
Robo3.2

Figure 1. Alternative Splicing of *Robo3* Generates Two Isoforms with Distinct Expression Patterns in Commissural Axons



Chen et al., Neuron, 2008

Figure 1. Alternative Splicing of *Robo3* Generates Two Isoforms with Distinct Expression Patterns in Commissural Axons



Models:

Robo3.1	⊣	Robo1/2 mediated repulsive response to slit
Robo3.2	→	Repulsive response to slit

Chen et al., Neuron, 2008

Alternative Splicing of the Robo3 Axon Guidance Receptor Governs the Midline Switch from Attraction to Repulsion

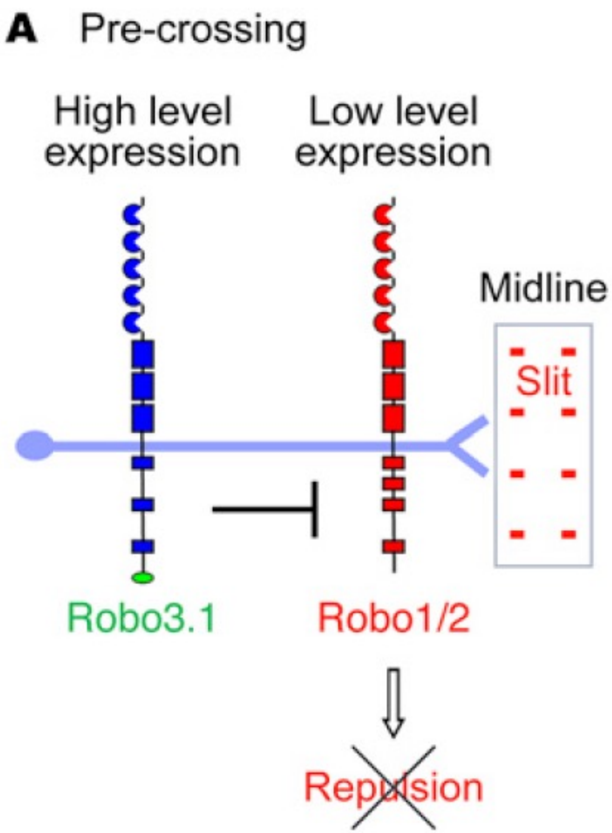


Figure 5. Model of Robo3 Activities in Commissural Axon Guidance at the Midline

Chen et al., Neuron, 2008

Alternative Splicing of the Robo3 Axon Guidance Receptor Governs the Midline Switch from Attraction to Repulsion

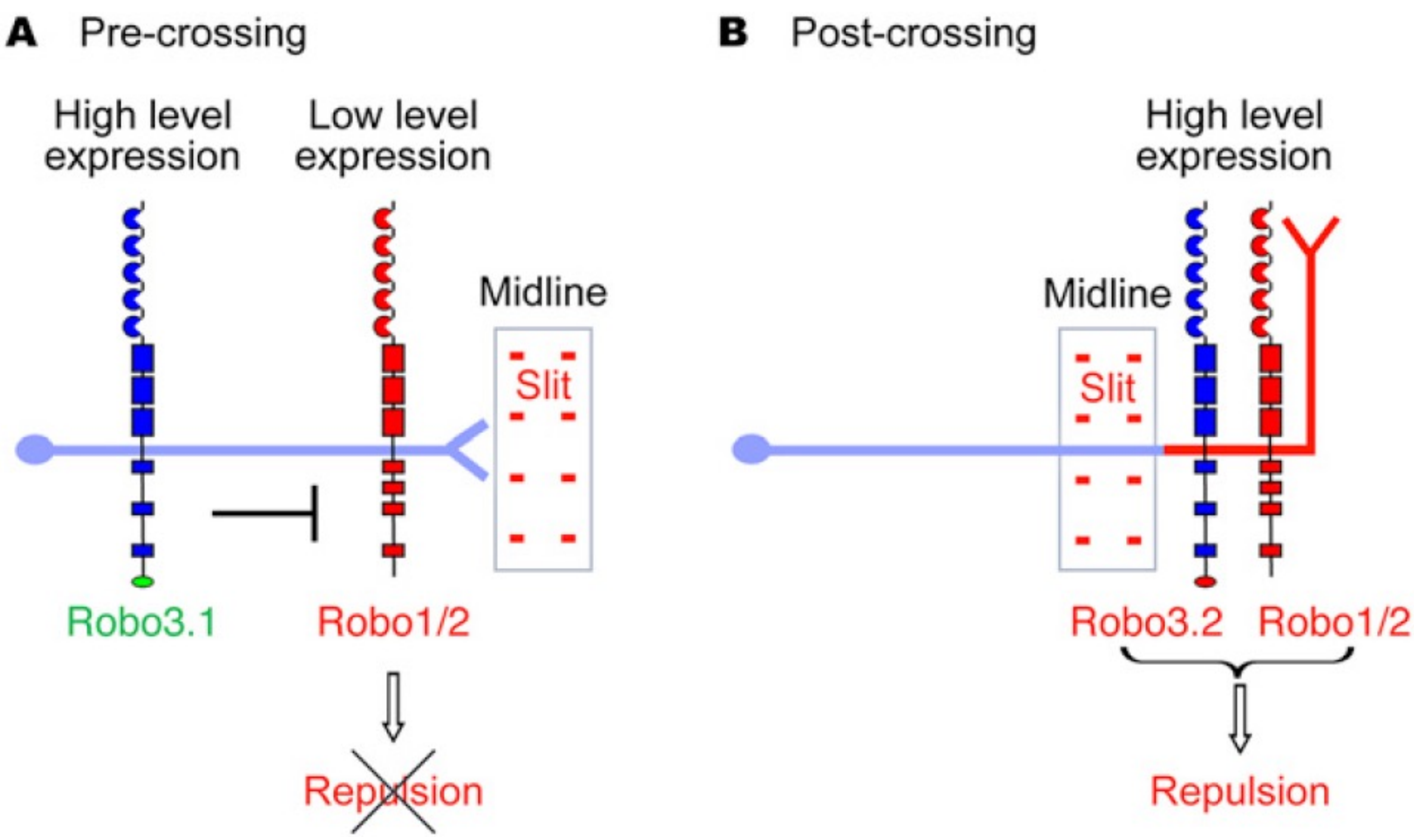
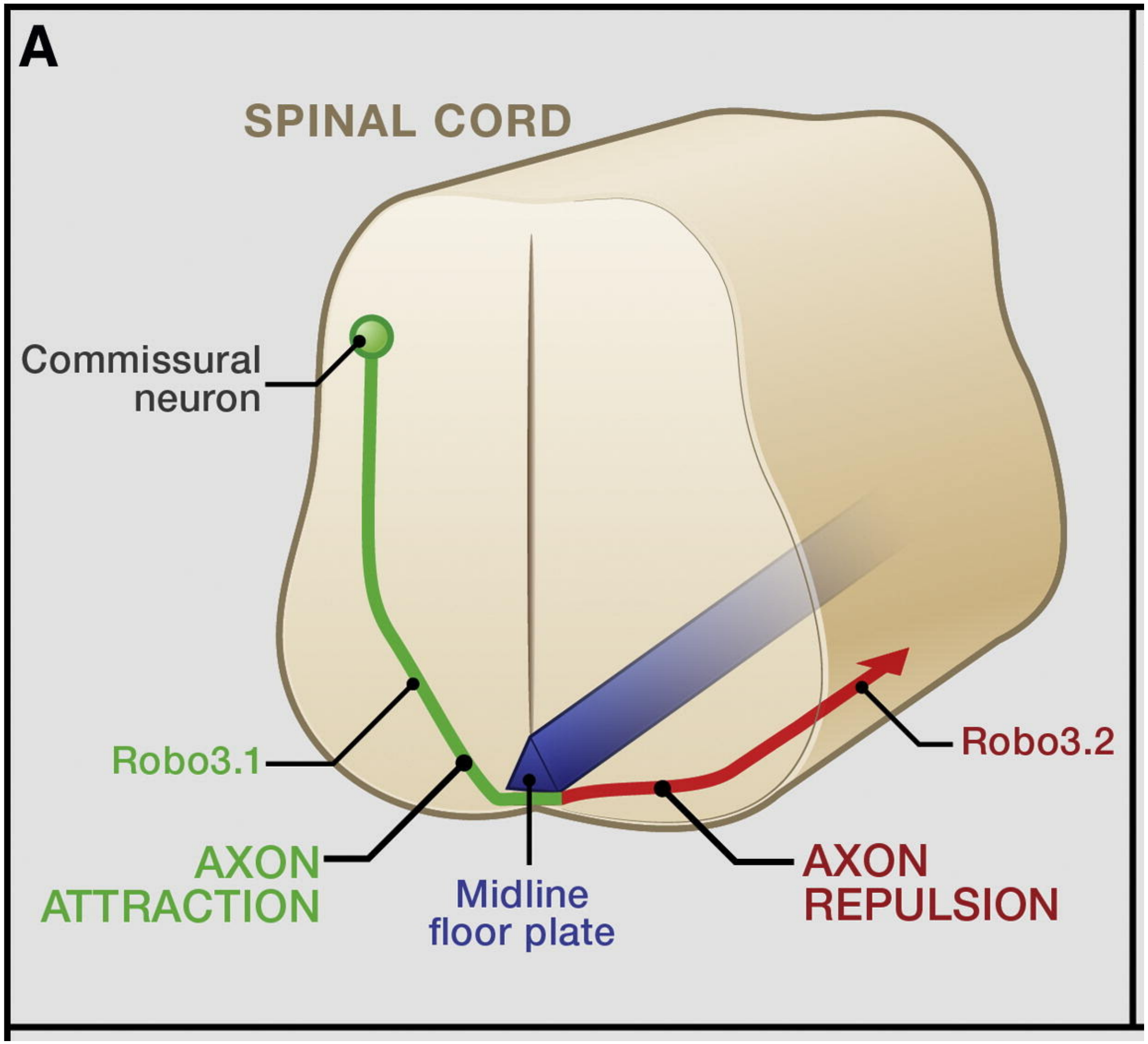


Figure 5. Model of Robo3 Activities in Commissural Axon Guidance at the Midline

Chen et al., Neuron, 2008

Regulation of Robo3.1 vs Rob3.2 expression?

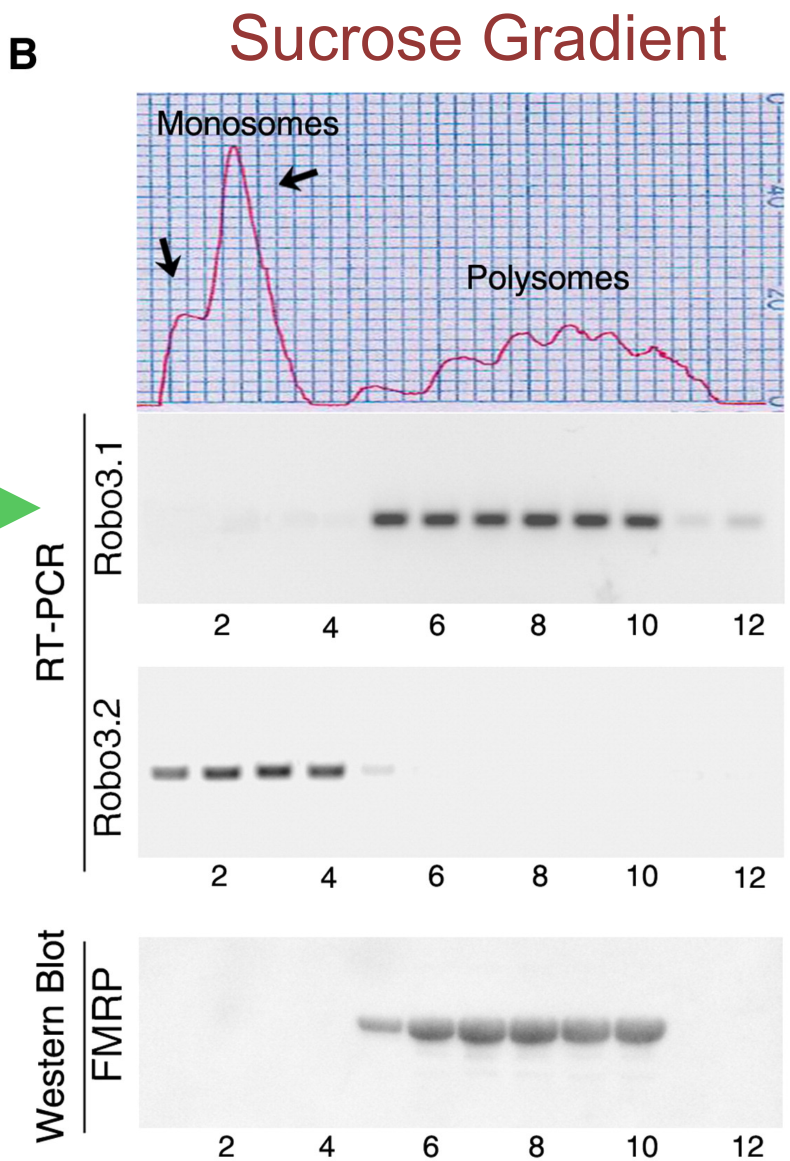
Robo3.1

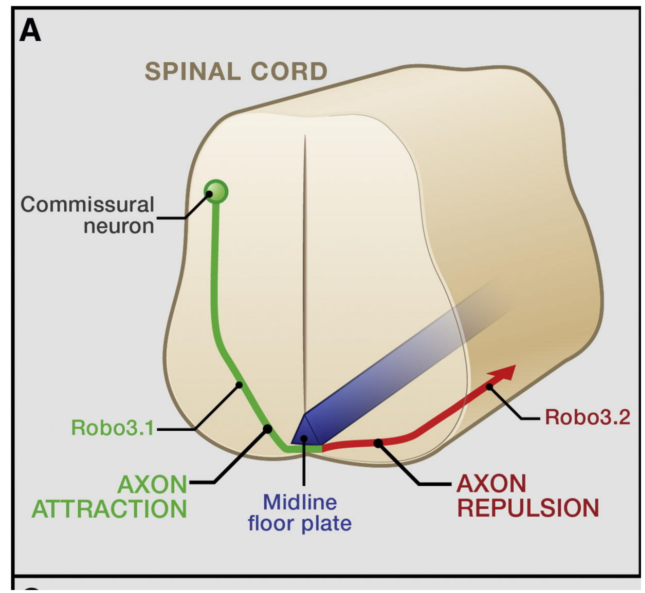


Robo3.2

Colak et al., 2013

Robo3.1 mRNA associated with polyribosomes in pre-crossing axons

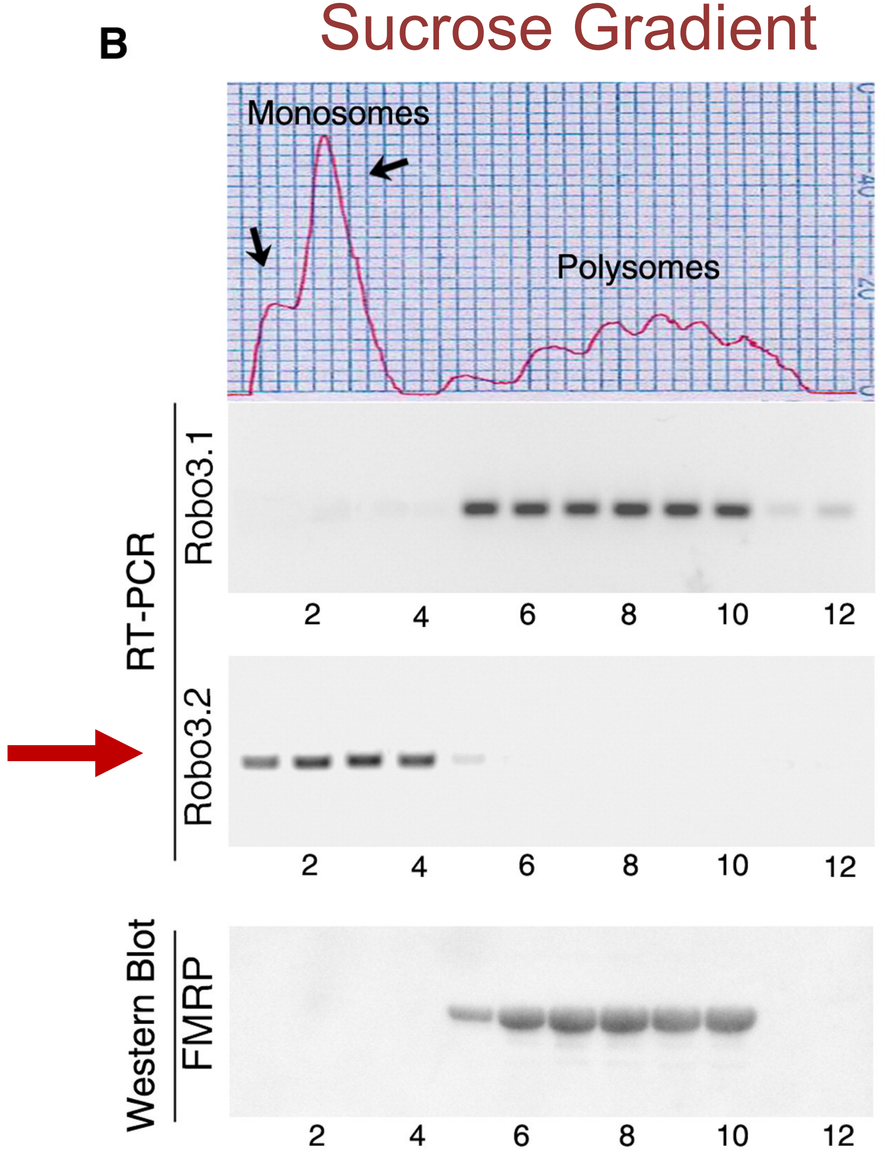


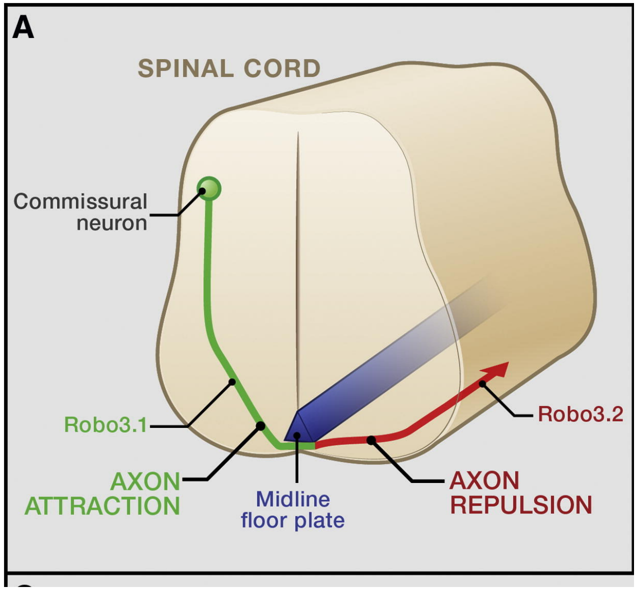


Positive Control

Colak et al., 2013

Robo3.2 is not translated in pre-crossing axons

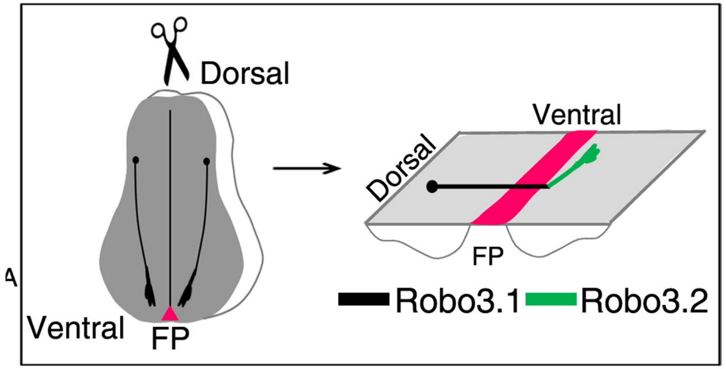




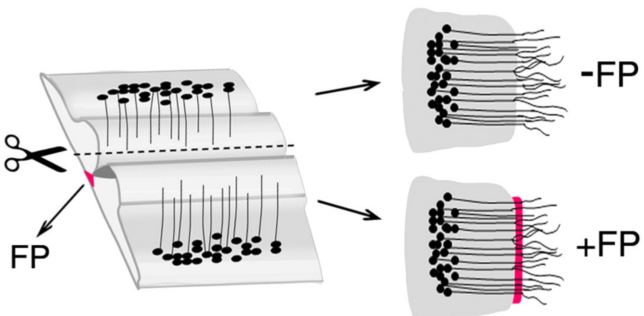
Positive Control

Colak et al., 2013

Hypothesis: Floorplate (FP) regulates expression of Robo3 isoforms

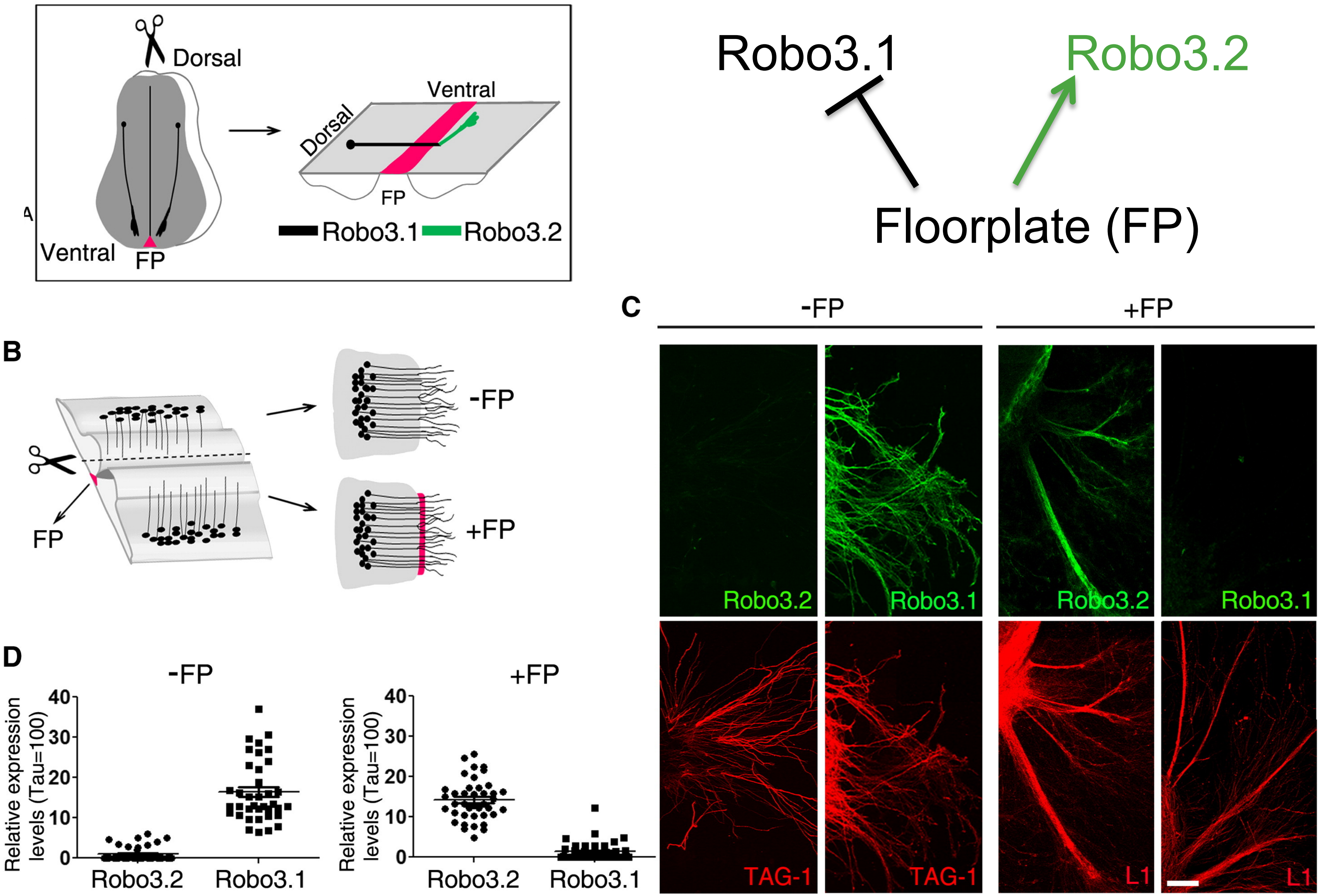


B



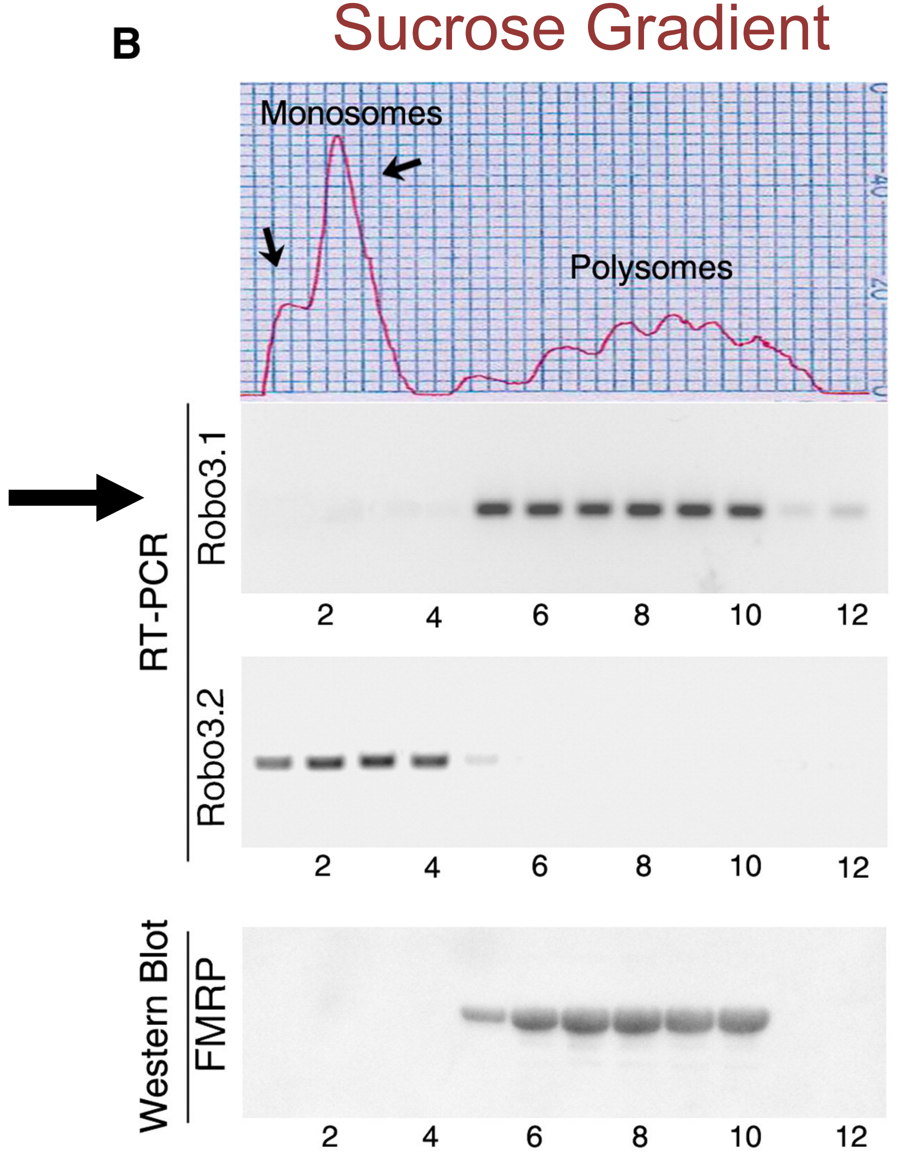
Colak et al., 2013

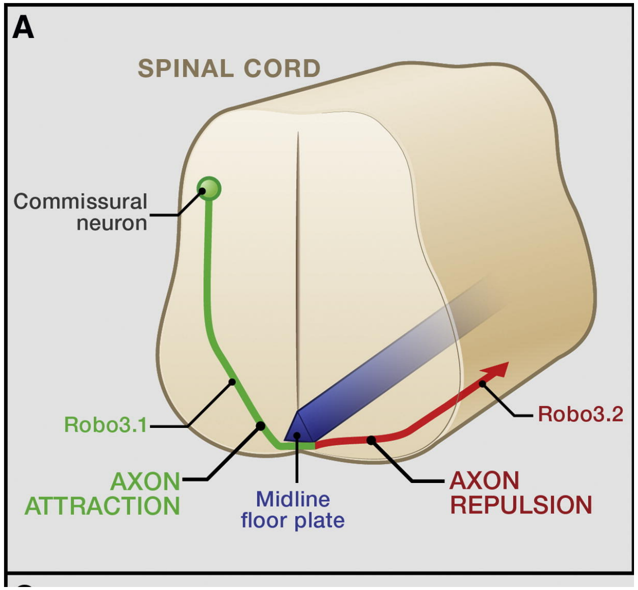
Floorplate (FP) regulates expression of Robo3 isoforms



Colak et al., 2013

Robo3.1 is translated in pre-crossing axons

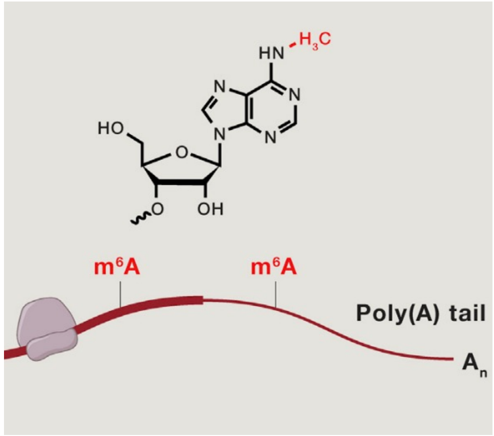




Positive Control

Colak et al., 2013

Robo3.1 translation enhanced in pre-crossing axons

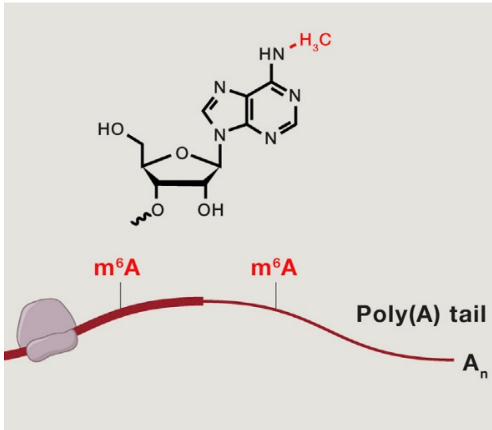


- Robo3.1 mRNA modified with m^6A
- YTHDF1 ”reader” protein binds m^6A
- YTHDF1 enhances Robo3.1 translation

Zhaung et al., NAR 2019

Wang et al., Cell, 2015

Robo3.1 translation enhanced in pre-crossing axons



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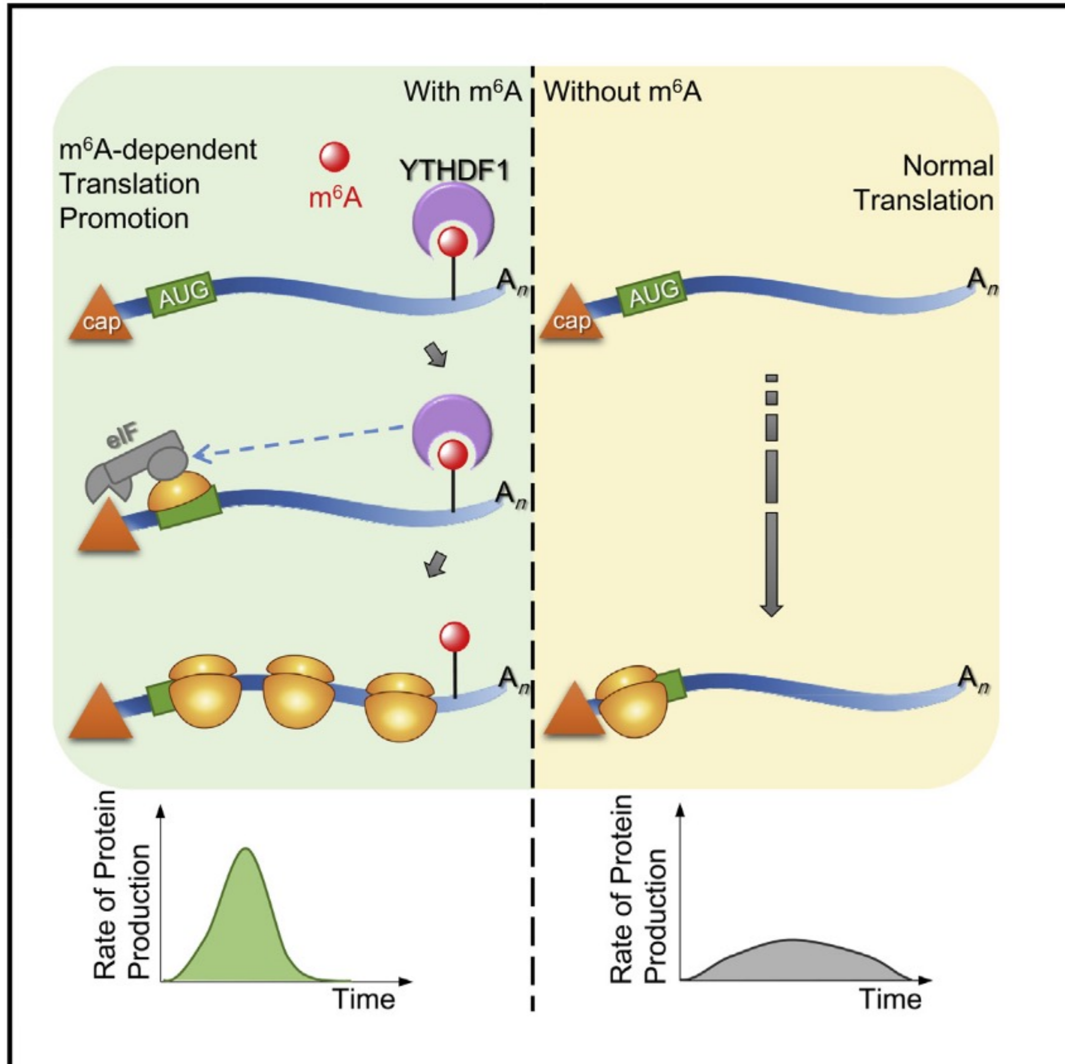
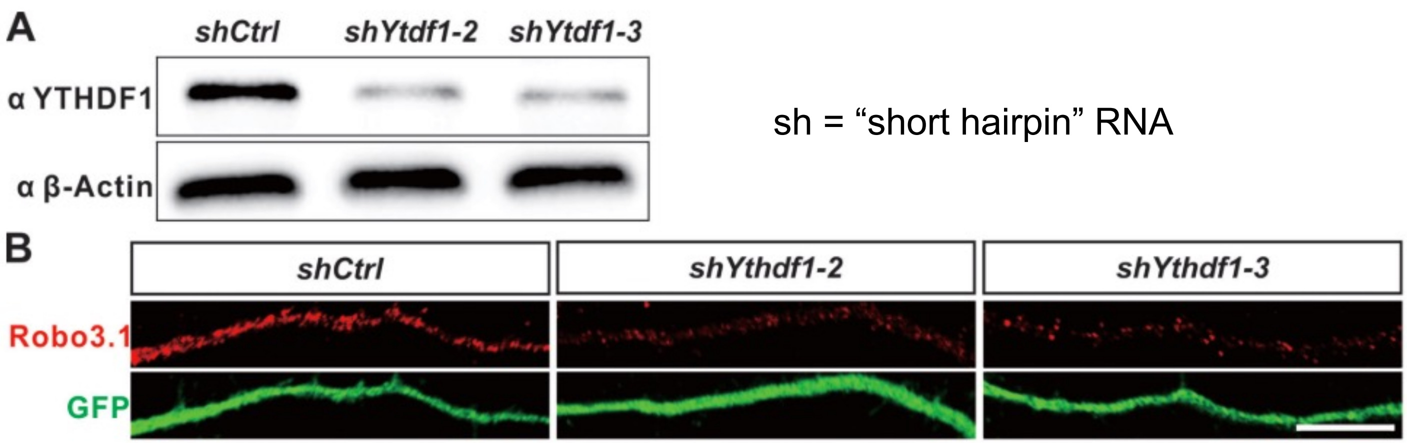


Zhaung et al., NAR 2019

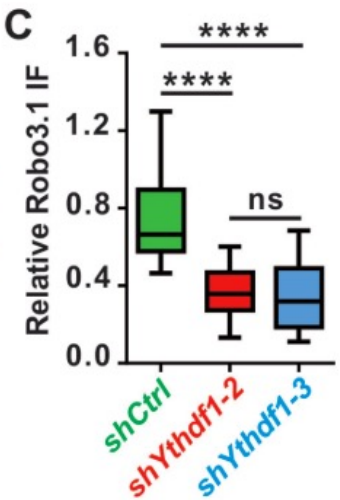
Wang et al., Cell, 2015

YTHDF1 is required for Robo3.1 translation

RNAi knockdown of Ytdf1 depletes Robo3.1

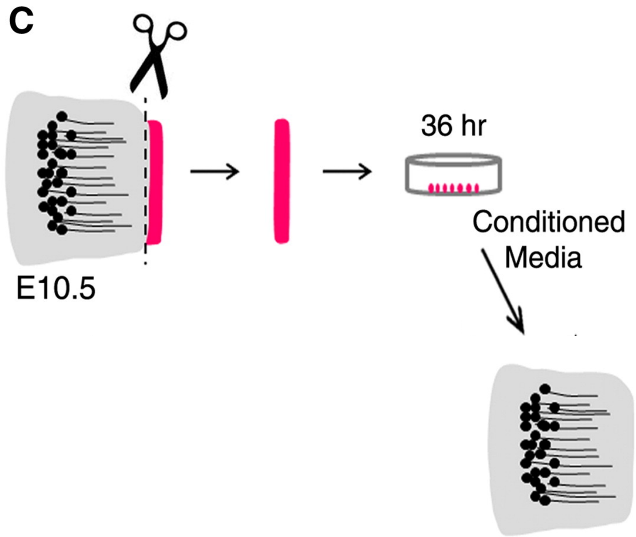


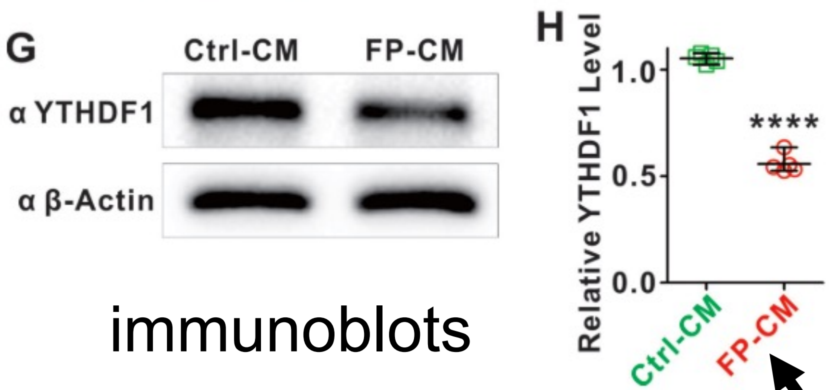
sh = “short hairpin” RNA



Zhaung et al., NAR 2019

Floor Plate signal reduces YTHDF1 expression





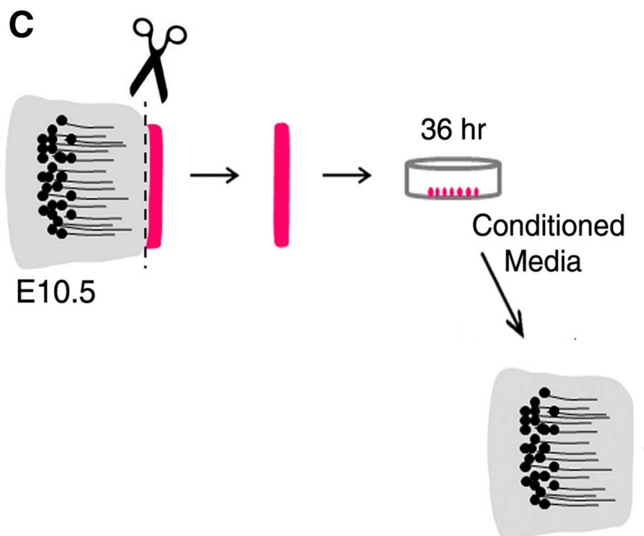
immunoblots

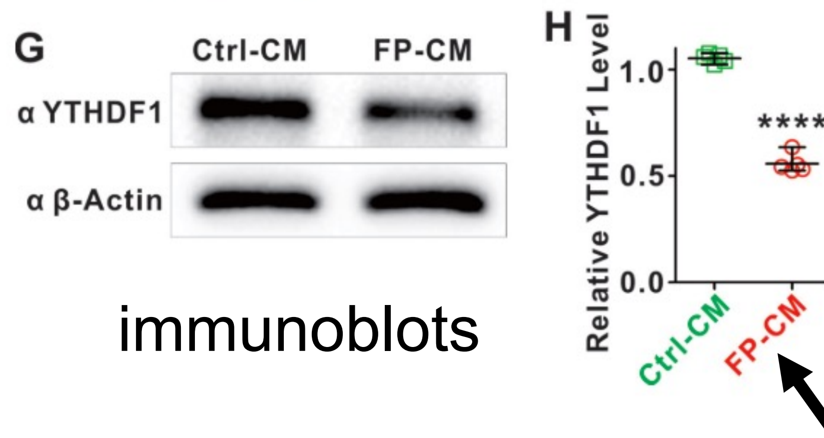
Floor Plate
Conditioned Media

Colak et al., 2013

Zhaung et al., NAR 2019

Floor Plate signal reduces YTHDF1 expression





immunoblots

Floor Plate
Conditioned Media

YTHDF1 → Robo3.1

⊣ ?

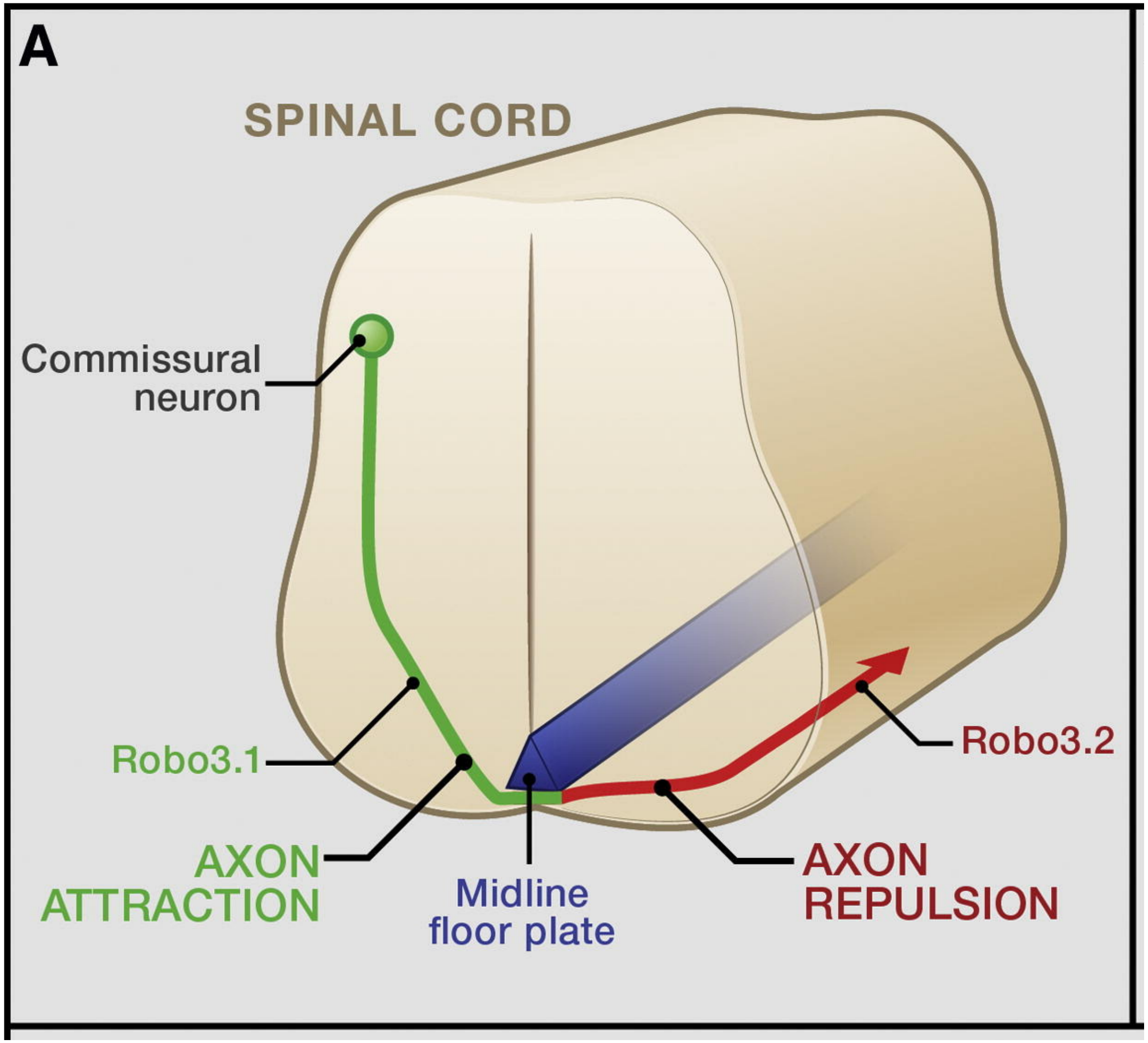
Floor Plate (FP)

Colak et al., 2013

Zhaung et al., NAR 2019

Floor Plate antagonizes Robo3.1 expression

Robo3.1



Robo3.2

Colak et al., 2013

Floorplate (FP) induces intra-axonal translation of Robo3.2

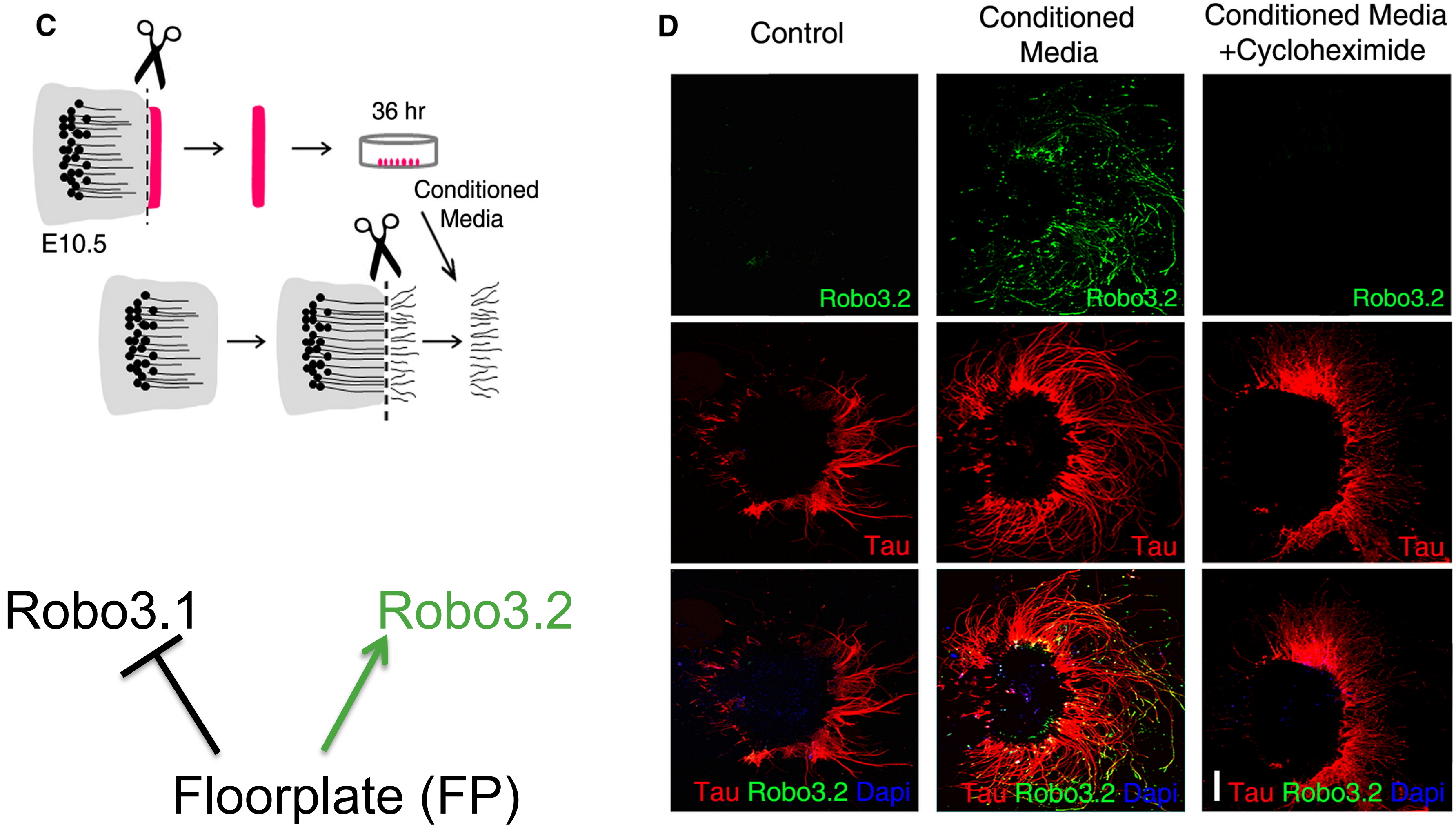
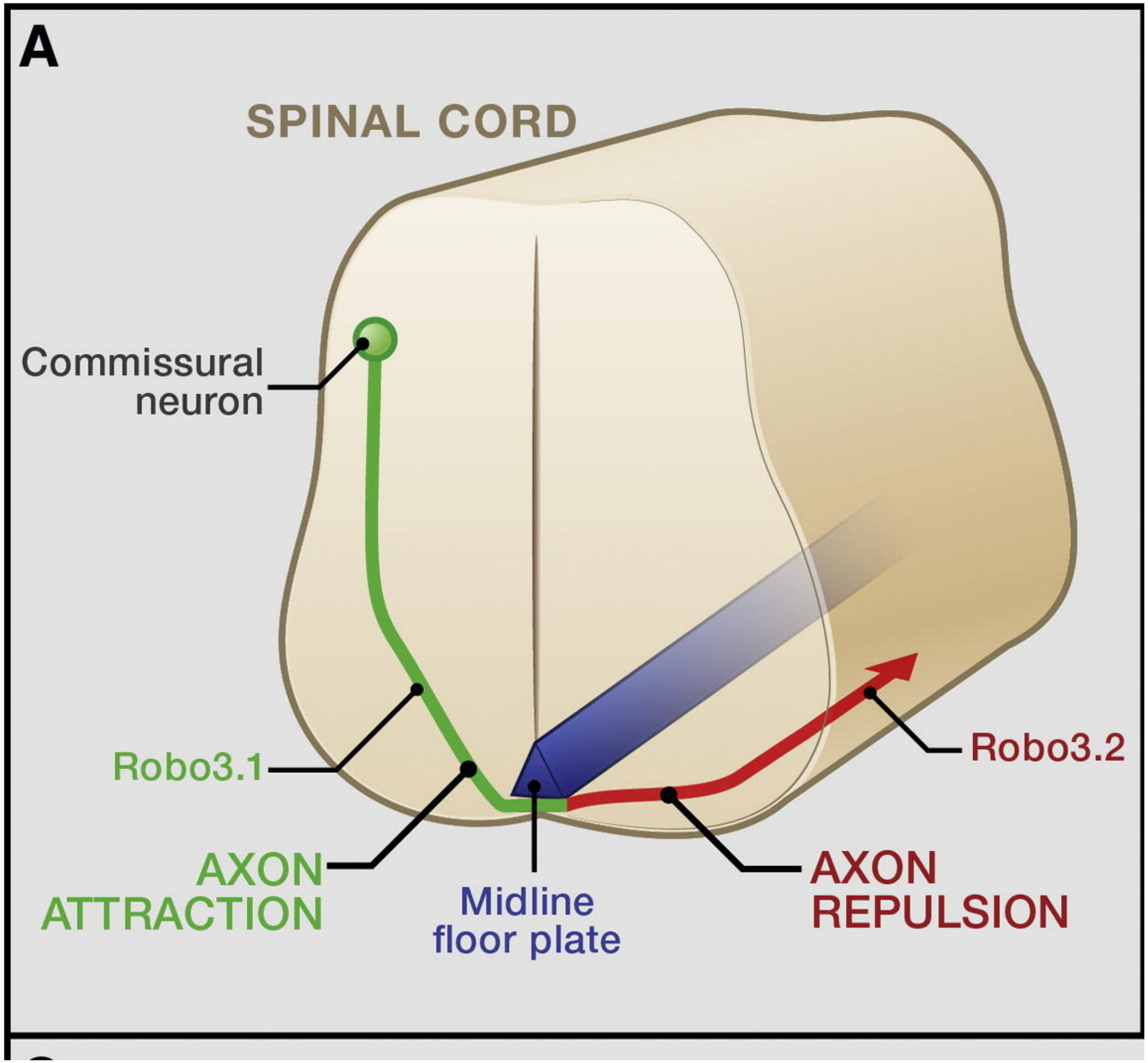


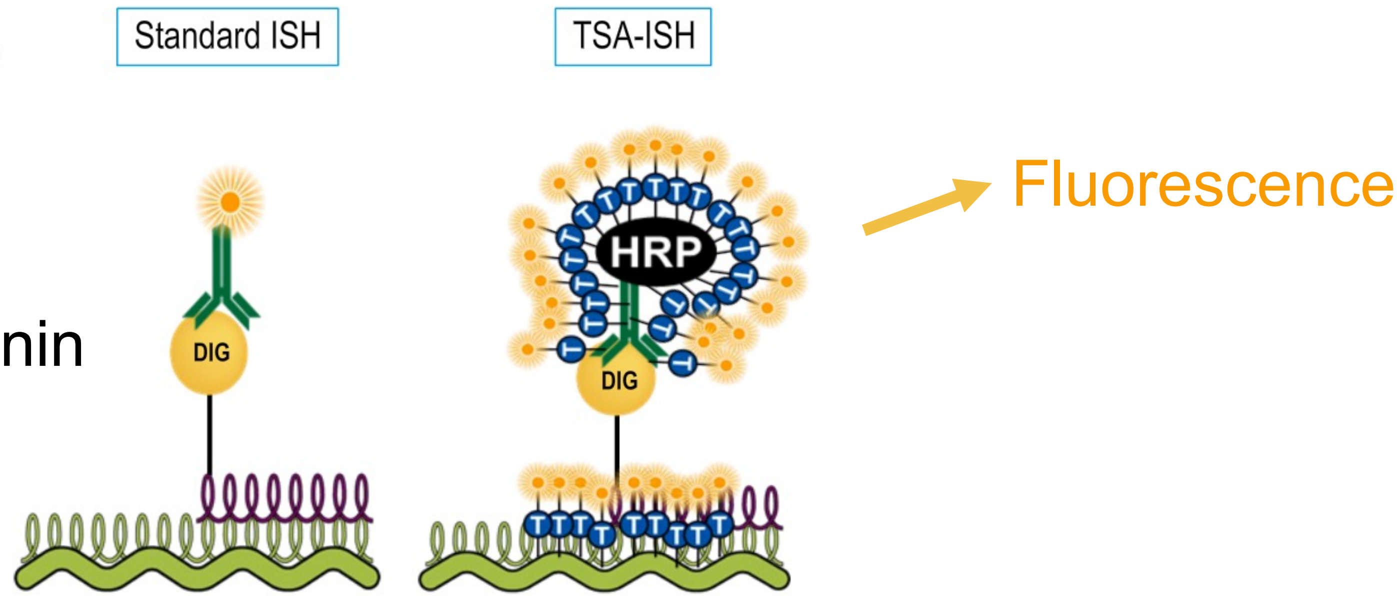
Figure 4

Robo3.2 is locally translated in post-crossing axons

Prediction: Robo3.2 mRNA in commissural axons

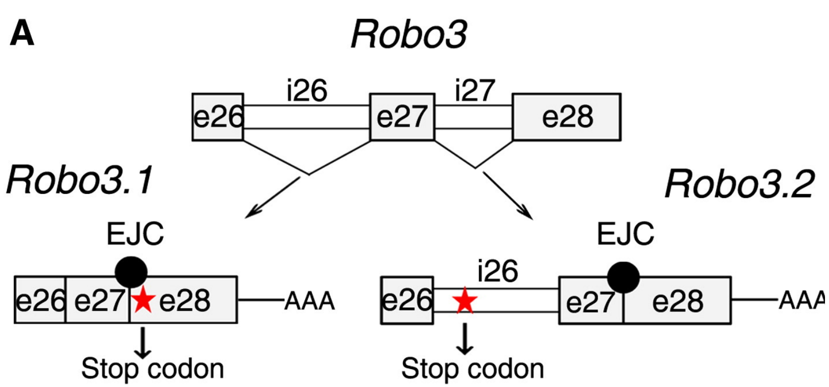


Fluorescence In Situ Hybridization (FISH)

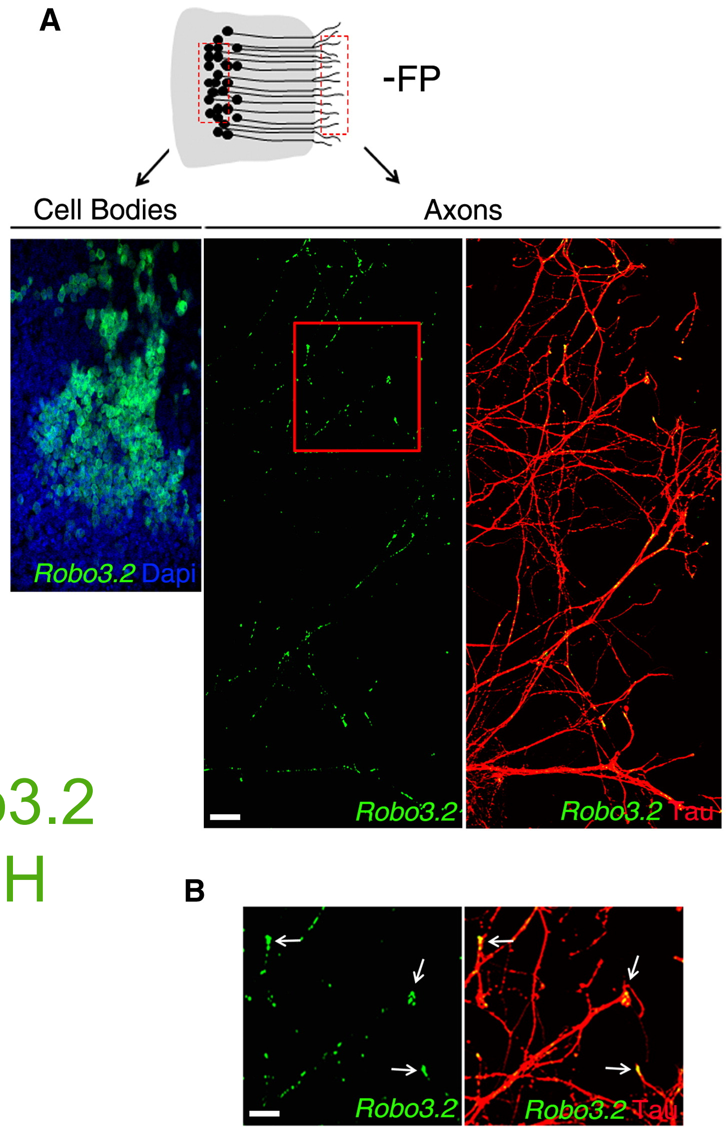


DIGoxygenin

How did they design Robo3.2-specific FISH probes?



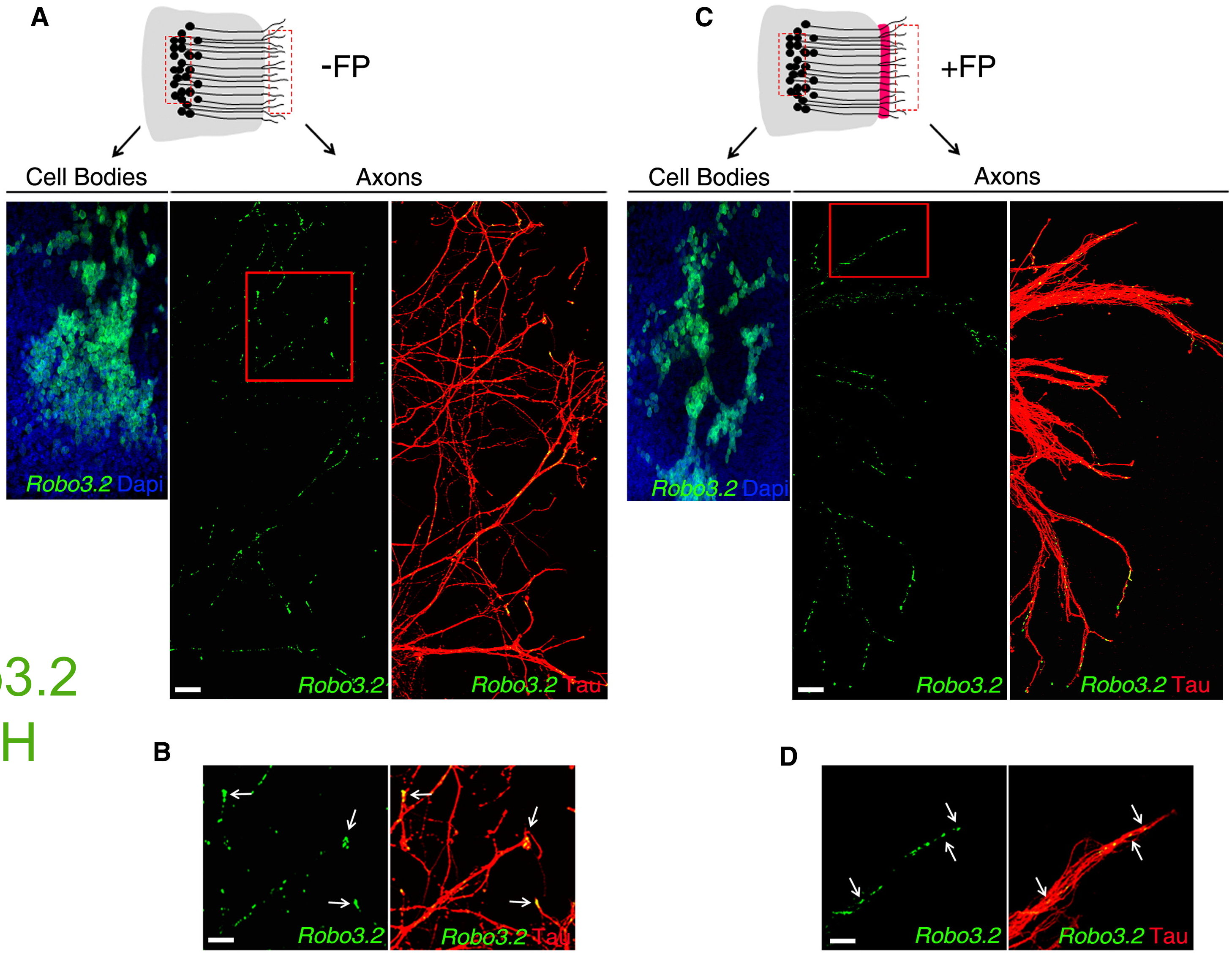
Robo3.2 mRNA is transported into pre-crossing axons



Robo3.2
FISH

Figure 3

Robo3.2 mRNA is transported into pre-crossing & post-crossing axons



Robo3.2 FISH

Figure 3

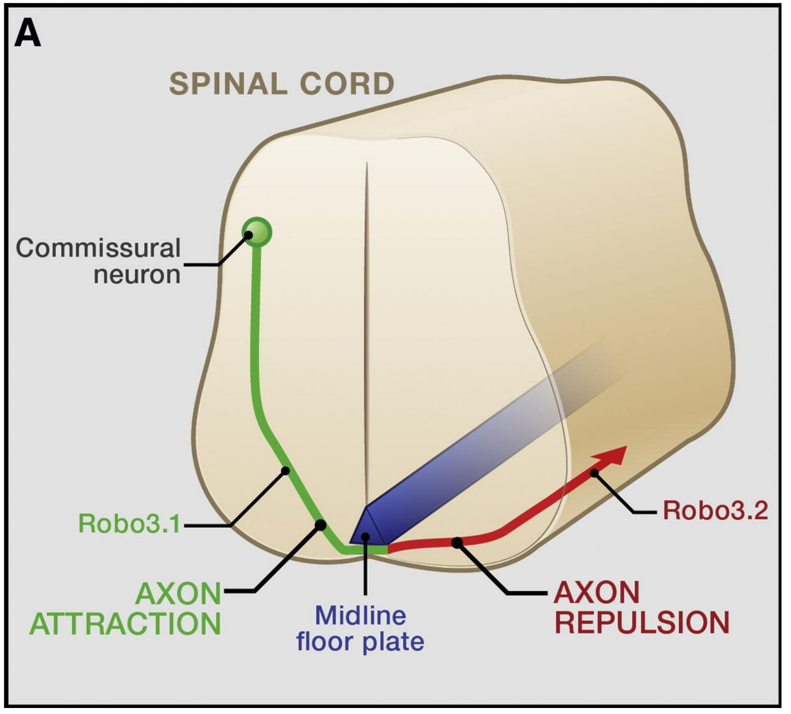
Cell

Regulation of Axon Guidance by Compartmentalized Nonsense-Mediated mRNA Decay

Dilek Colak,[1] Sheng-Jian Ji,[1] Bo T. Porse,[2,3,4] and Samie R. Jaffrey[1,*]
[1]Department of Pharmacology, Weill Medical College, Cornell University, New York, NY 10065, USA
[2]The Finsen Laboratory, Rigshospitalet, Faculty of Health Sciences
[3]Biotech Research and Innovation Center (BRIC)
[4]Danish Stem Cell Centre (DanStem), Faculty of Health Sciences
University of Copenhagen, Copenhagen 2200, Denmark
*Correspondence: srj2003@med.cornell.edu
http://dx.doi.org/10.1016/j.cell.2013.04.056

SUMMARY

Growth cones enable axons to navigate toward their targets by responding to extracellular signaling molecules. Growth-cone responses are mediated in part by the local translation of axonal messenger RNAs (mRNAs). However, the mechanisms that regulate local translation are poorly understood. Here we show that Robo3.2, a receptor for the Slit family of guidance cues, is synthesized locally within axons of commissural neurons. *Robo3.2* translation is induced by floor-plate-derived signals as axons cross the spinal cord midline. *Robo3.2* is also a predicted target of the nonsense-mediated mRNA decay (NMD) pathway. We find that NMD regulates Robo3.2 synthesis by inducing the degradation of *Robo3.2* transcripts in axons that encounter the floor plate. Commissural neurons deficient in NMD proteins exhibit aberrant axonal trajectories after crossing the midline, consistent with misregulation of Robo3.2 expression. These data show that local translation is regulated by mRNA stability and that NMD acts locally to influence axonal pathfinding.



Cell

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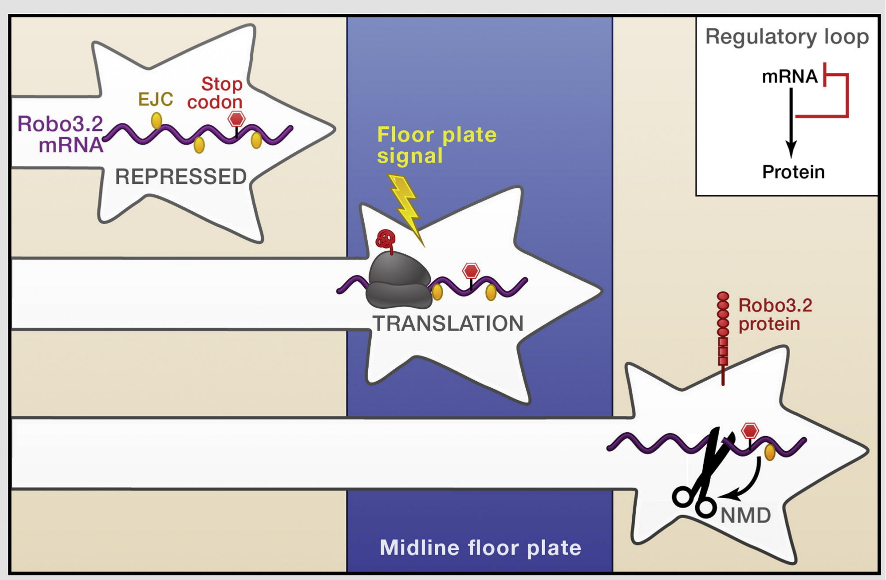

Conclusions

1. Robo3.2 mRNA is translocated to axons
2. Robo3.2 translation is repressed in pre-crossing axons
3. Floorplate signal induces Robo3.2 translation
4. NMD removes Robo3.2 mRNA from post-crossing axons

Cell

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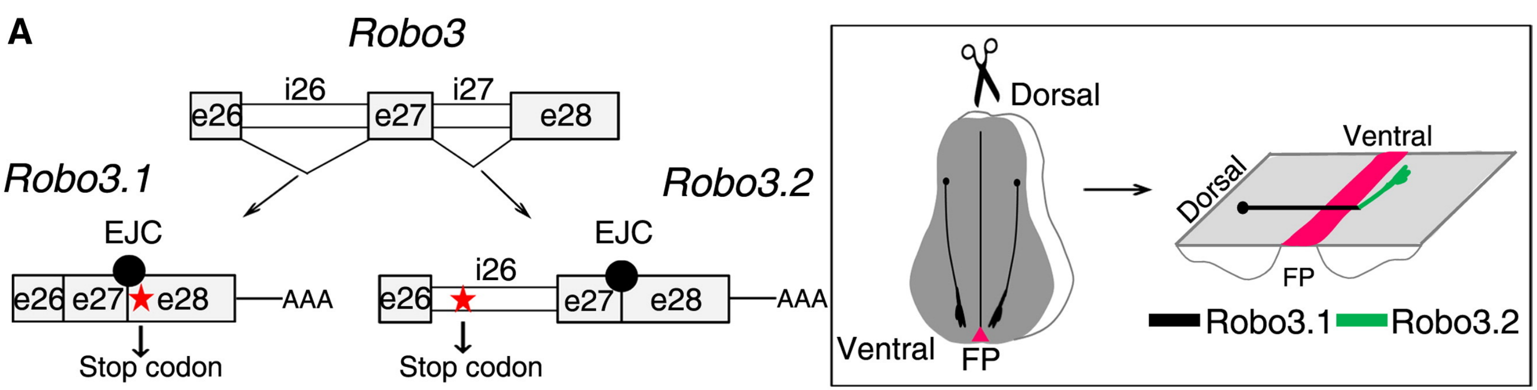
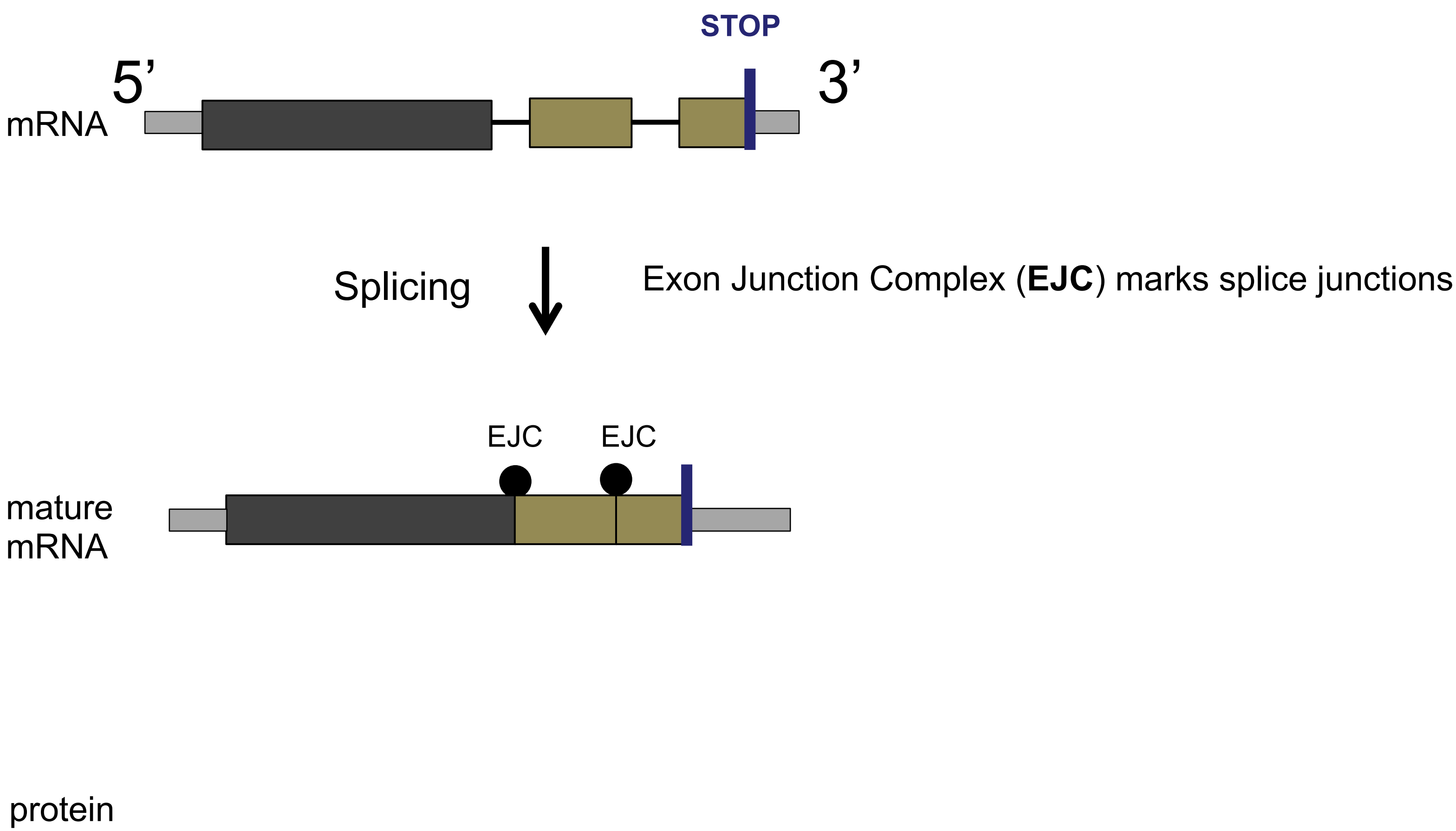
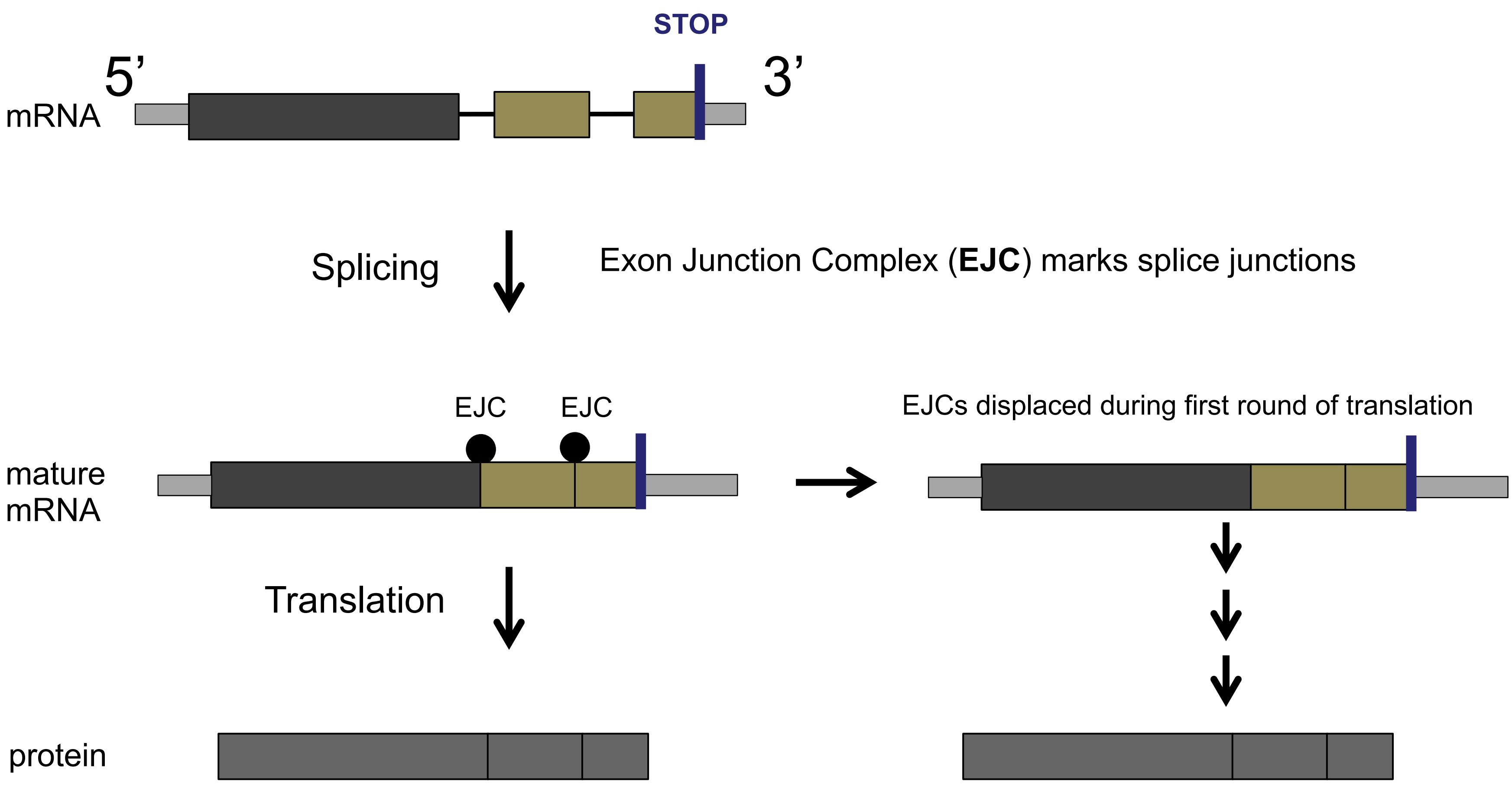


Figure 1

Translation



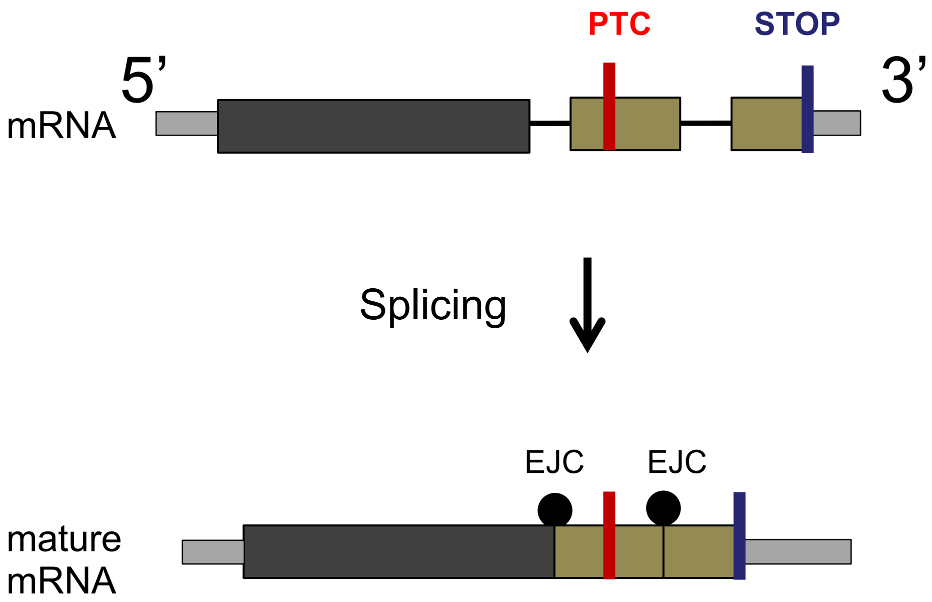
Translation



Nonsense Mediated Decay (NMD) RNA Surveillance

NMD degrades mRNA with premature stop codon

PTC – Premature Termination Codon



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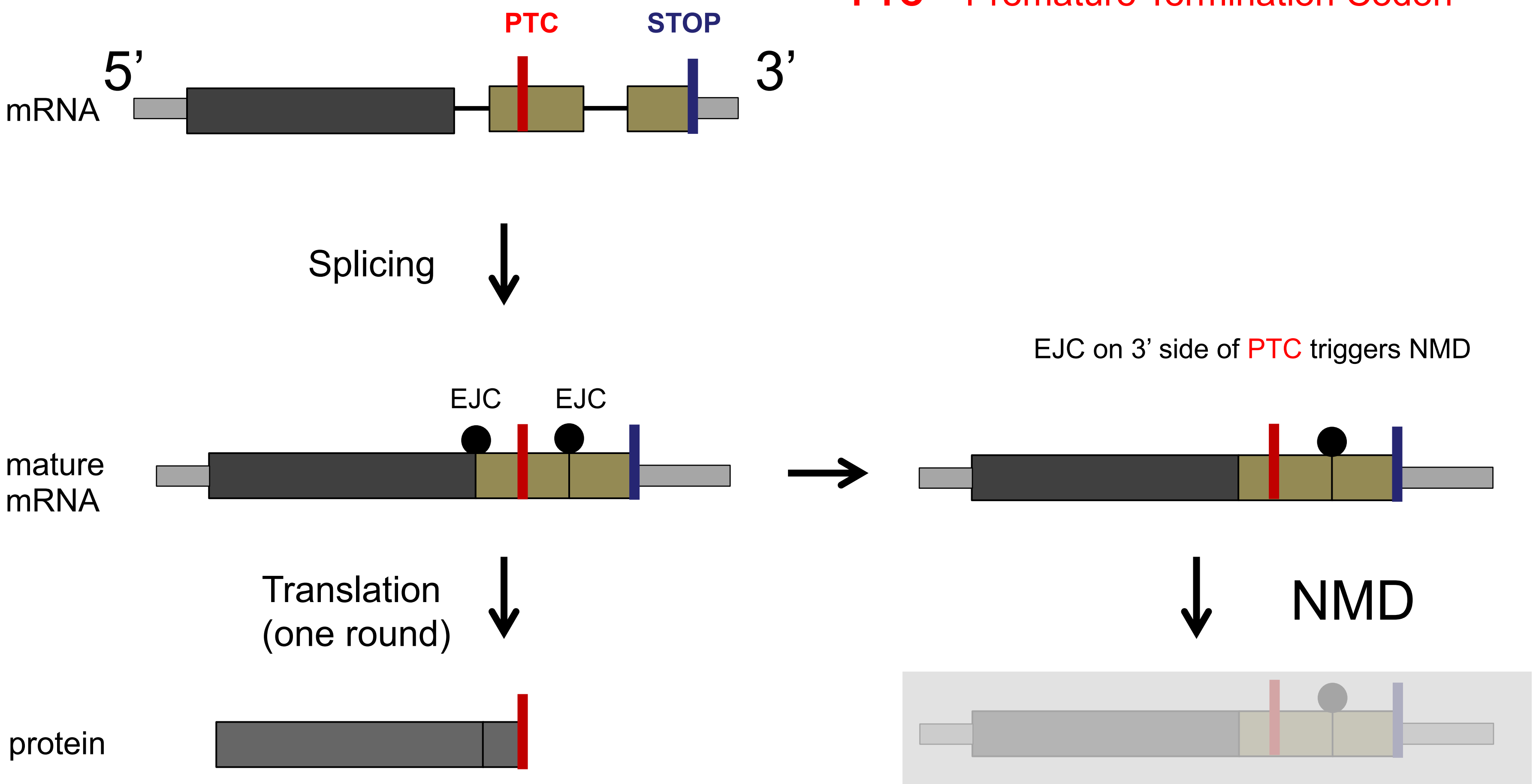
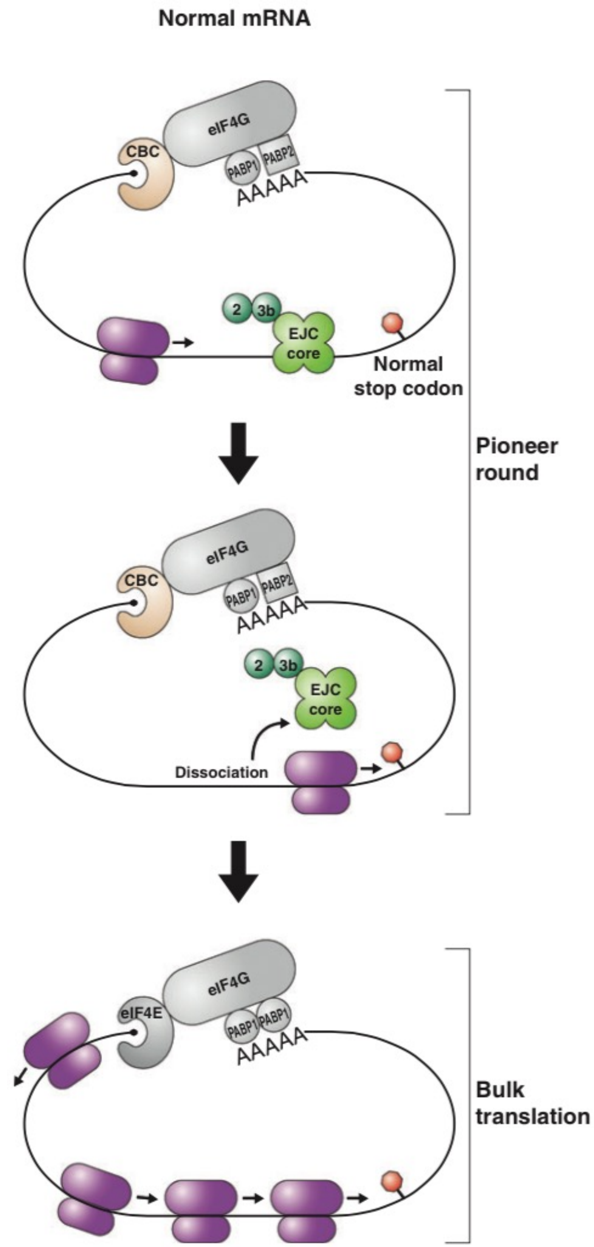


Nonsense Mediated Decay (NMD) RNA Surveillance

- 2 UPF2
- 3b UPF3b



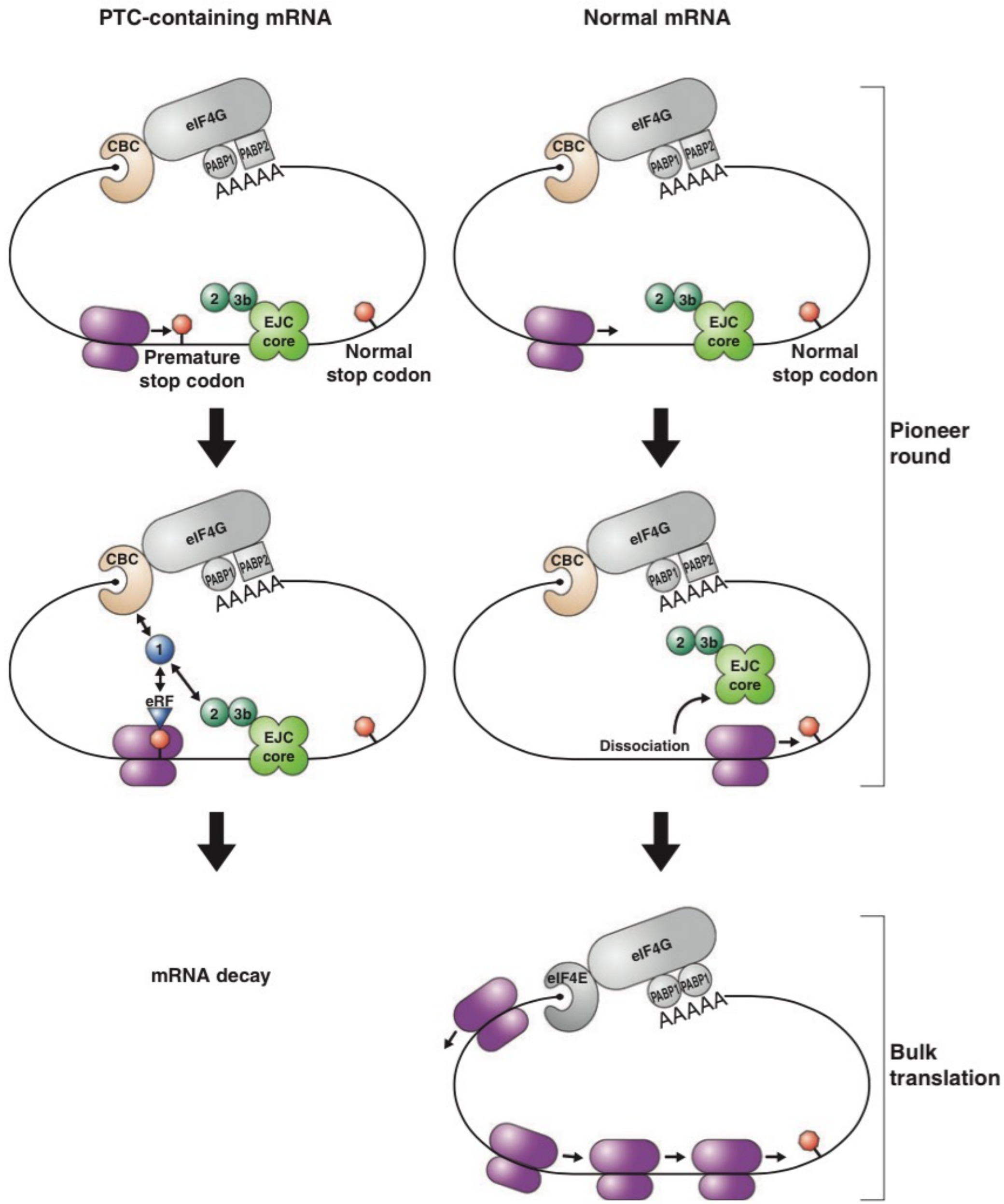
Annu. Rev. Biochem. 2007.76:51-74

Nonsense Mediated Decay (NMD) RNA Surveillance Pathway

2 UPF2

3b UPF3b

1 UPF1



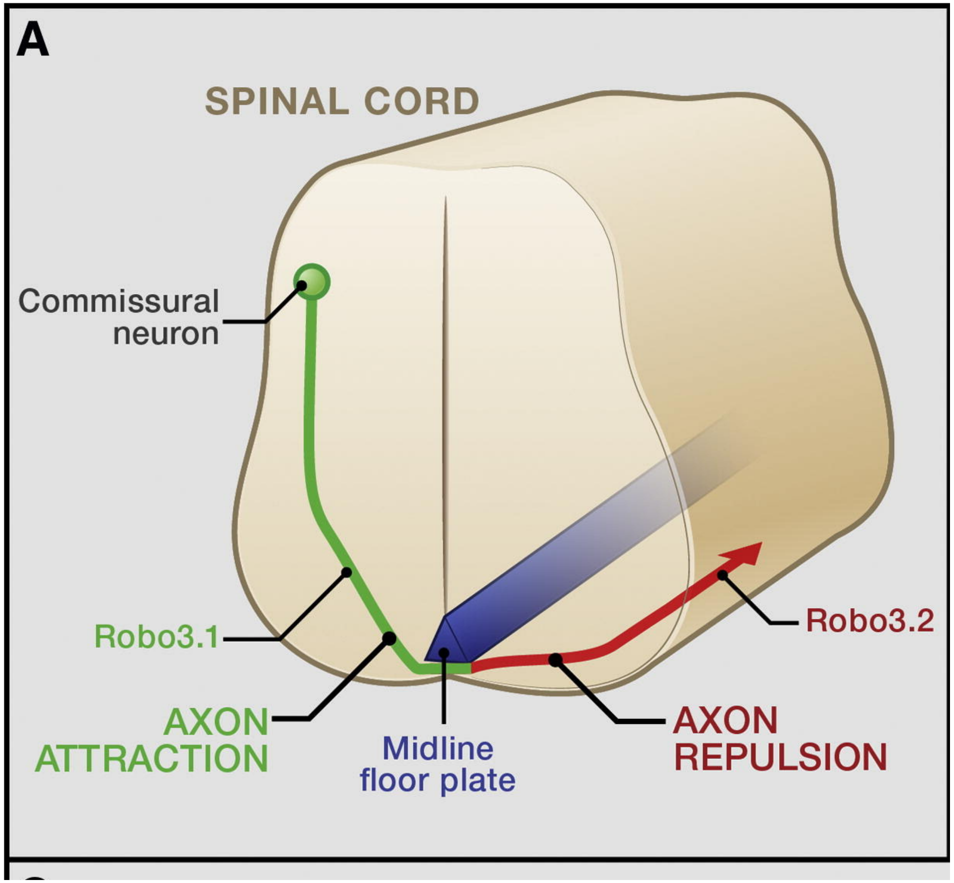
Annu. Rev. Biochem. 2007.76:51-74

Robo3.2 is degraded by NMD in **post-crossing axons**?

NMD

? ⊥

Robo3.2 mRNA

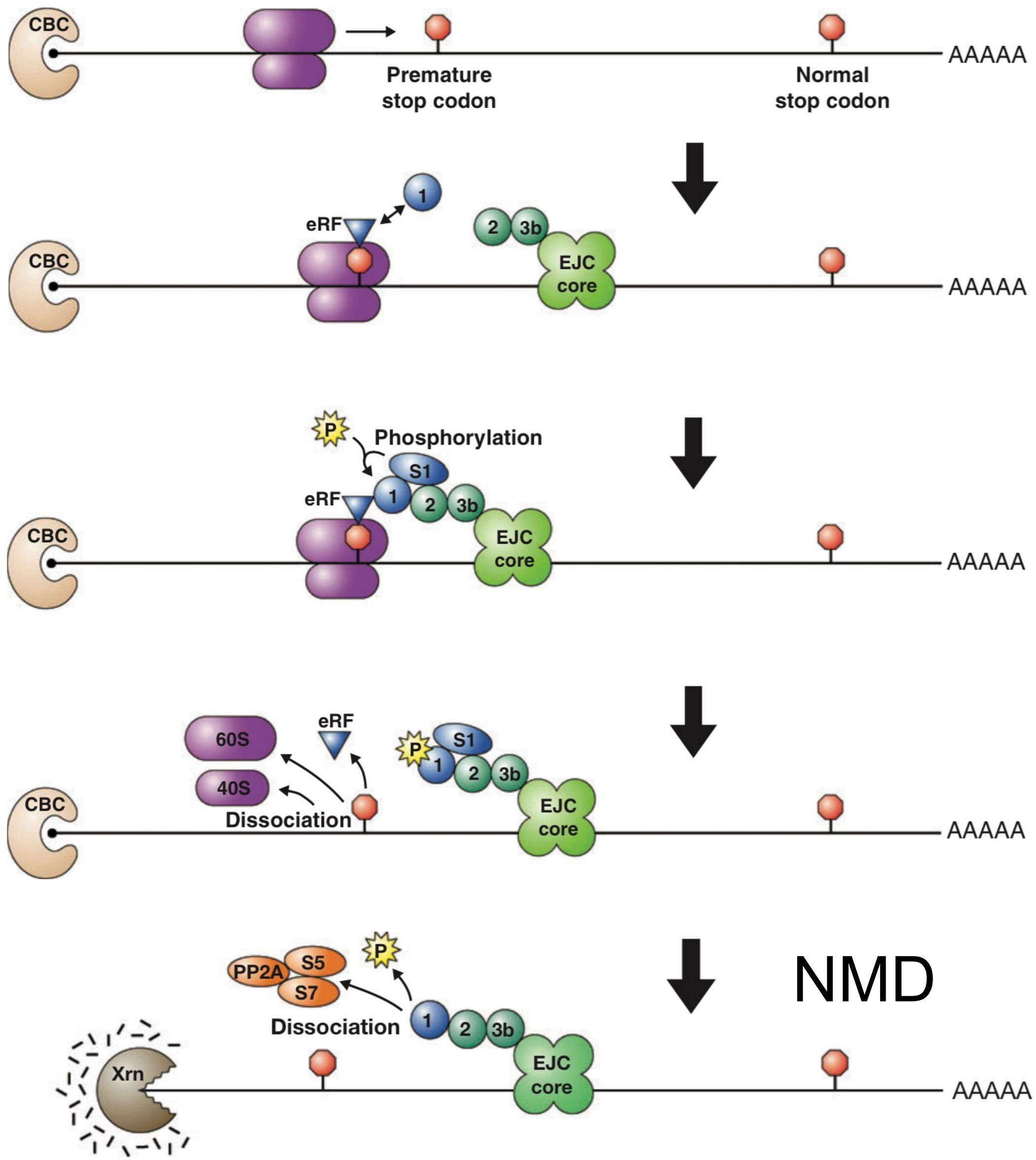


Nonsense Mediated Decay (NMD) RNA Surveillance Pathway

2 Upf2

1 Upf1

S1 Smg1



Annu. Rev. Biochem. 2007.76:51-74

NMD components are localized to axon tips

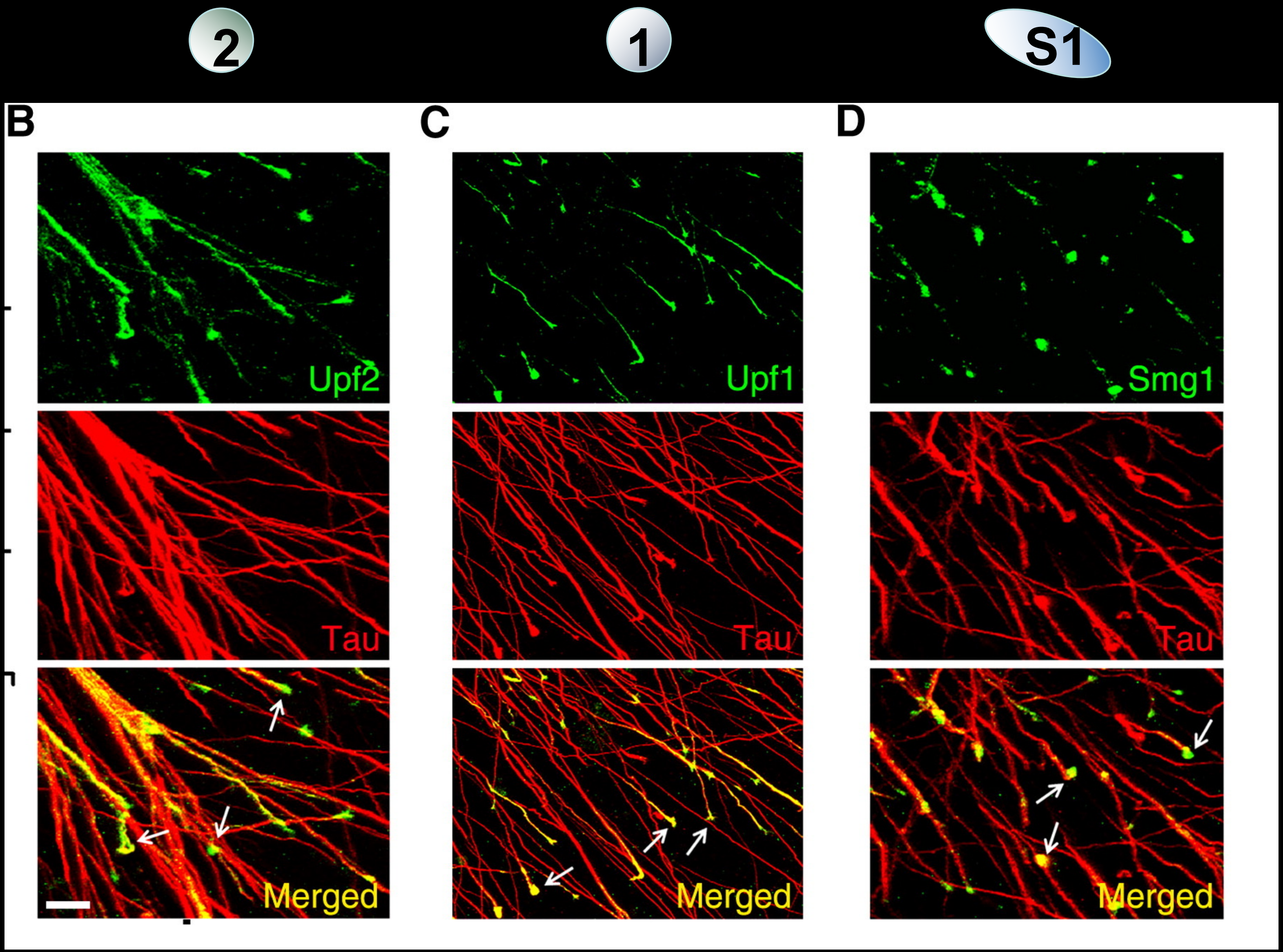
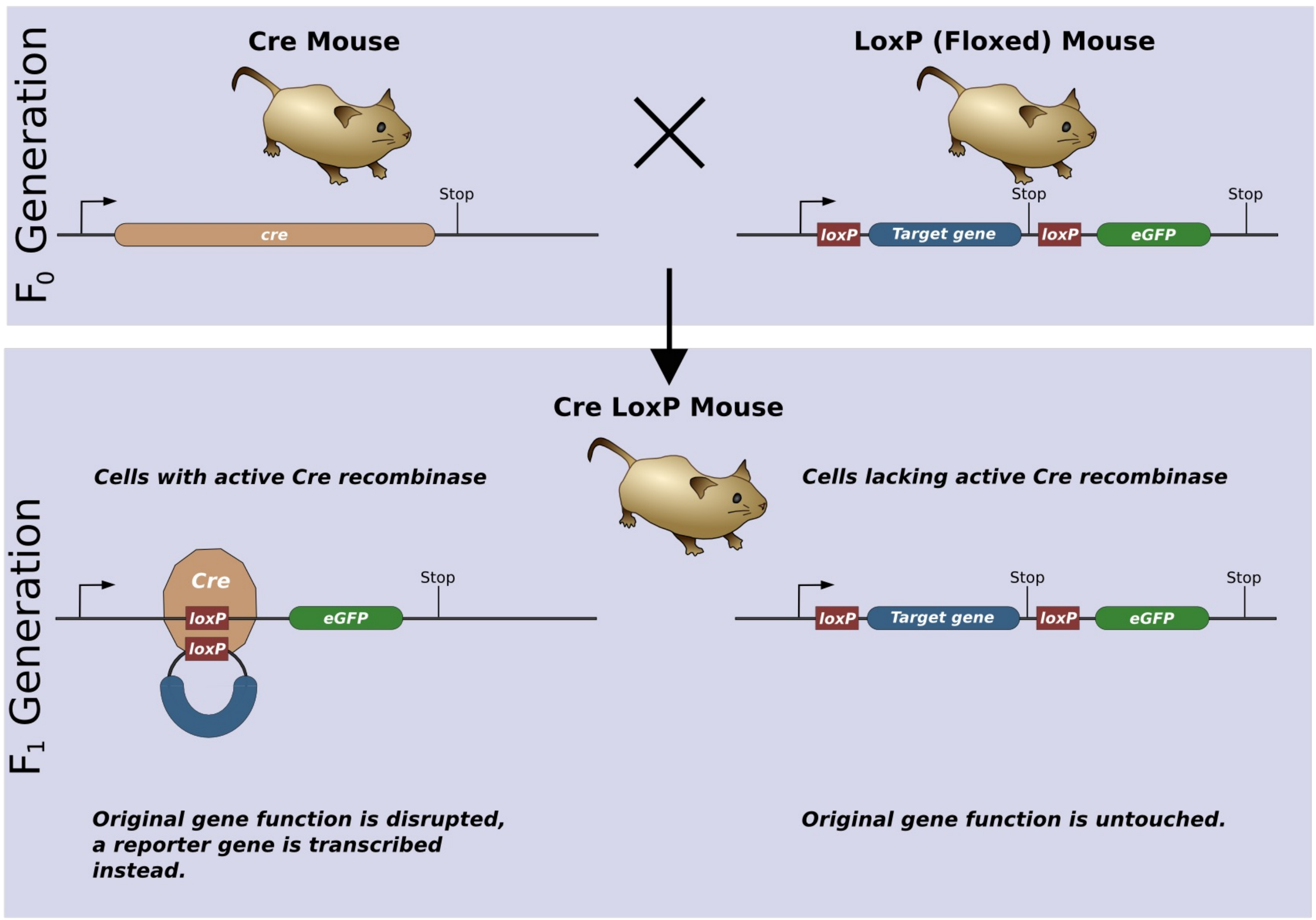
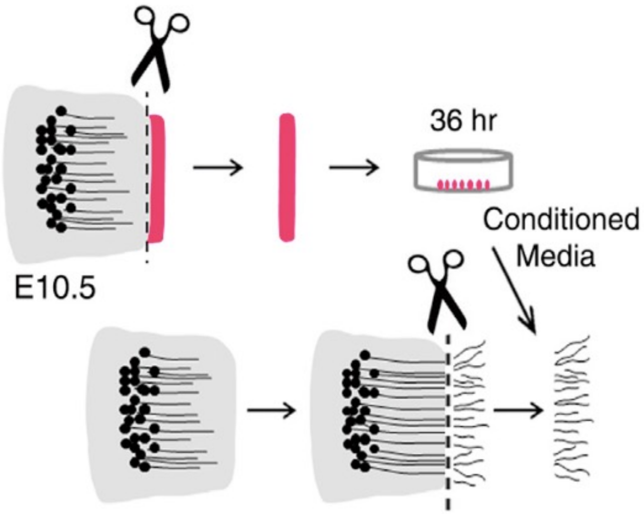


Figure 5

Upf2 conditional knockout



NMD regulates Robo3.2 mRNA levels in post-crossing axons



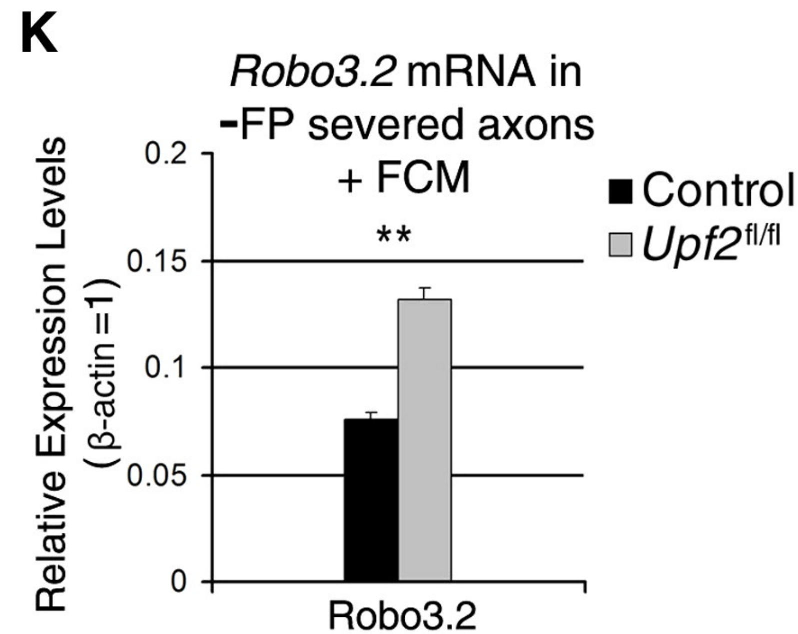
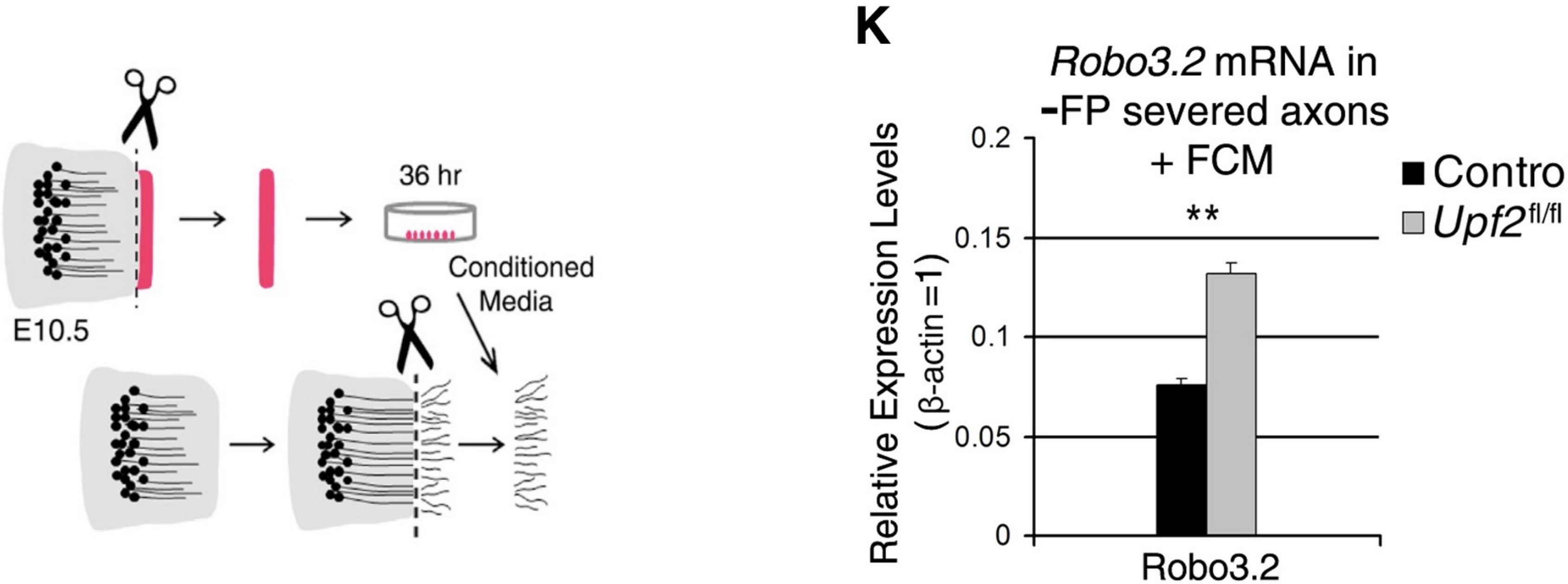


Figure 5

NMD regulates Robo3.2 mRNA levels in post-crossing axons



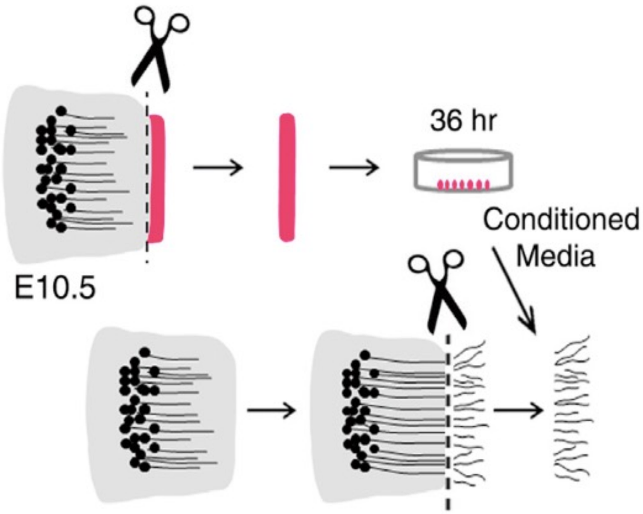
Robo3.2 mRNA → translation → Robo3.2 protein → repulsion

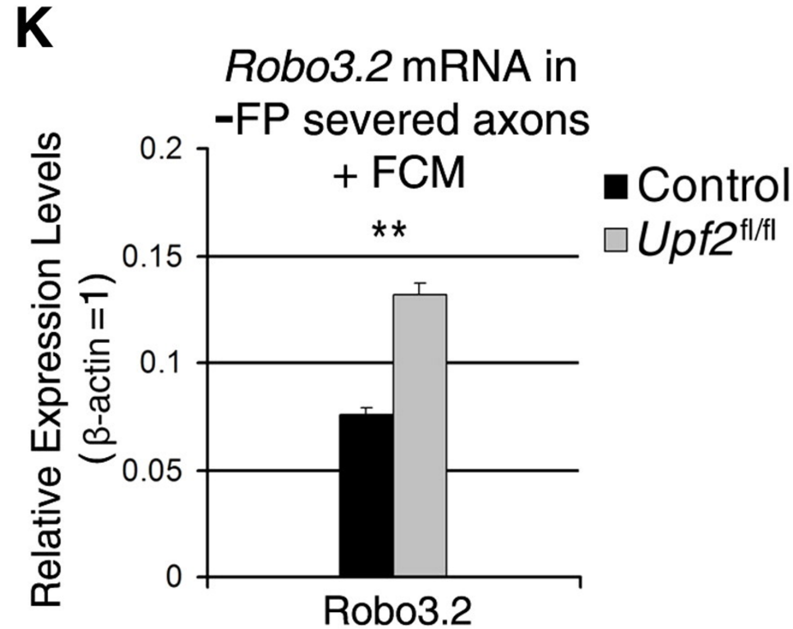
↑ ?

Floorplate (FP)

Figure 5

NMD regulates Robo3.2 mRNA levels in post-crossing axons





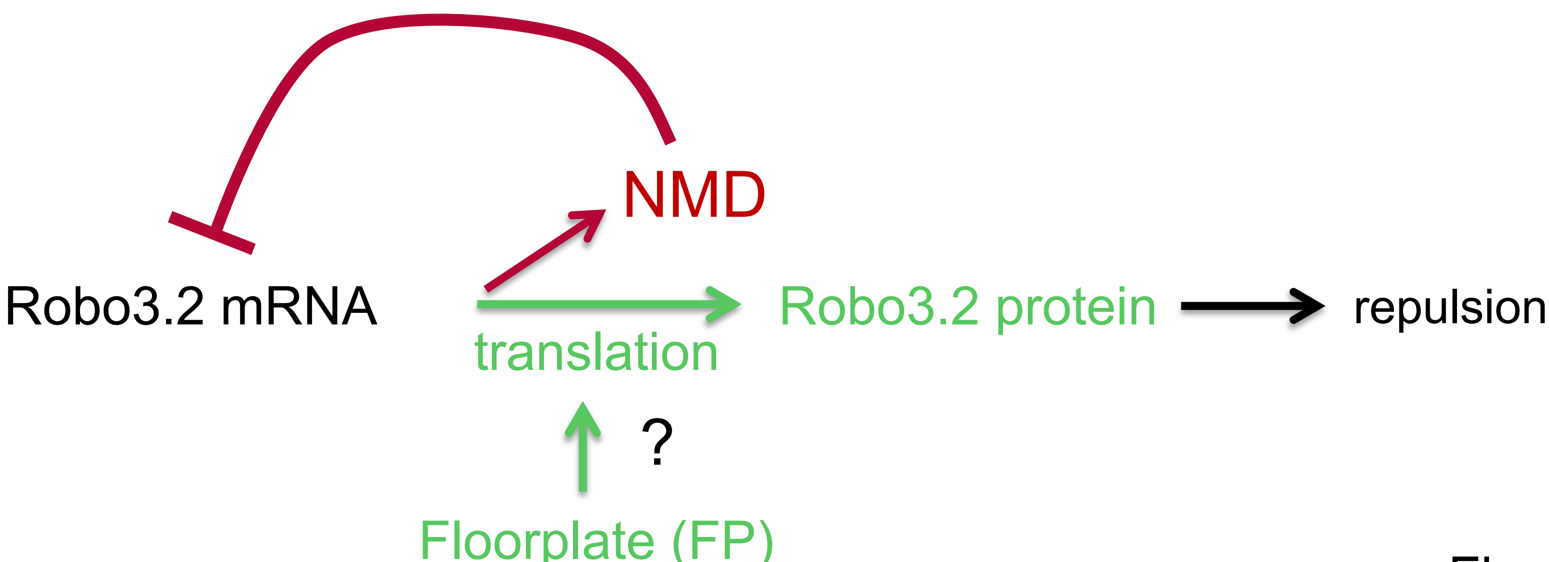


Figure 5

NMD regulates post-crossing axon behavior

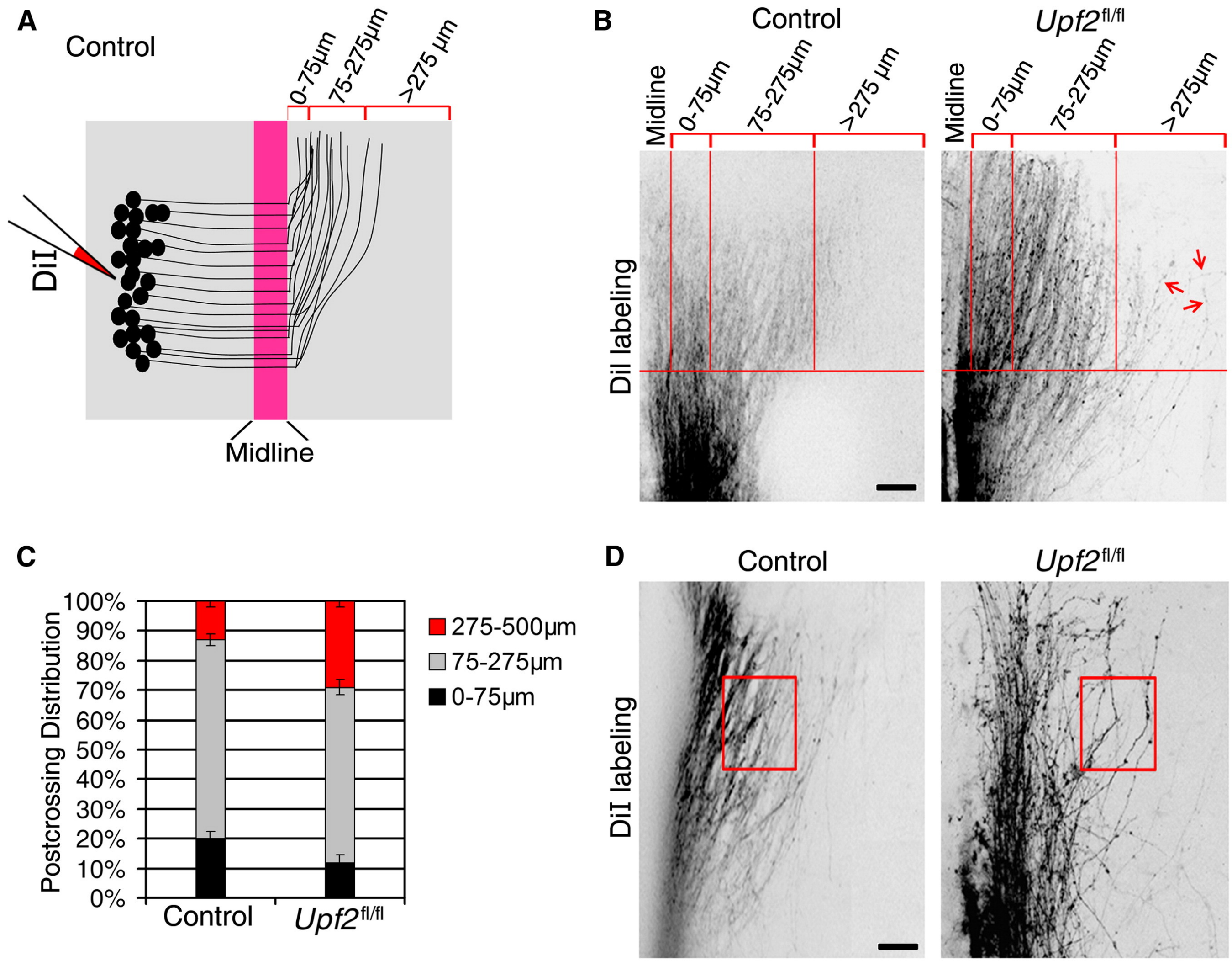


Figure 6

NMD machinery is localized to growth cones of several types of neurons

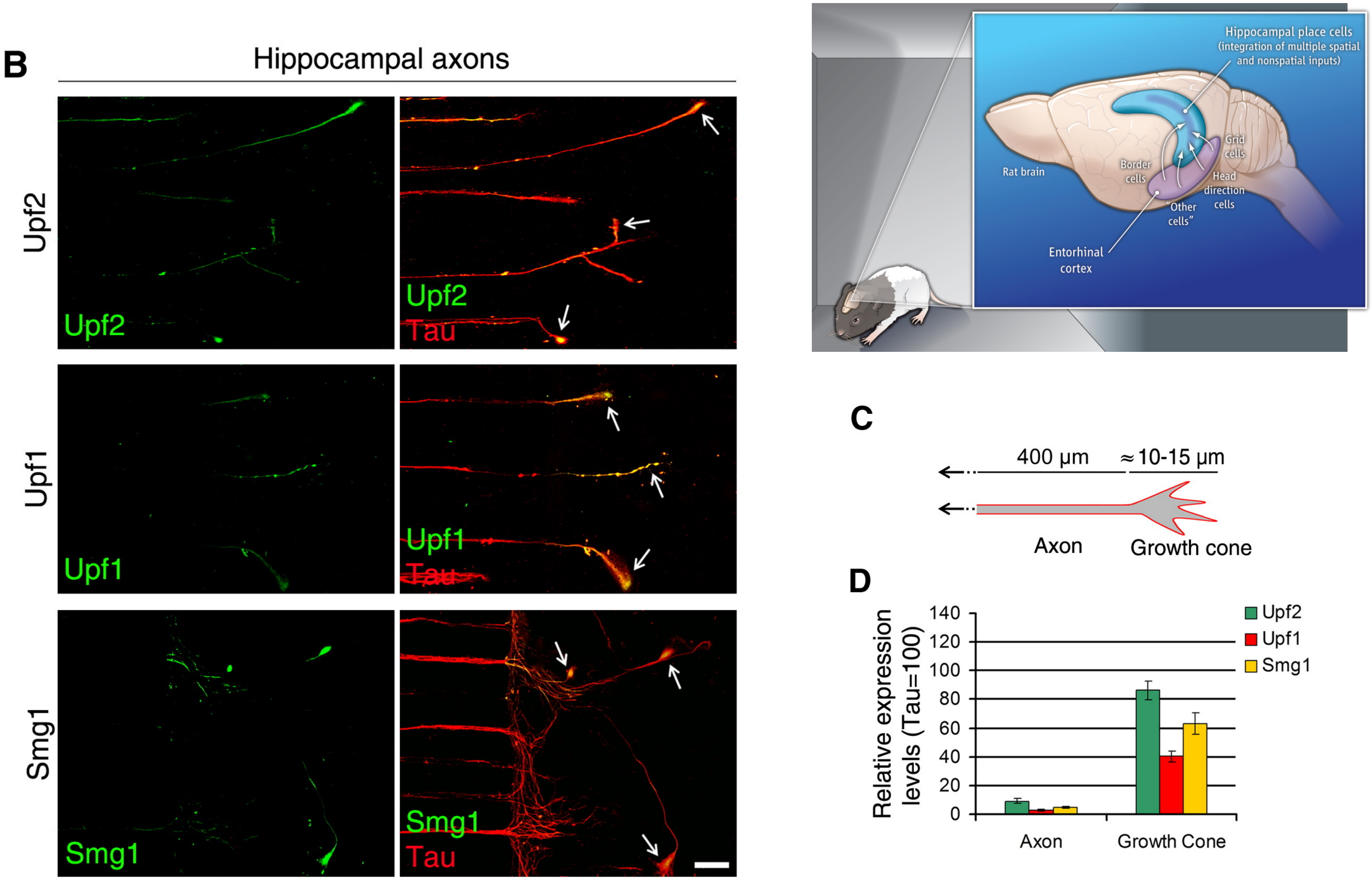
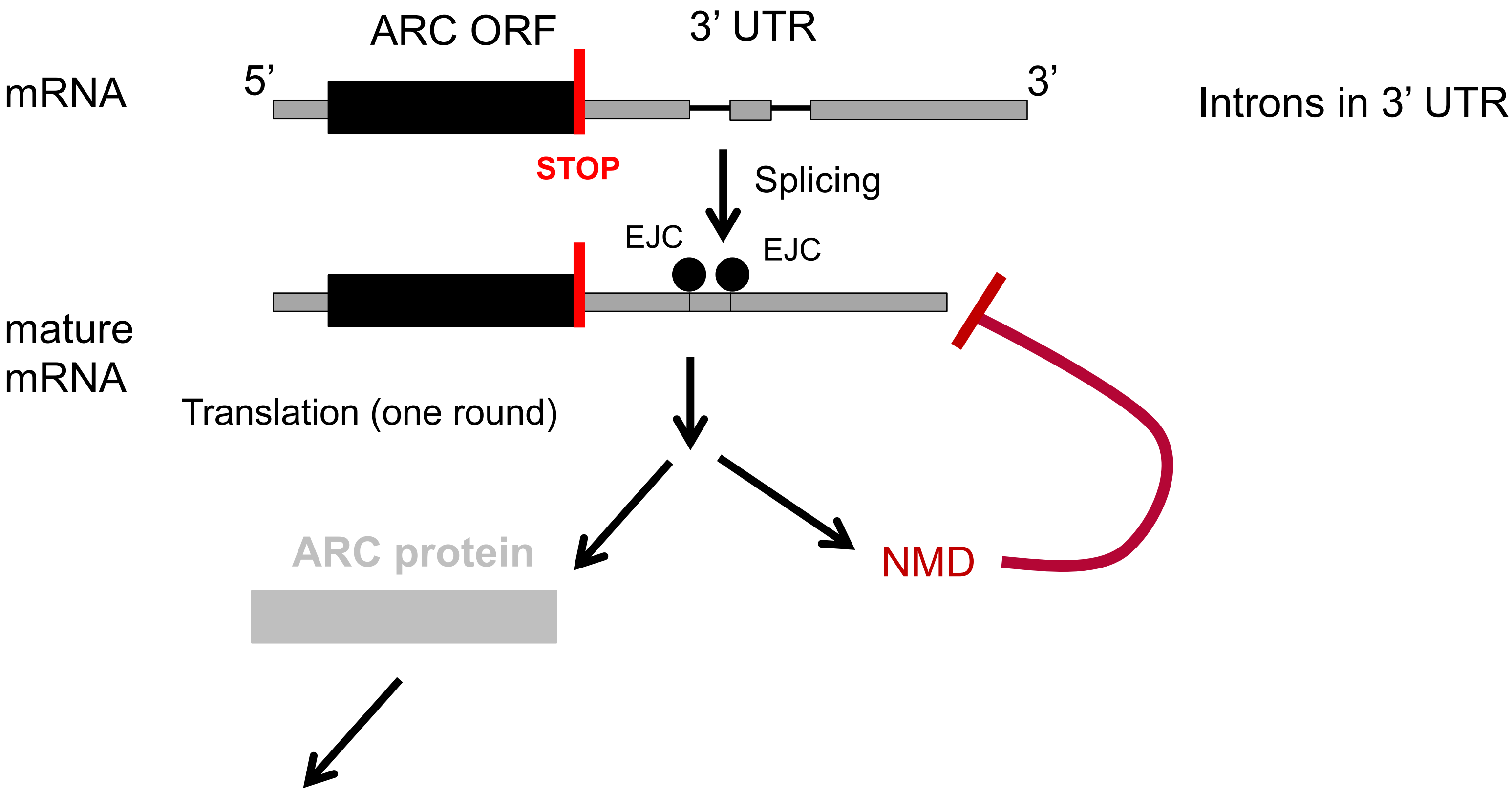


Figure 7

NMD puts the brakes on protein synthesis in neurons



Activity-dependent synaptic plasticity (e.g., LTP)

~150 genes with 3’ UTR introns

Giorgi et al, 2007

Cell

Regulation of Axon Guidance by Compartmentalized Nonsense-Mediated mRNA Decay

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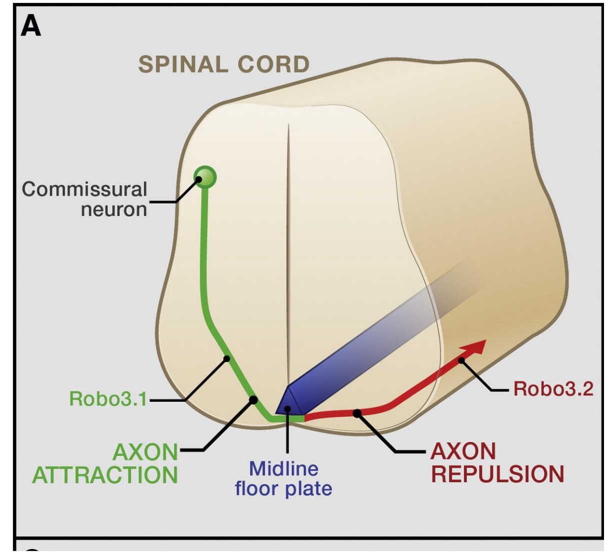

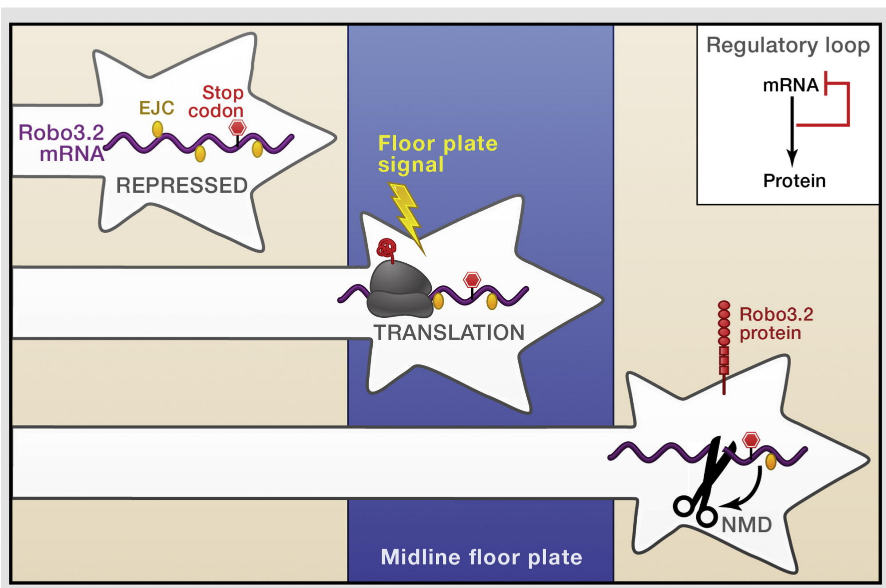

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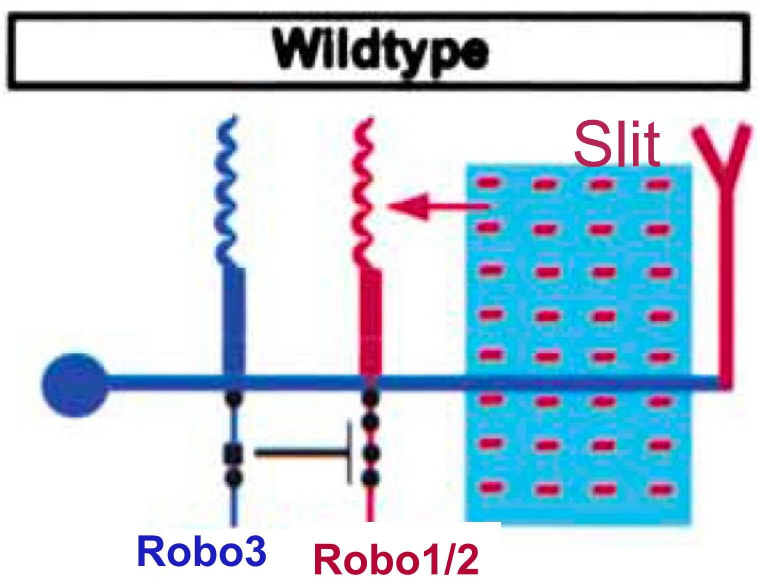
The Journal of Neuroscience, July 14, 2010 • 30(28):9445–9453 • 9445

Development/Plasticity/Repair

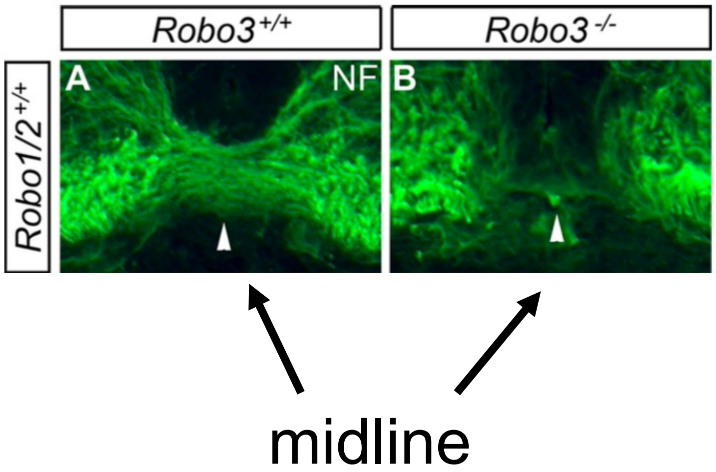
Collaborative and Specialized Functions of Robo1 and Robo2 in Spinal Commissural Axon Guidance

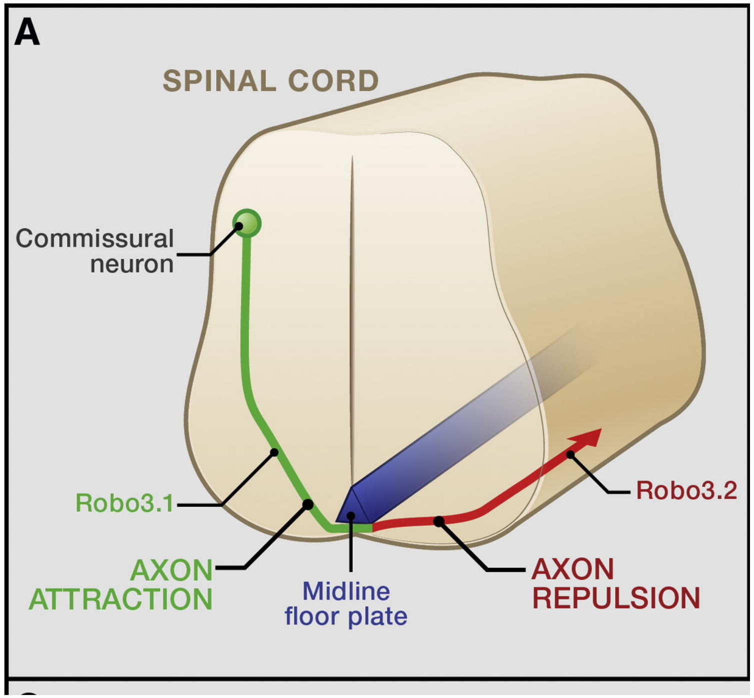
Alexander Jaworski, Hua Long, and Marc Tessier-Lavigne
Division of Research, Genentech Inc., South San Francisco, California 94080



Robo3 = Attraction (by blocking repulsive response to slit)

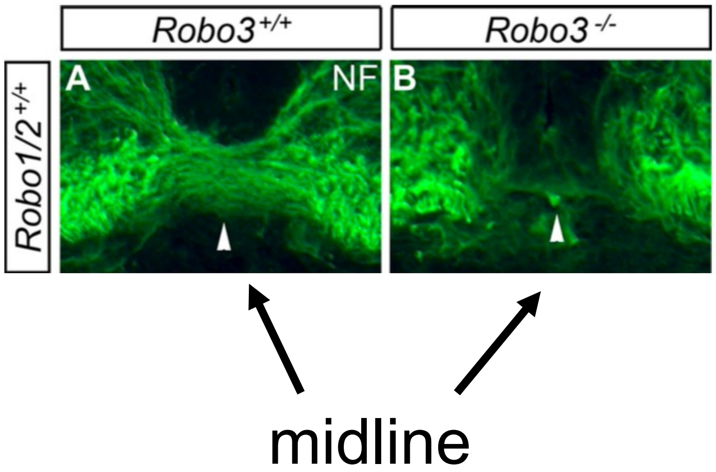
Commissural neurons fail to cross midline in *Robo3(-/-)*

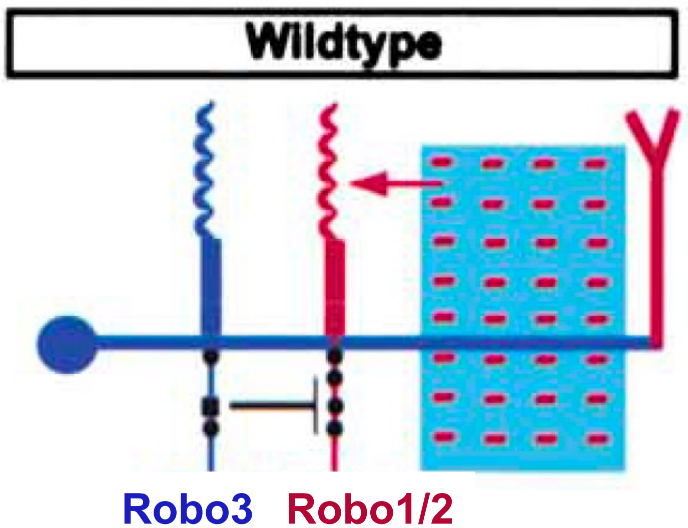




Jaworski et al., J. Neurosci. (2010)

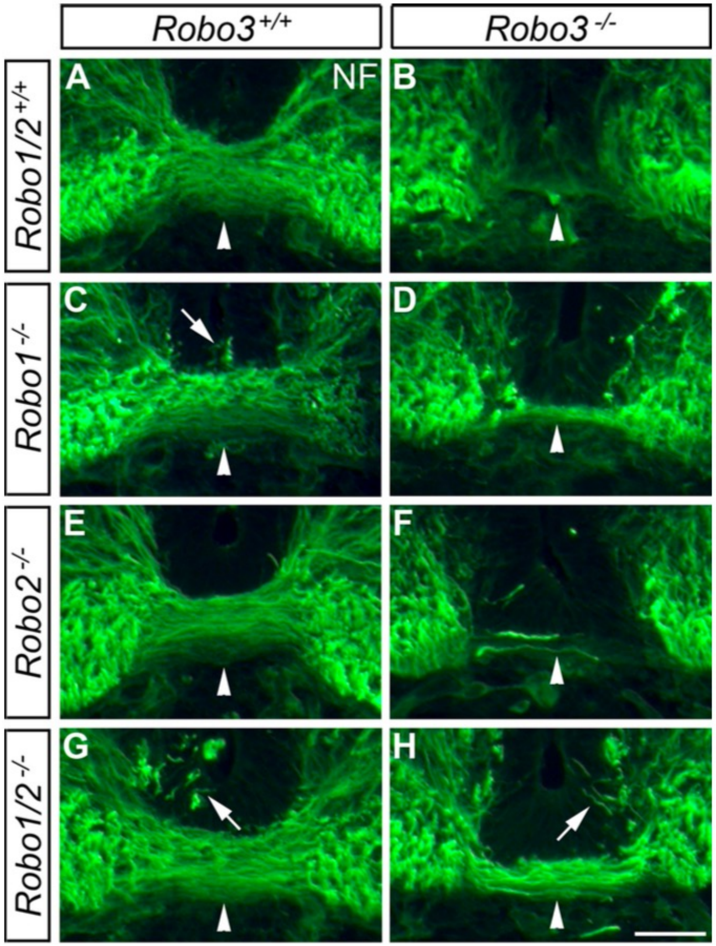
Commissural neurons fail to cross midline in *Robo3(-/-)*

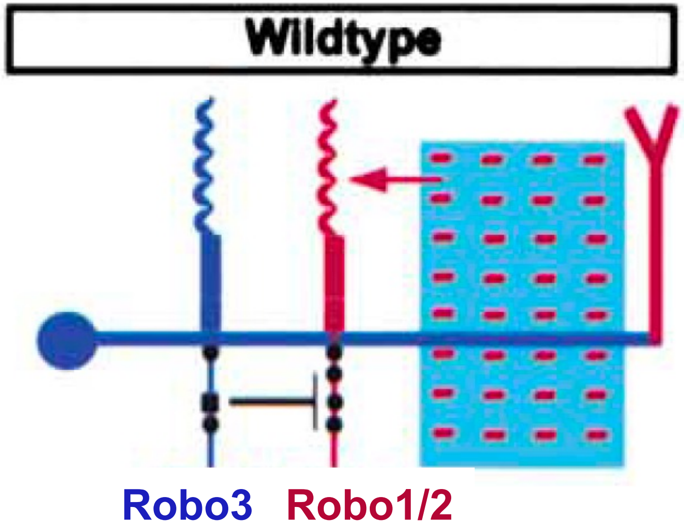




Jaworski et al., J. Neurosci. (2010)

Loss of *Robo1* and *Robo2* does not fully rescue midline crossing in *Robo3* mutants





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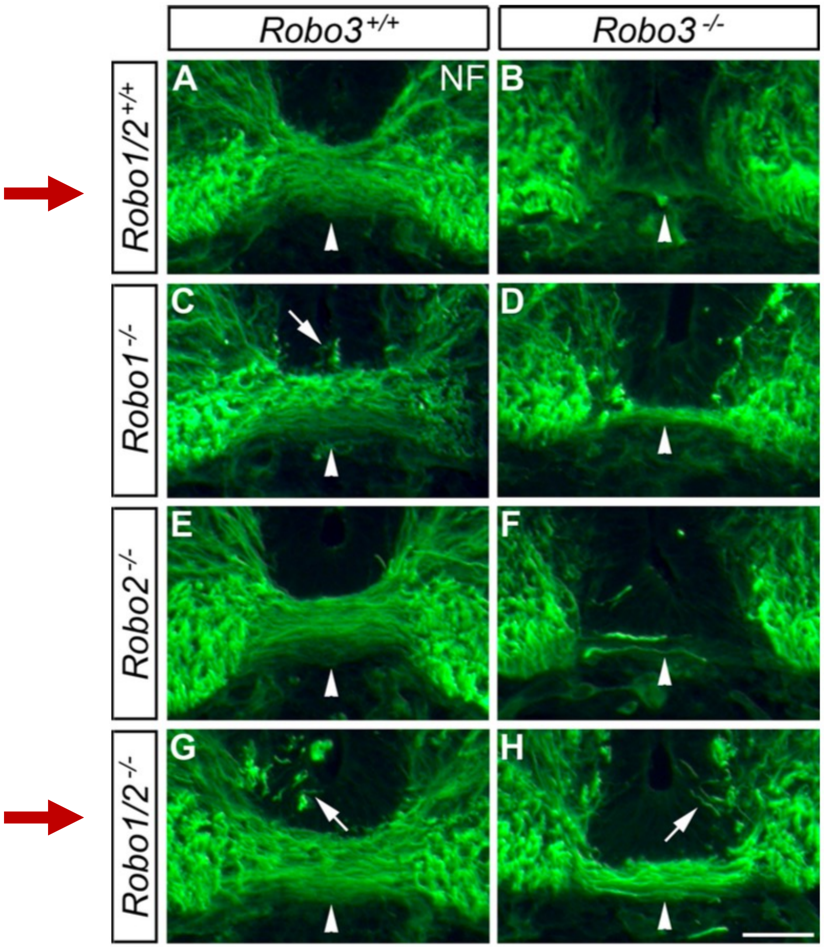

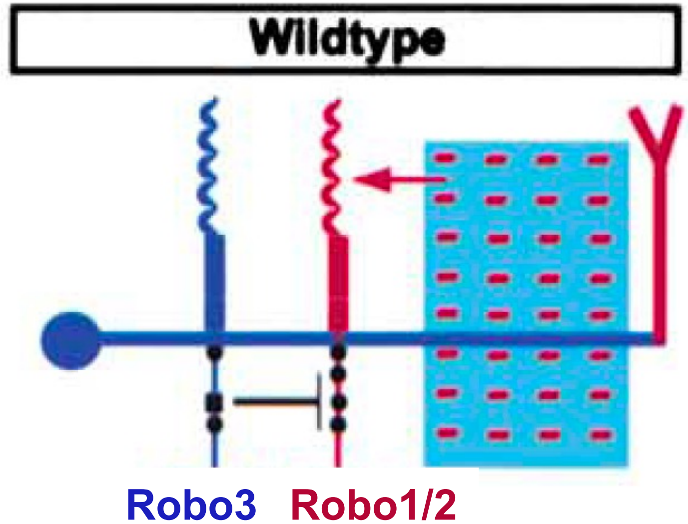

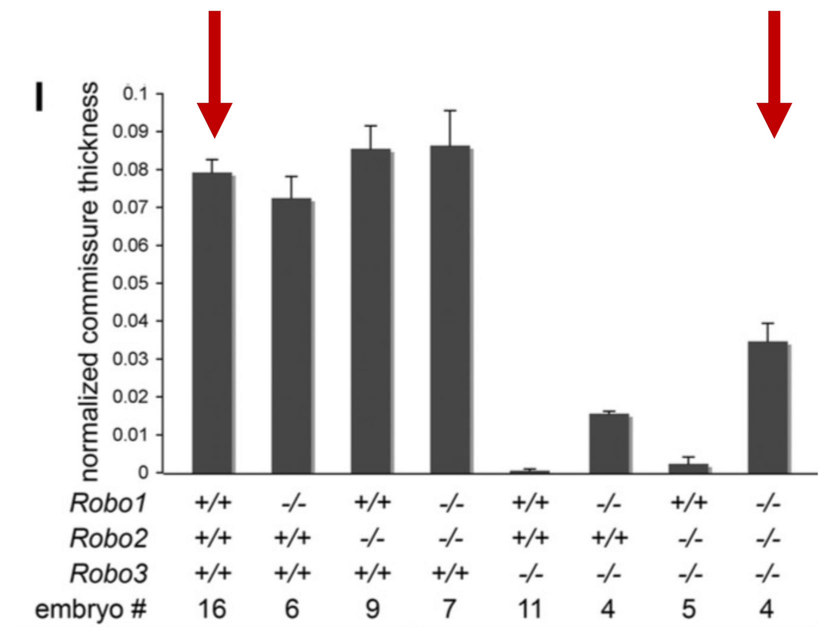

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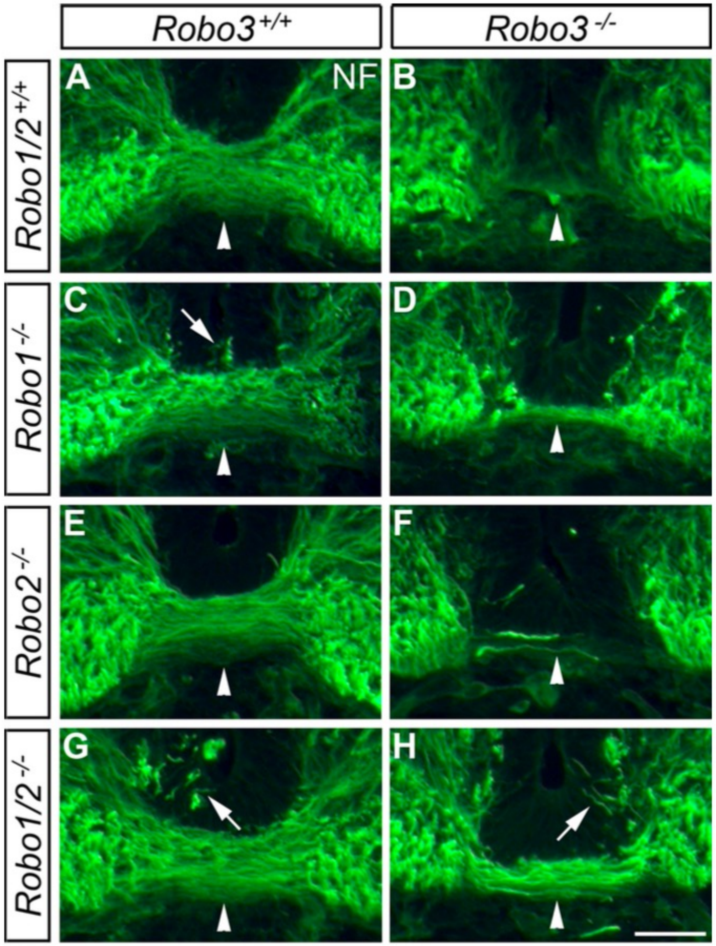

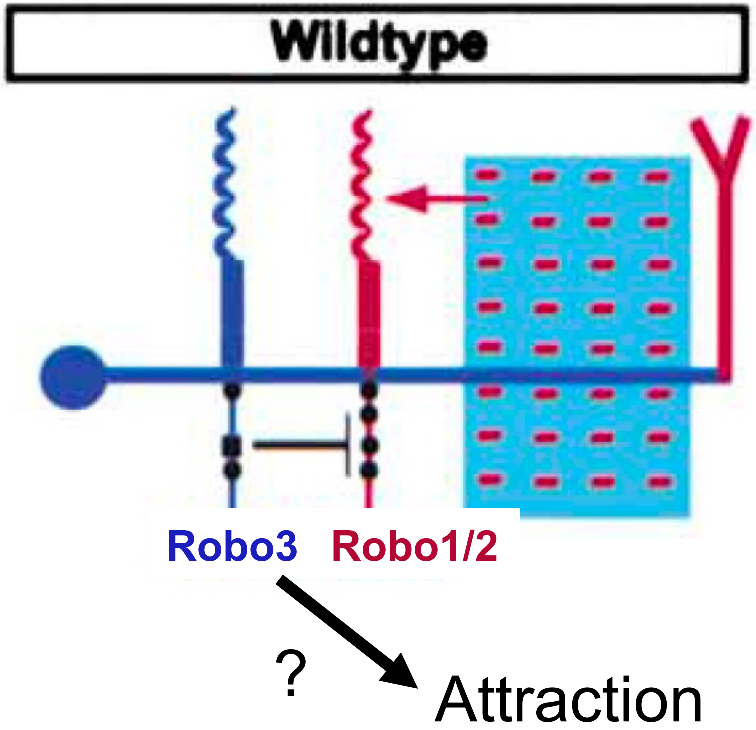

Jaworski et al., J. Neurosci. (2010)

Neuron
Article

Signaling Switch of the Axon Guidance Receptor Robo3 during Vertebrate Evolution

Pavol Zelina,[1,2,3,11] Heike Blockus,[1,2,3,11] Yvrick Zagar,[1,2,3,11] Amélie Péres,[4,5,6] François Friocourt,[1,2,3] Zhuhao Wu,[7] Nicolas Rama,[1,2,3] Coralie Fouquet,[8] Erhard Hohenester,[9] Marc Tessier-Lavigne,[7] Jörn Schweitzer,[10] Hugues Roest Crollius,[4,5,6] and Alain Chédotal[1,2,3,*]
[1]INSERM, UMRS U968, Institut de la Vision, Paris 75012, France
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[3]CNRS, UMR 7210, Paris 75012, France
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[5]CNRS, UMR 8197, Paris 75005, France
[6]INSERM, U1024, Paris 75005, France
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[9]Imperial College London, Department of Life Sciences, London SW7 2AZ, UK
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SUMMARY

Development of neuronal circuits is controlled by evolutionarily conserved axon guidance molecules, including Slits, the repulsive ligands for roundabout (Robo) receptors, and Netrin-1, which mediates attraction through the DCC receptor. We discovered that the Robo3 receptor fundamentally changed its mechanism of action during mammalian evolution. Unlike other Robo receptors, mammalian Robo3 is not a high-affinity receptor for Slits because of specific substitutions in the first immunoglobulin domain. Instead, Netrin-1 selectively triggers phosphorylation of mammalian Robo3 via Src kinases. Robo3 does not bind Netrin-1 directly but interacts with DCC. Netrin-1 fails to attract pontine neurons lacking Robo3, and attraction can be restored in *Robo3*$^{-/-}$ mice by expression of mammalian, but not nonmammalian, Robo3. We propose that Robo3 evolution was key to sculpting the mammalian brain by converting a receptor for Slit repulsion into one that both silences Slit repulsion and potentiates Netrin attraction.

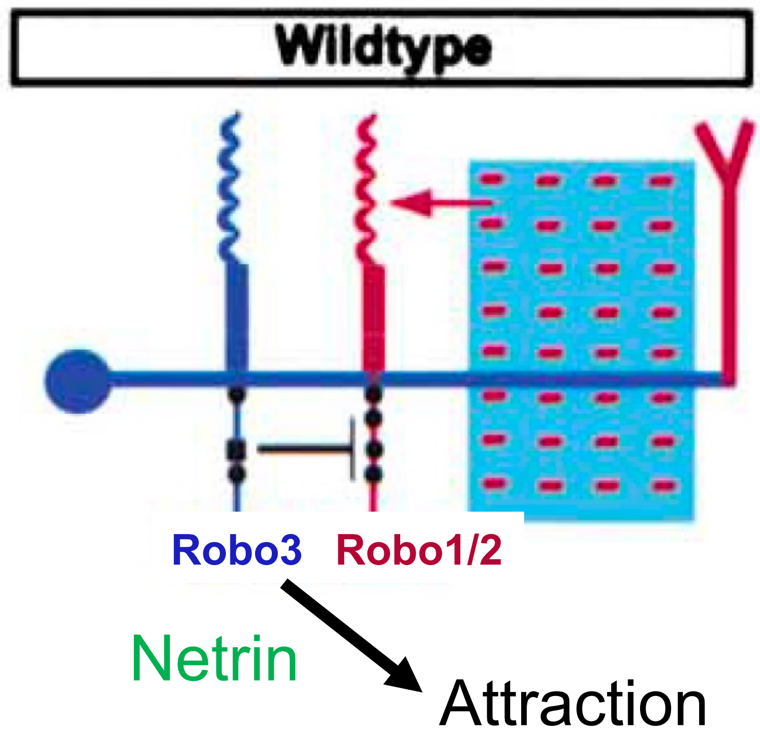

Figure 2

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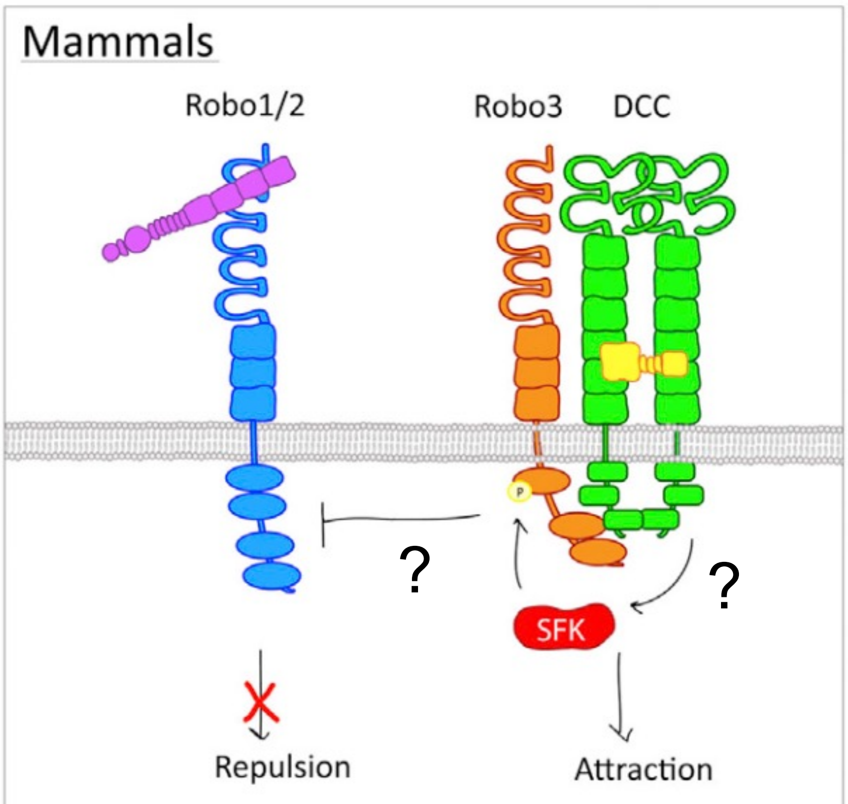

SFK = Src-Family Kinase

- Mammalian Robo3 Interacts with DCC receptor for chemoattraction to Netrin

Beamish and Kennedy, Dev Cell (2014)

Neuron

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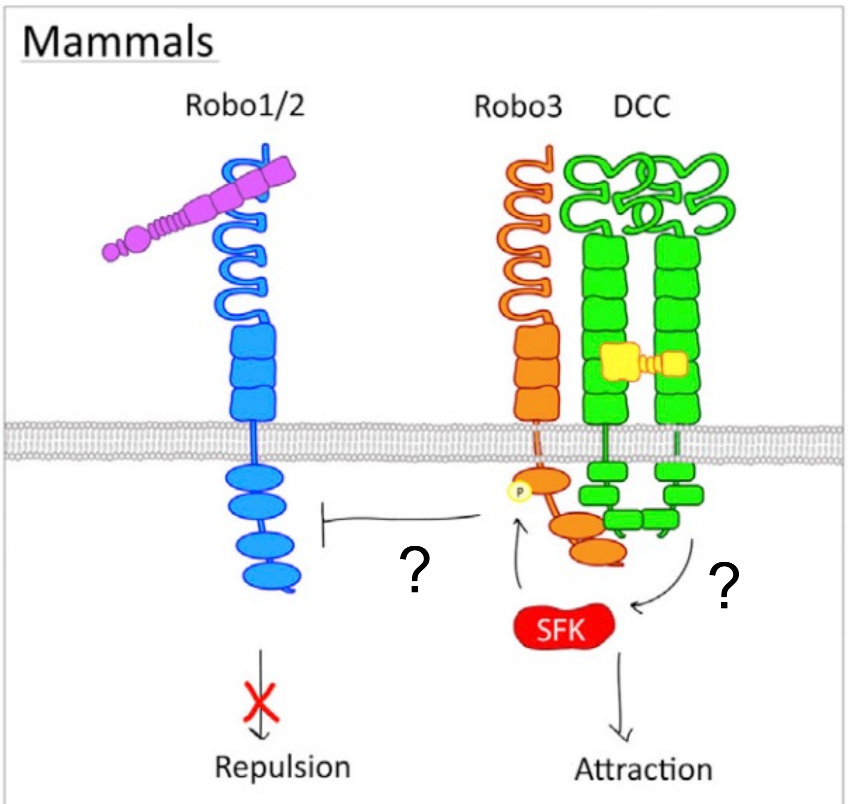


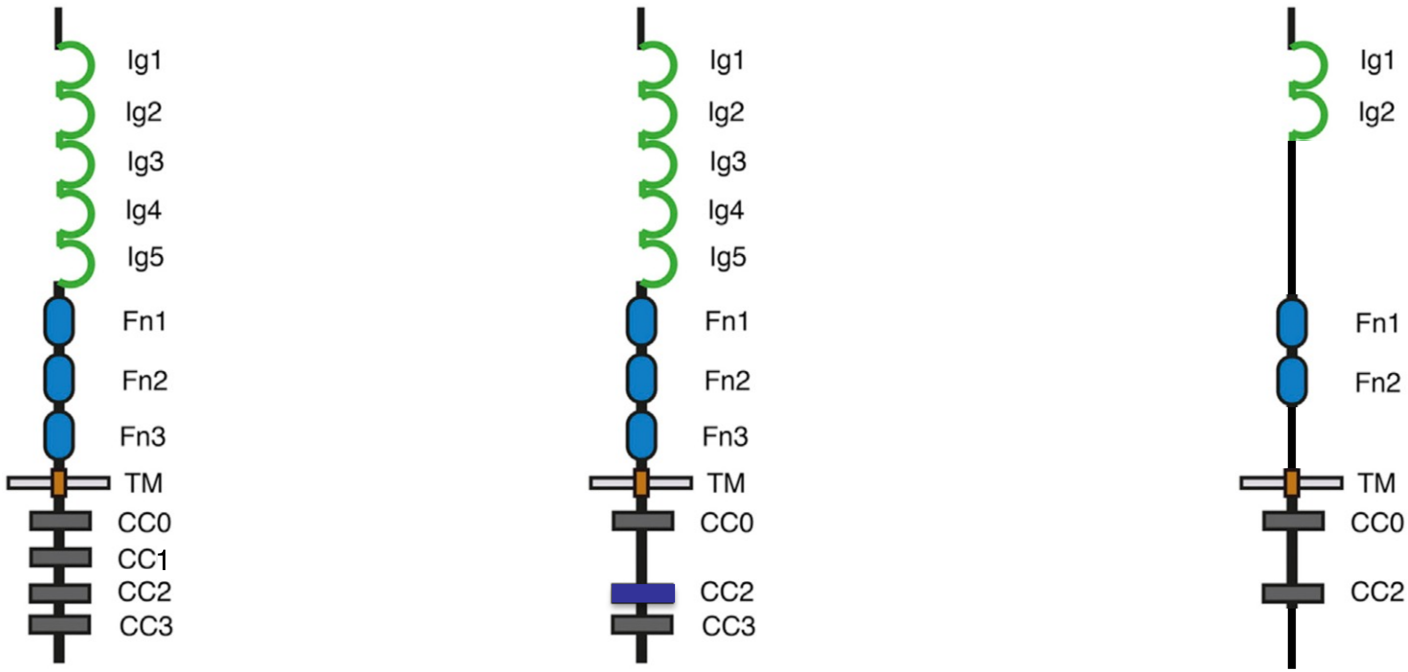
SFK = Src-Family Kinase

- Mammalian Robo3 Interacts with DCC receptor for chemoattraction to Netrin
- Mammalian Robo3 does not bind slit
- Evolution of mammalian brain

Beamish and Kennedy, Dev Cell (2014)

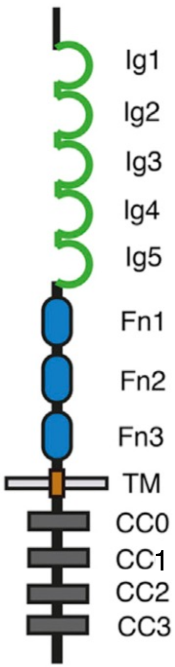
Four Mammalian Robo genes

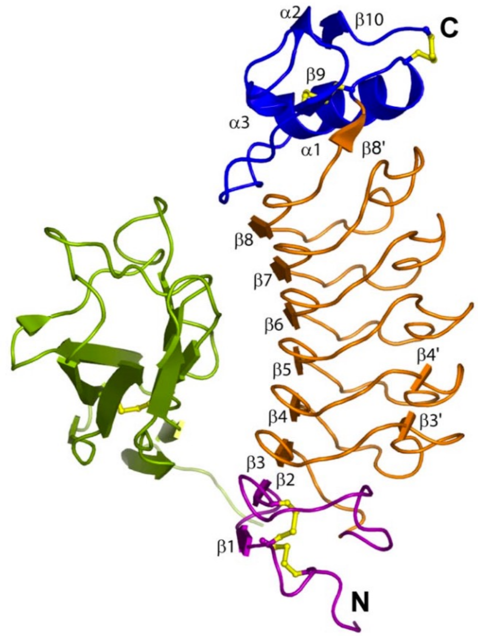
Robo1/2	Robo3	Robo 4
Repulsion	Attraction & Repulsion	Angiogenesis



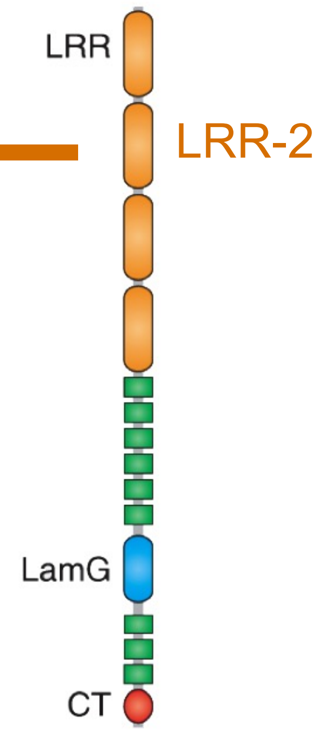
Robo1/2 Ig1 binds Slit LRR-2 domain

Robo1/2
Repulsion





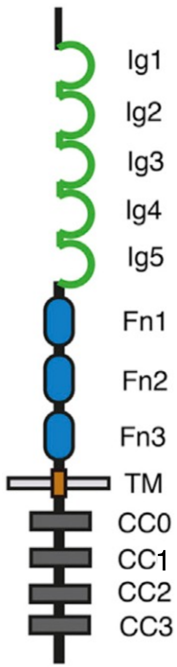
Slit

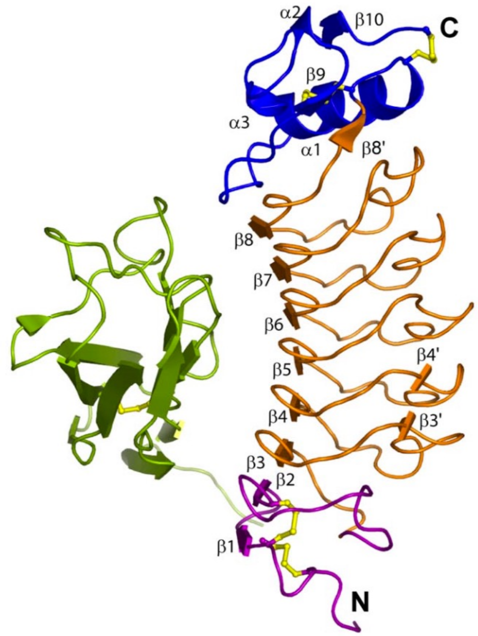


Morlot et al. PNAS (2007)

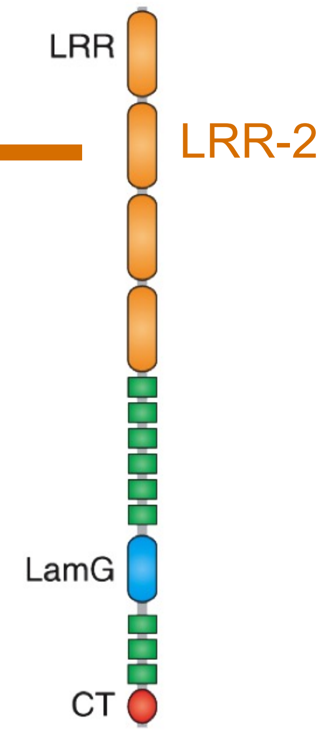
Robo1/2 Ig1 binds Slit LRR-2 domain

Robo1/2
Repulsion

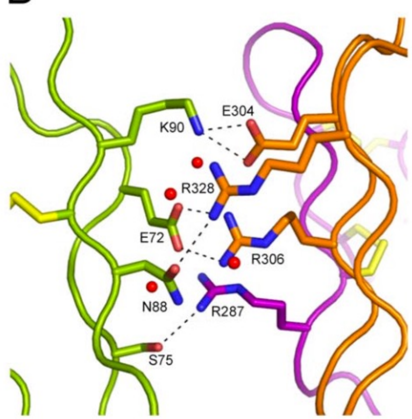




Slit



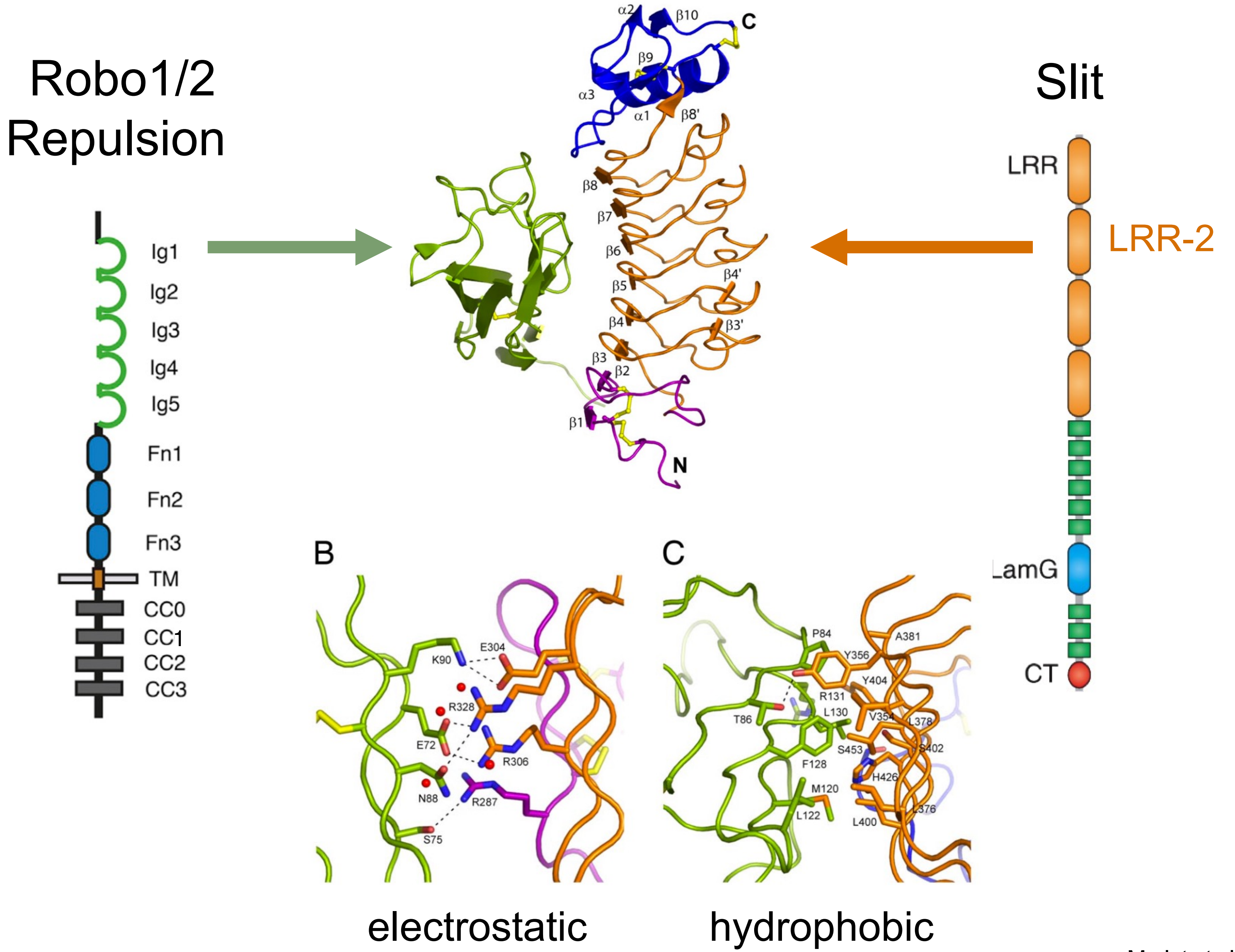
B



electrostatic

Morlot et al. PNAS (2007)

Robo1/2 Ig1 binds Slit LRR-2 domain



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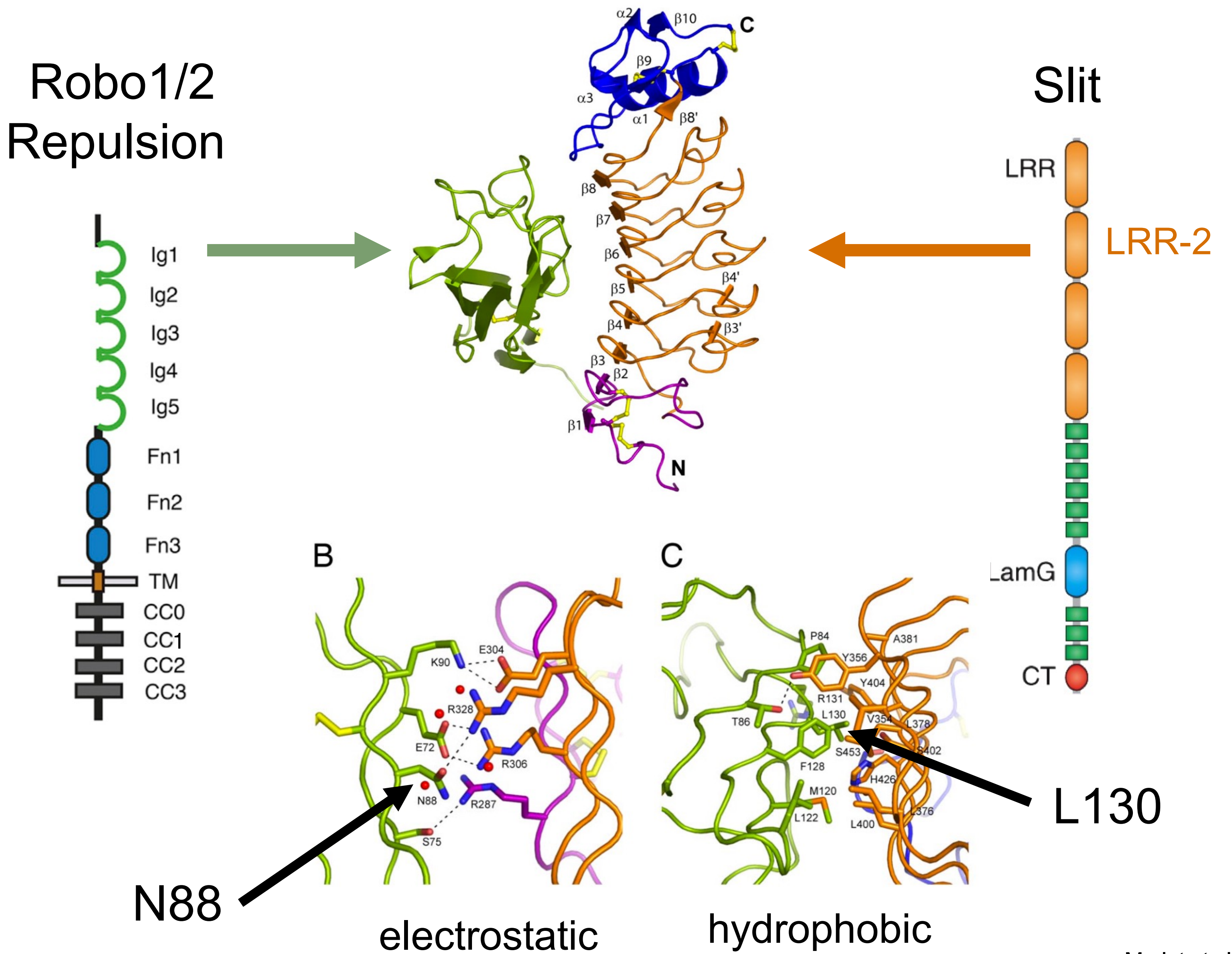
Robo1/2 Ig1 binds Slit LRR-2 domain



Morlot et al. PNAS (2007)

Mammalian-specific structure of Robo3 Ig1 domain

Zelina et al., 2014

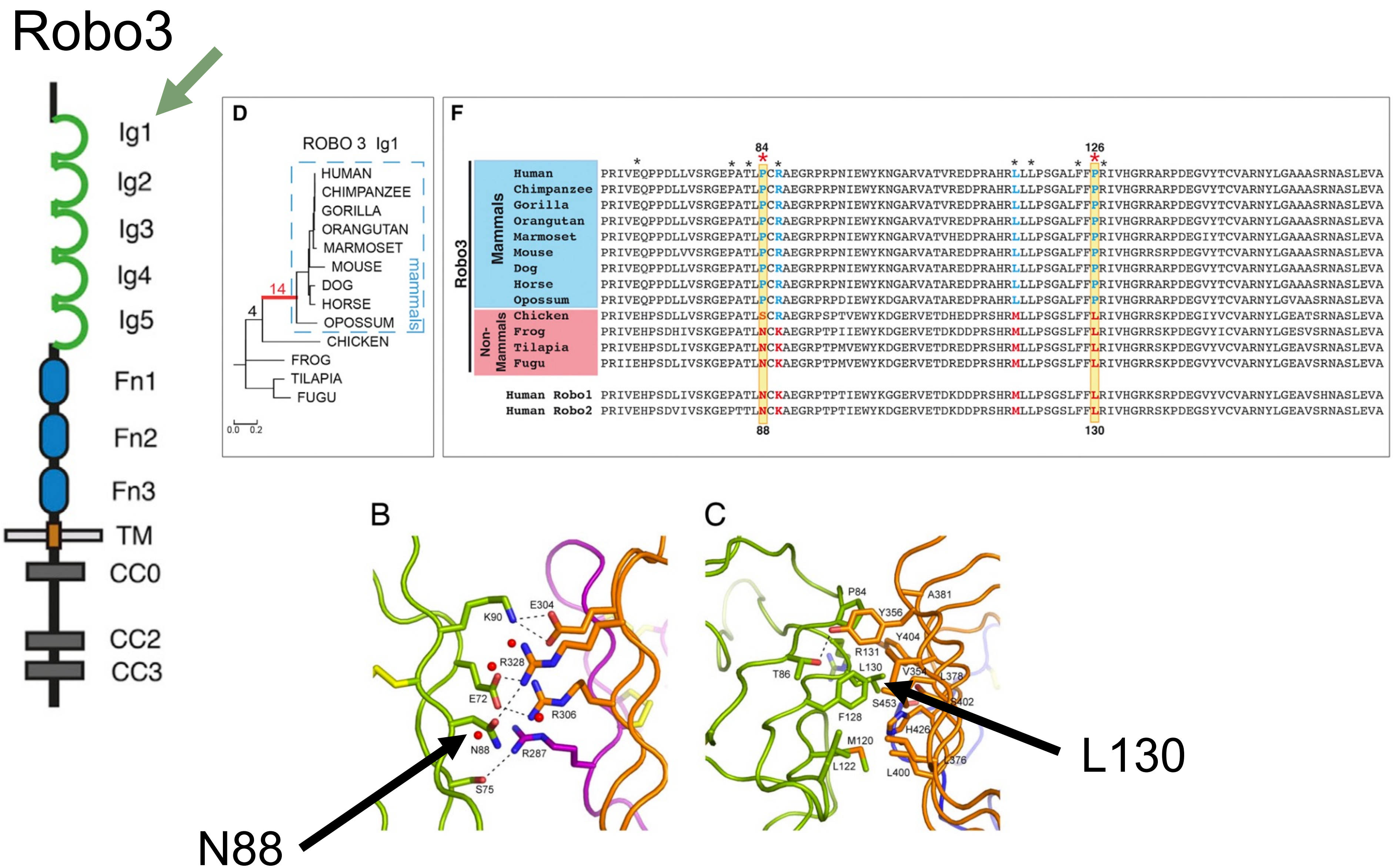


Figure 1

Mammalian-specific structure of Robo3 Ig1 domain

Zelina et al., 2014

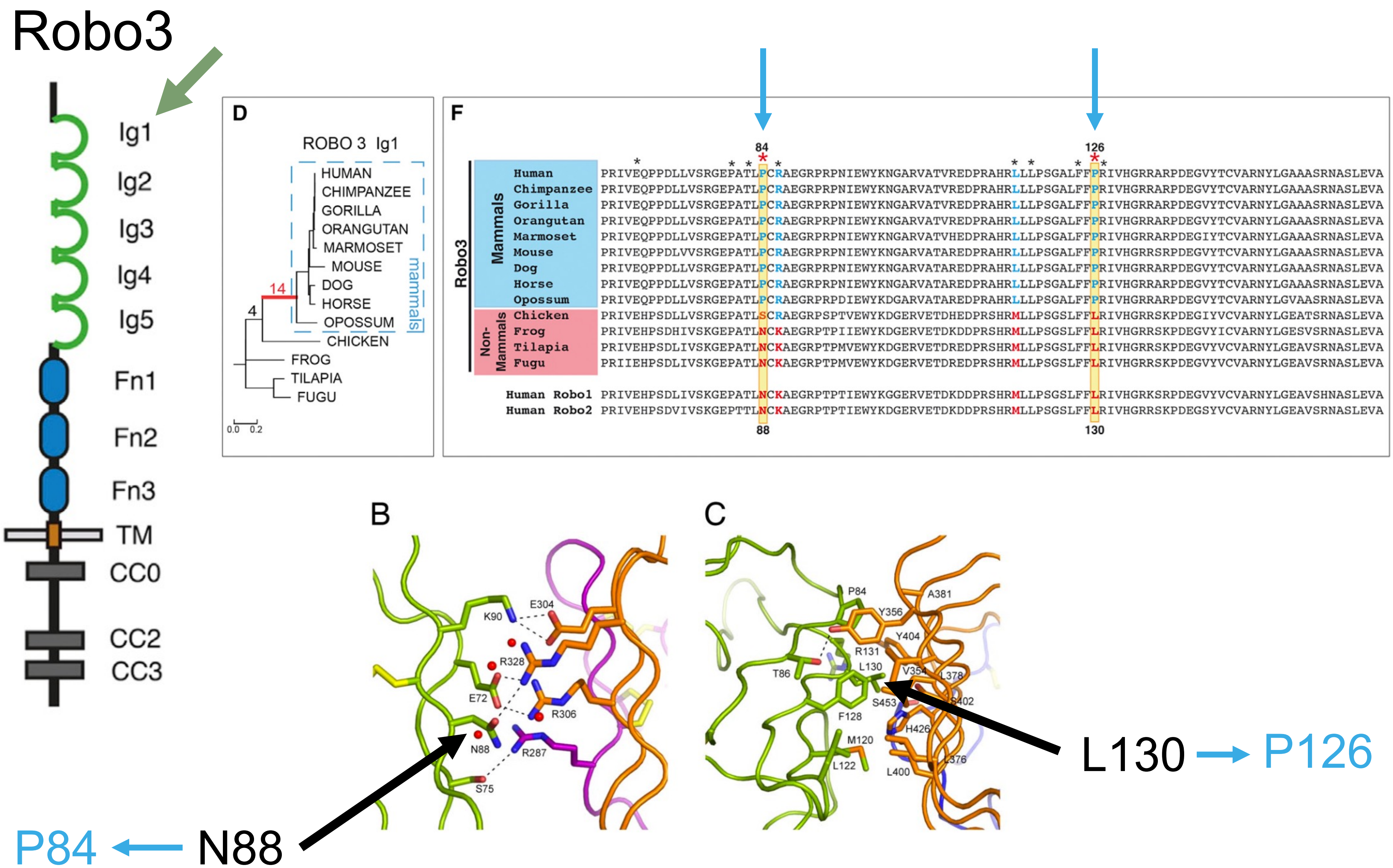


Figure 1

Mammalian Robo3 does NOT bind Slit

Zelina et al., 2014

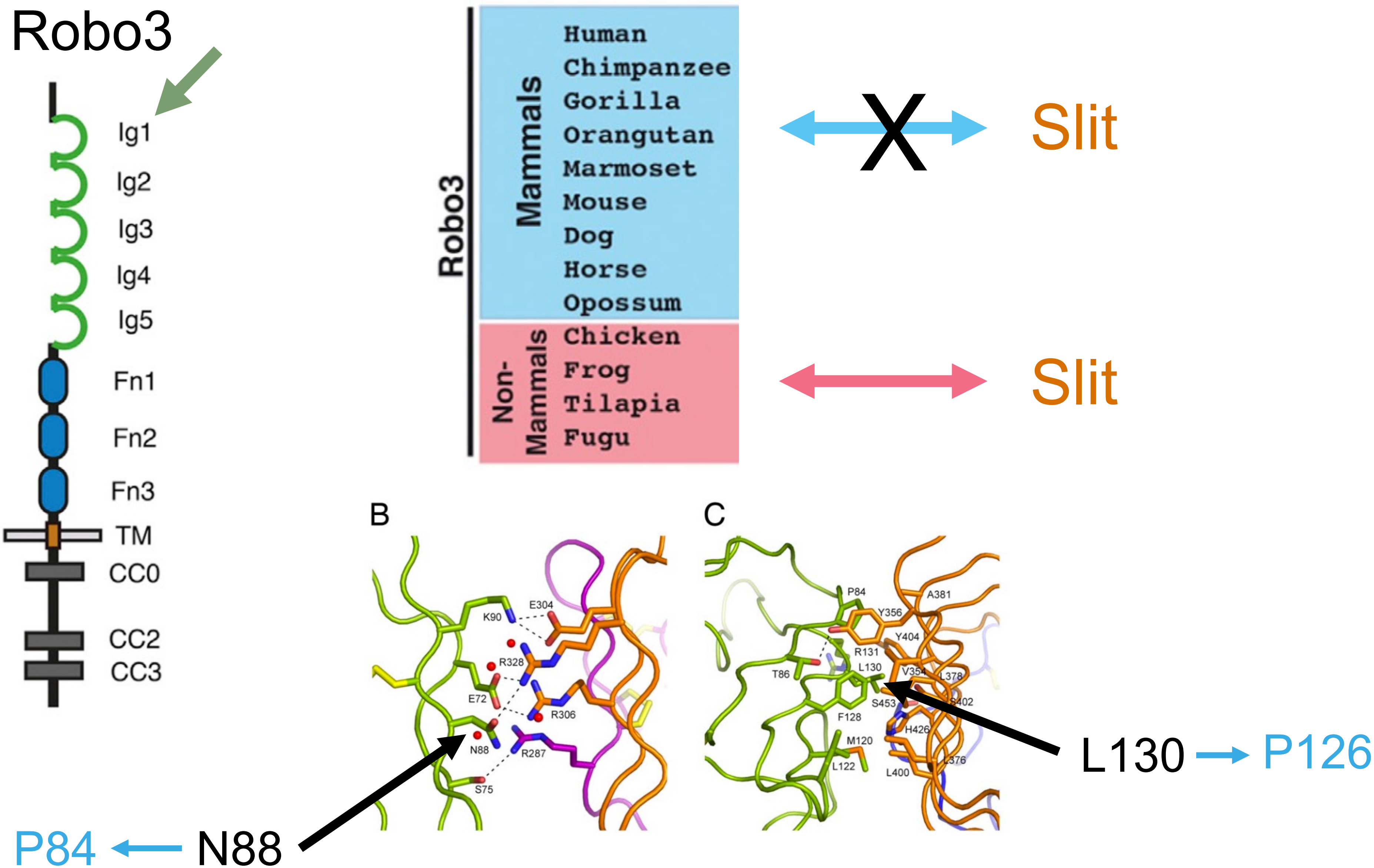


Figure 1

CellPress

Neuron

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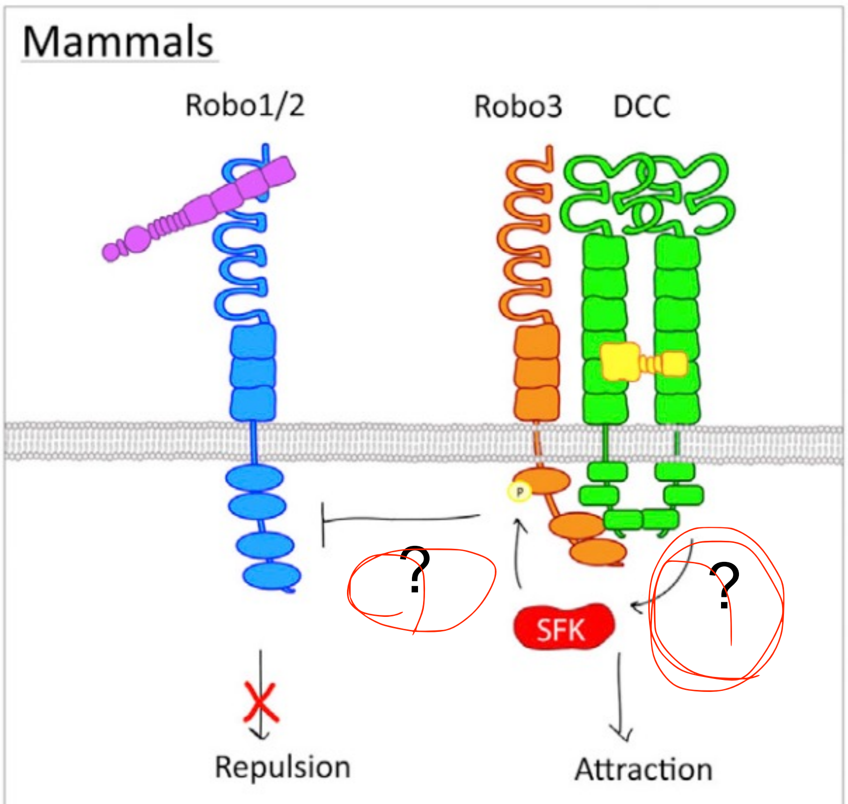

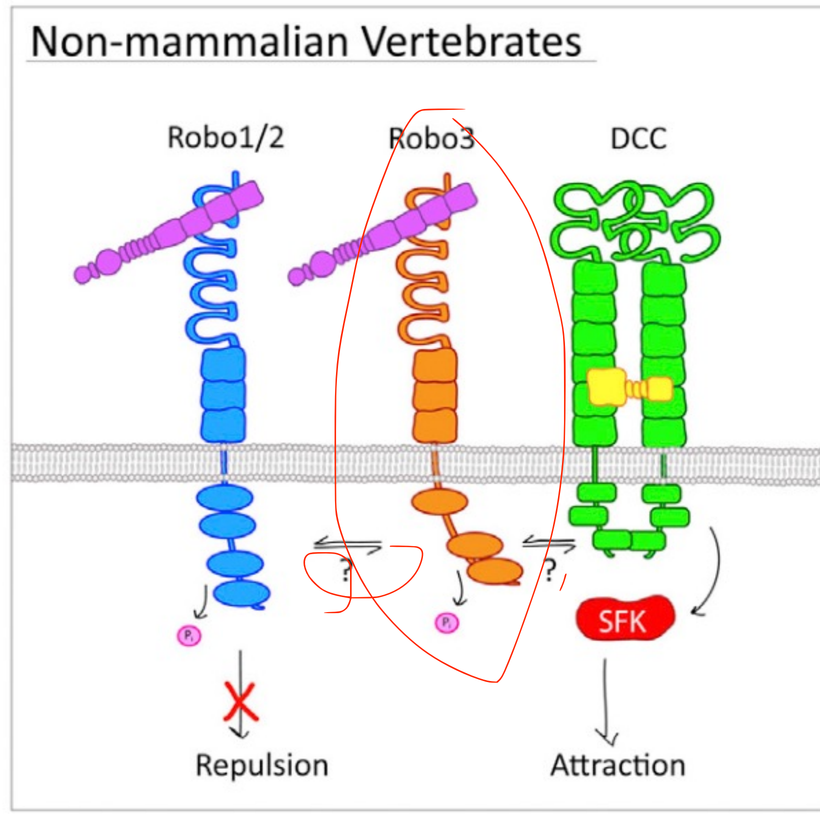

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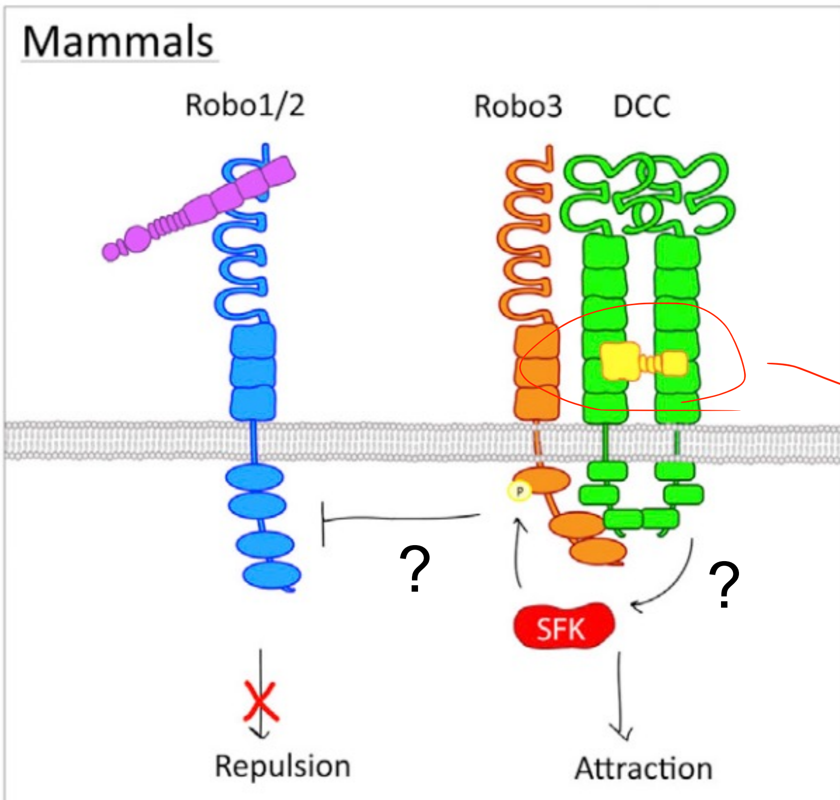


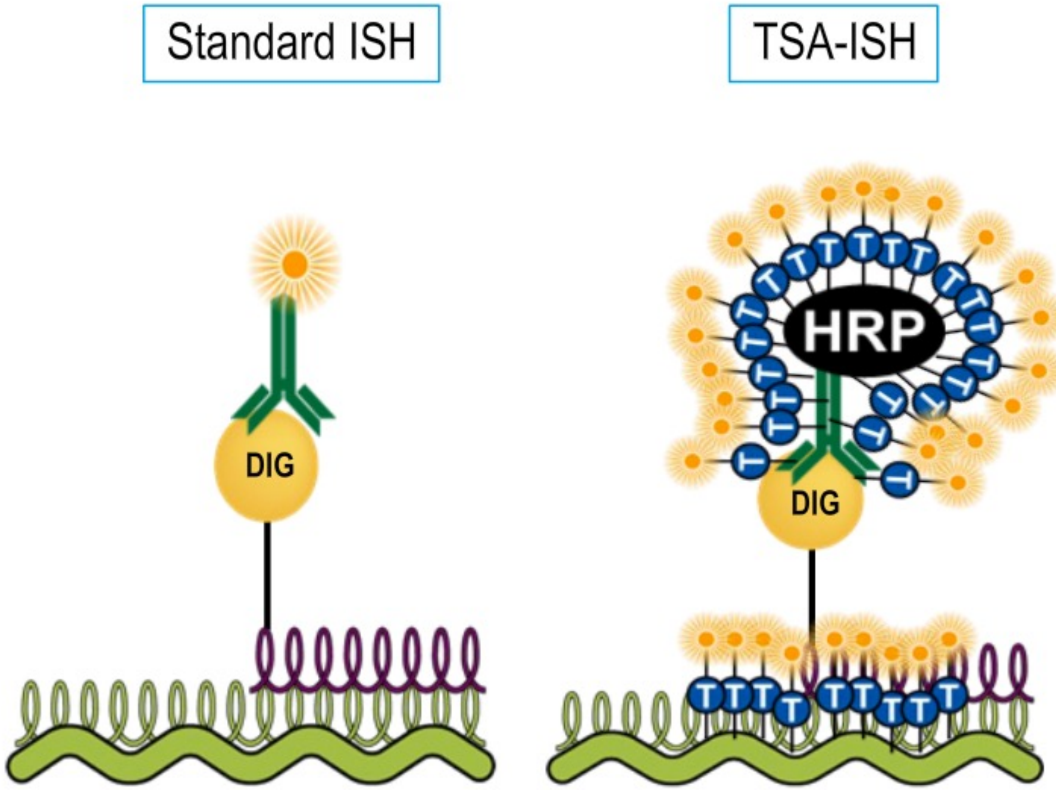
SFK = Src-Family Kinase

QUESTIONS

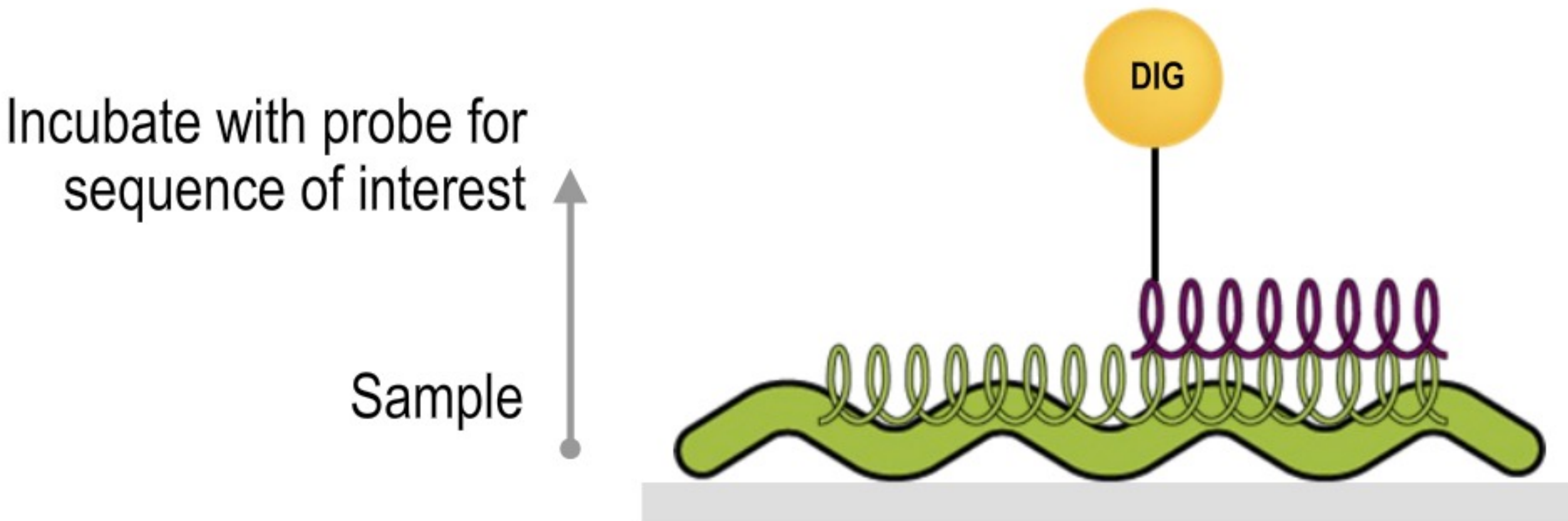
- How does Robo3 antagonize Robo1/2 response to slit?
- How does Robo3.2 enhance repulsion on contralateral side? (since Robo3.2 does not bind slit)
- Netrin/DCC →? Robo3

Beamish and Kennedy, Dev Cell (2014)

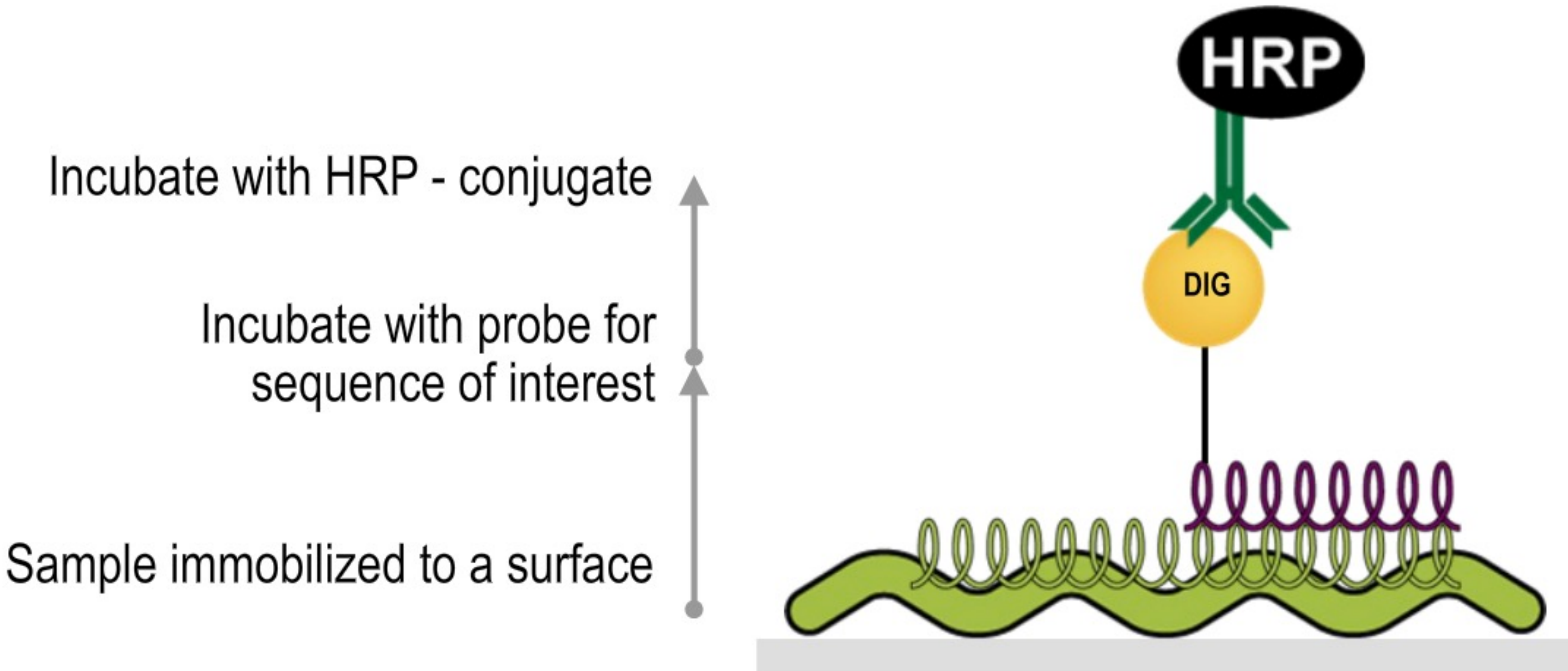
TSA *in situ* hybridization assay



https://www.youtube.com/watch?v=wMcYNN9_4HQ


DIG
Incubate with probe for sequence of interest
Sample

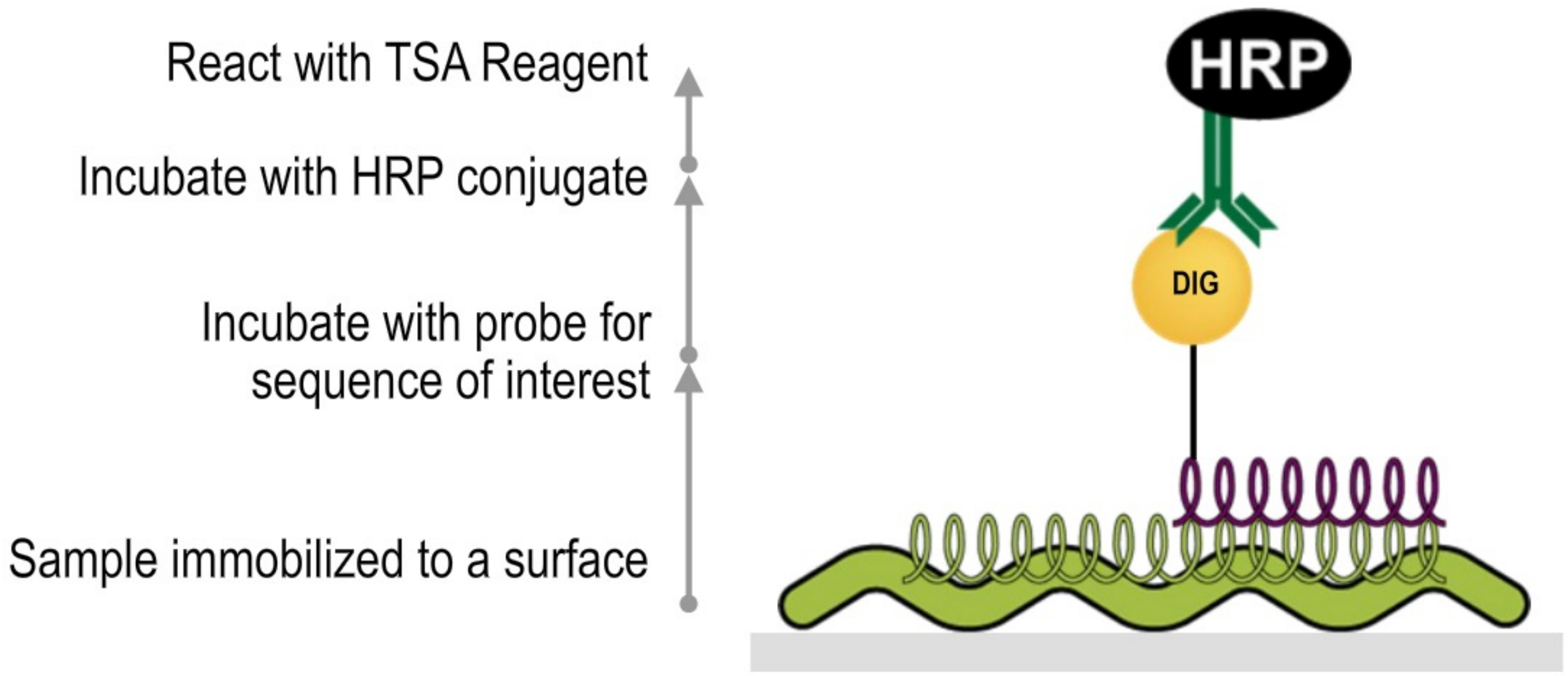
Horseradish peroxidase (HRP) is required for TSA.



Introduction of TSA reagent

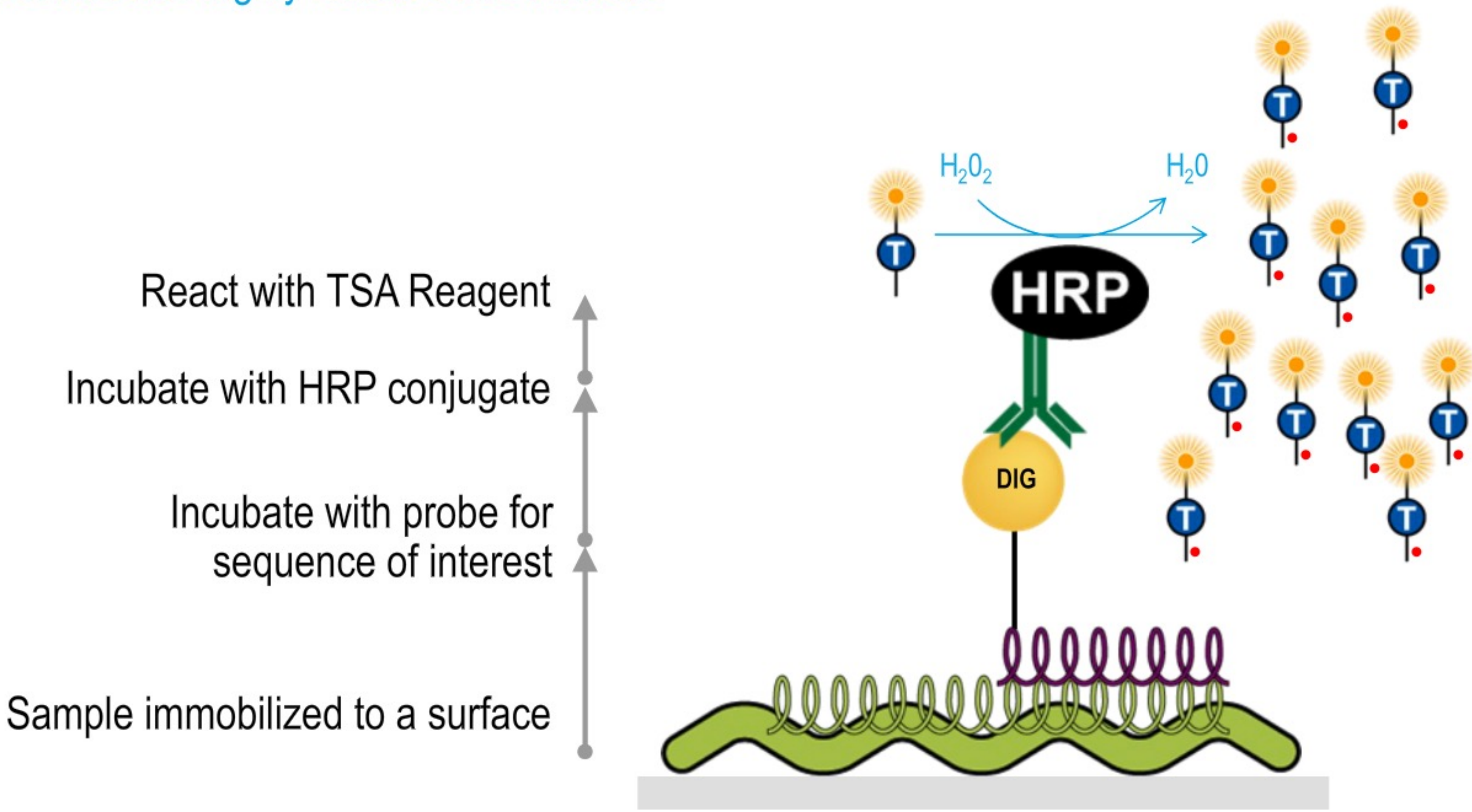
TSA reagent may be labeled with:

- Fluorophores for direct fluorescence detection
- Haptens for chromogenic or indirect fluorescence detection
- Use **TSA Plus** for maximum sensitivity



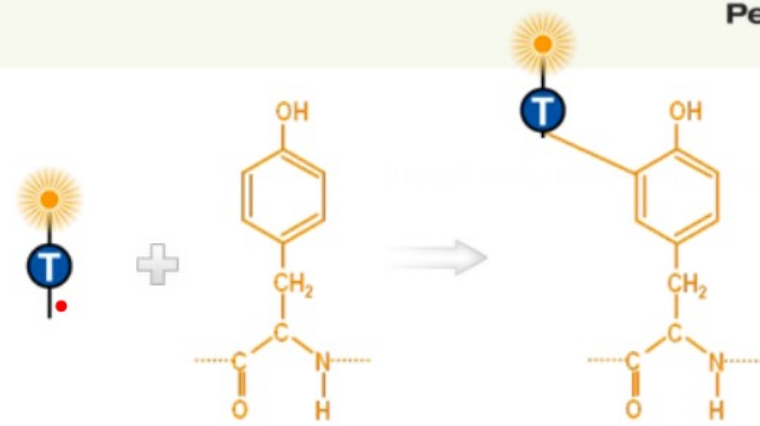
Introduction of TSA reagent

HRP catalyzes conversion of tyramide molecules into highly reactive free radicals



Increasing Signal…

Free radicals form covalent bonds with tyrosine in adjacent proteins



Signal amplification

React with TSA Reagent

Incubate with HRP conjugate

Incubate with probe for sequence of interest

Sample immobilized to a surface