

C. elegans L1 cell adhesion molecule functions in axon guidance



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Developmental Biology Center

C. elegans embryogenesis



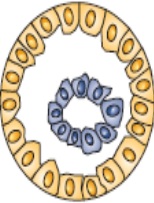
Goldstein lab, UNC-Chapel Hill

CELL ADHESION

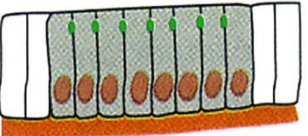
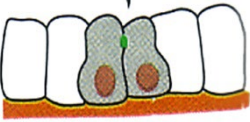
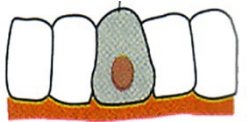
tissue boundaries



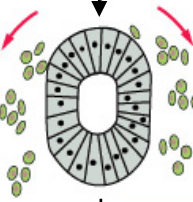
cell sorting



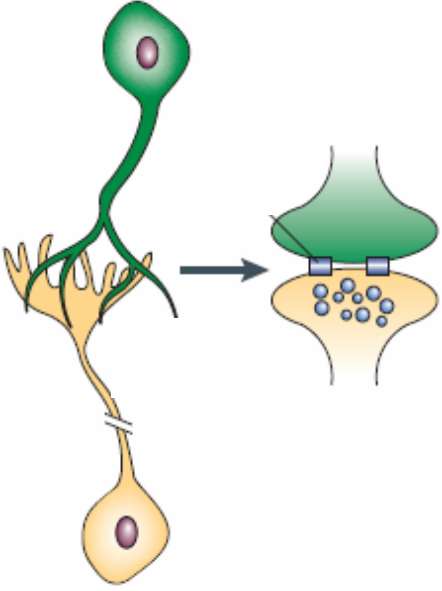
epithelial adherens junctions



cell migration



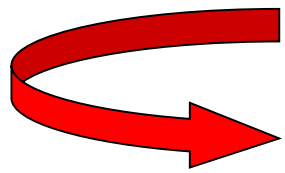
axon guidance
synapse targeting & adherens



Adapted from Molecular Biology of the Cell, Alberts et al.; Gumbiner et al., 2005, Nature review Mol Cell Bio.

IMPAIRED CELL ADHESION

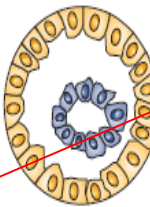
DEVELOPMENTAL DISORDERS



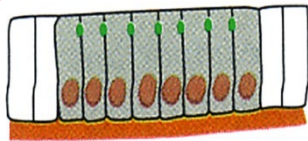
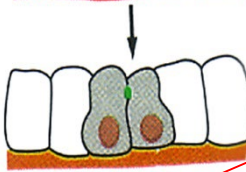
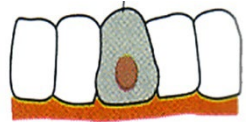
tissue boundaries



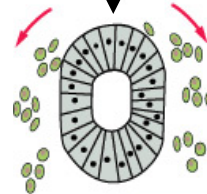
cell sorting



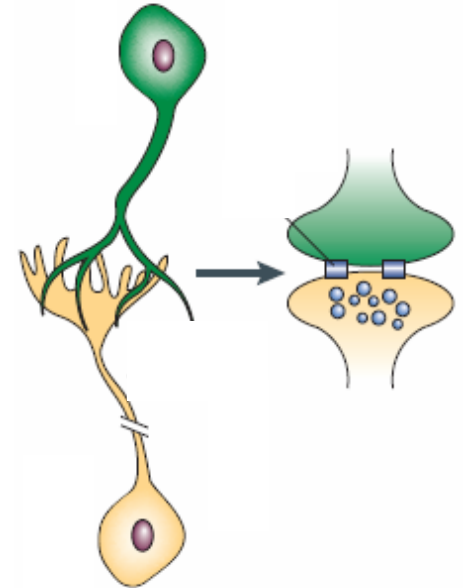
epithelial adherens junctions



cell migration



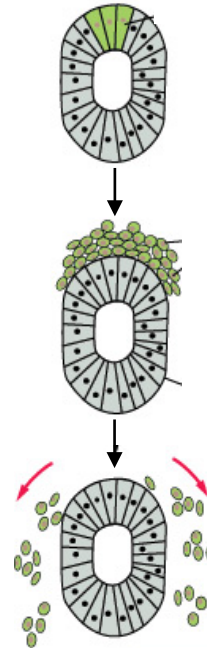
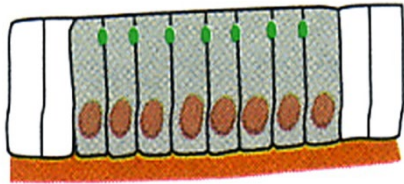
axon guidance
synapse targeting & adherens



IMPAIRED CELL ADHESION

Loss of cell adhesion
Cell delamination

→ inappropriate cell migration → METASTASIS

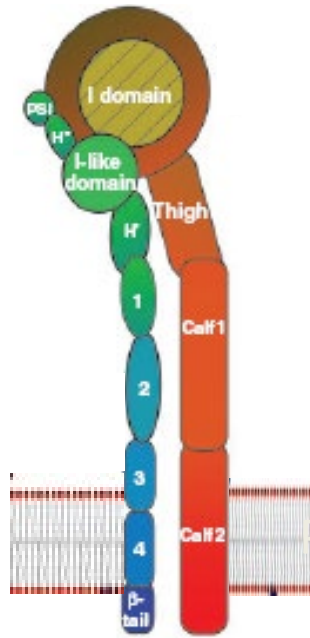


Cell Adhesion Receptors

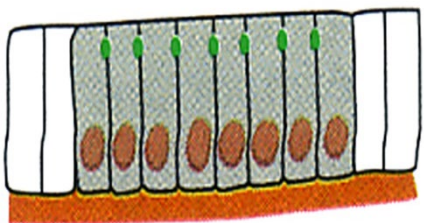
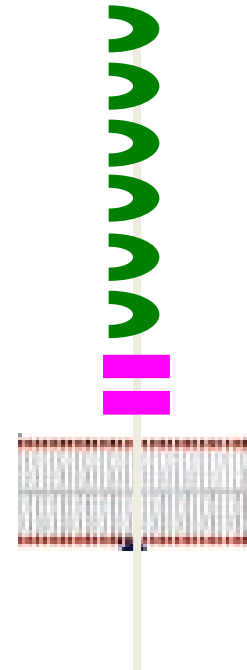
CADHERIN



INTEGRIN



IgCAM

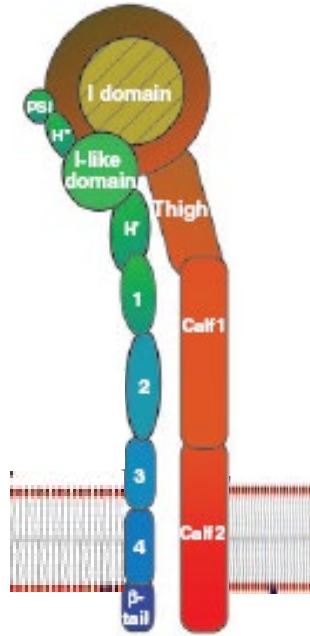


Cell Adhesion Receptors

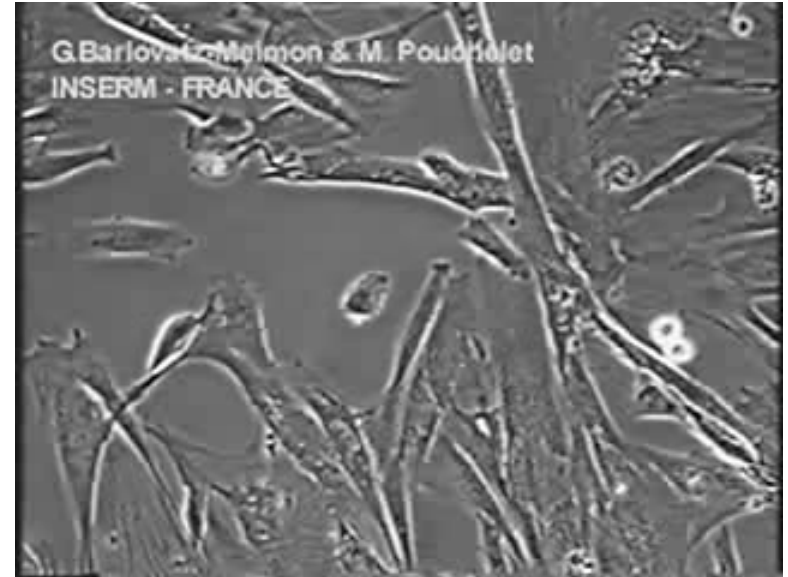
CADHERIN



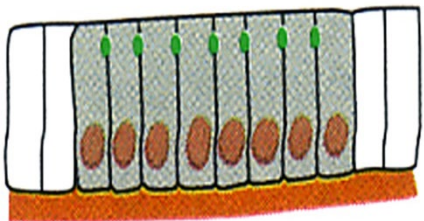
INTEGRIN



Barlovatz Meimon & Pouchelet, INSERM, FRANCE



migrating cultured muscle cells

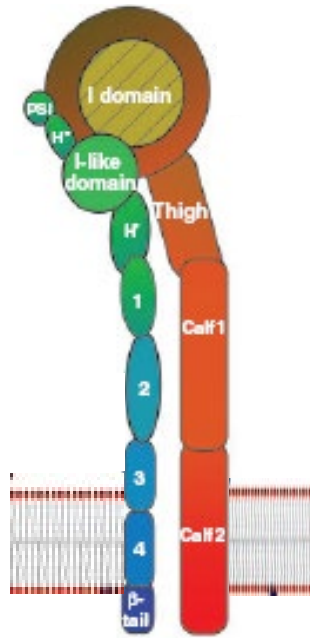


Cell Adhesion Receptors

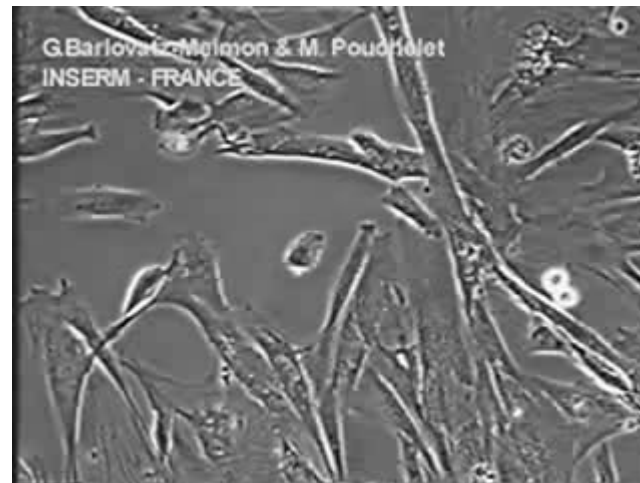
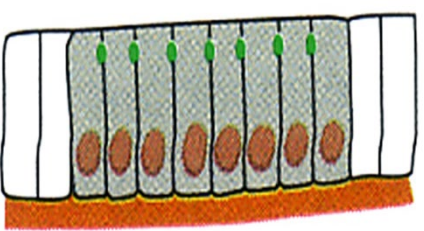
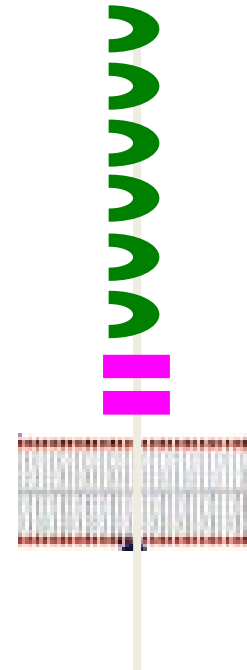
CADHERIN



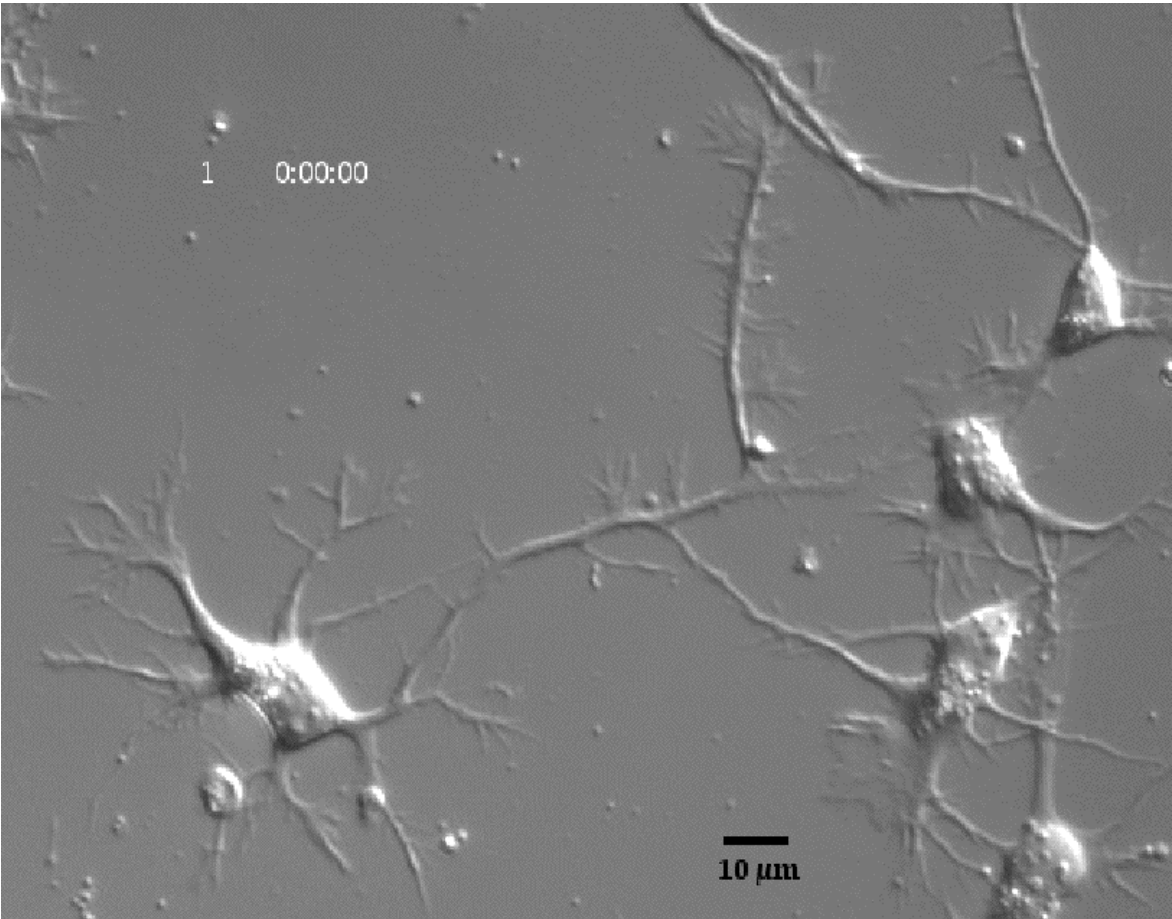
INTEGRIN



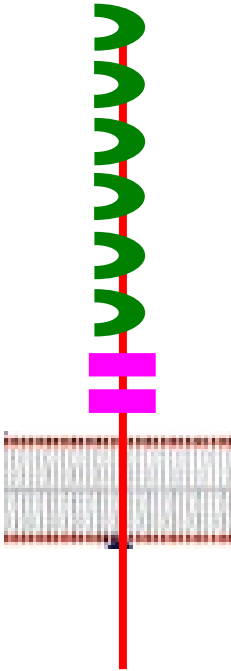
IgCAM



Cultured rat hippocampal neurons



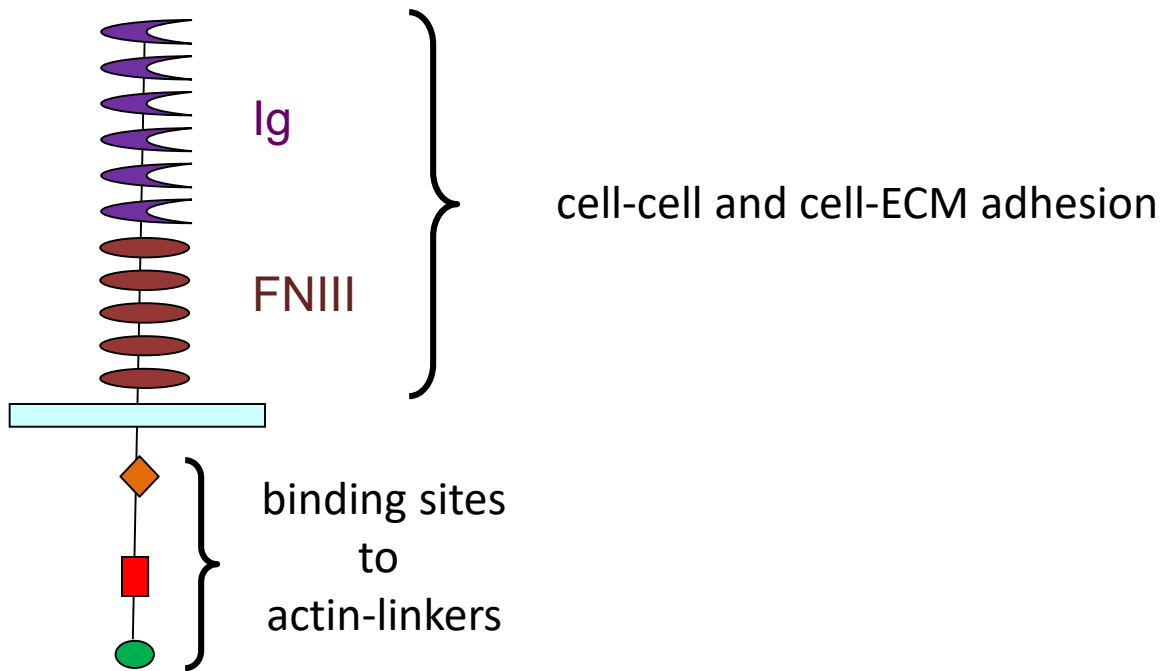
IgCAM



Dr. Lorene Lanier
UMN Neuroscience

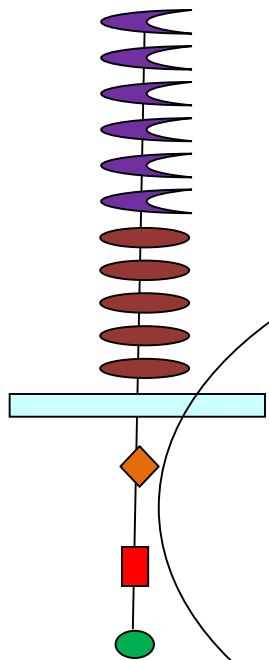
mammalian L1CAMs

L1 NrCAM CHL1 neurofascin



Neuronal role for mammalian L1CAMs

L1 NrCAM CHL1 neurofascin



L1 syndrome

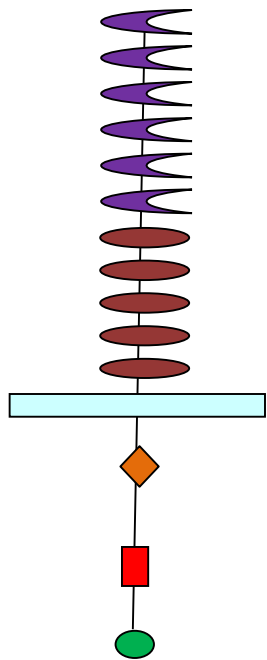
Corpus callosum hypoplasia
Retardation/intellectual disability
Adducted thumbs
Spastic paraplegia
Hydrocephalus



Fransen et al., 1994. Hum. Mol. Gen.
Vits et al., 1994. Nat. Genetics

Neuronal role for mammalian L1CAMs

L1 NrCAM CHL1 neurofascin



autism
addiction

Sakurai et al., 2006. Psych. Genetics
Yoo et al., 2012. Psych. Invest.

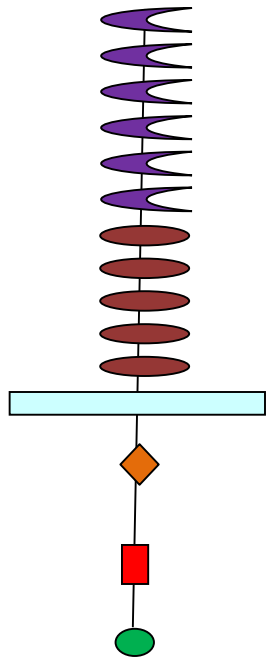
Corpus callosum hypoplasia
Retardation/intellectual disability
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Fransen et al., 1994. Hum. Mol. Gen.
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Neuronal role for mammalian L1CAMs

L1 NrCAM CHL1 neurofascin



schizophrenia

autism
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Chu & Liu, 2010. J. Human Genetics

Sakurai et al., 2006. Psych. Genetics
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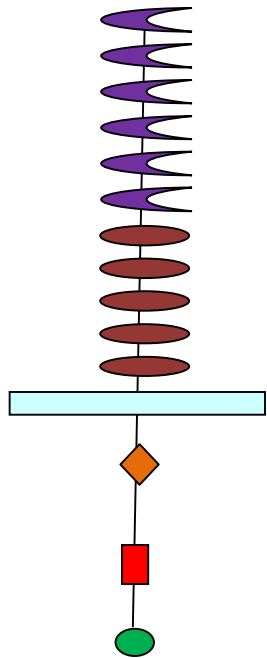
Corpus callosum hypoplasia
Retardation/intellectual disability
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Fransen et al., 1994. Hum. Mol. Gen.
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Neuronal role for mammalian L1CAMs

L1 NrCAM CHL1 neurofascin



autism
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schizophrenia

Chu & Liu, 2010. J. Human Genetics

Corpus callosum hypoplasia
Retardation/intellectual disability
Adducted thumbs
Spastic paraplegia
Hydrocephalus

Neurodevelopment

neurite outgrowth
axon guidance
Myelination



1. External embryonic development
2. Large embryos
3. Amenable to manipulation
4. Early chick embryonic structures are very similar to those of the human embryo.



1. Mammal
2. Reverse genetics



1. External embryonic development
2. Large number of transparent embryos
3. generation time ~3 months but quick embryonic development



1. Genetically accessible
2. Short generation time
~ 2 weeks, 3.5 days
3. Small genomes
4. Small size



Caenorhabditis elegans

Nobel Prize in Physiology or Medicine 2002

for their discoveries concerning “genetic regulation of organ development and programmed cell death



Invariant cell lineage: 302 neurons
Transparent



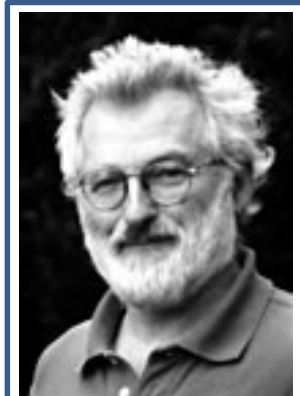
**Sydney
Brenner**



How does the nervous system
develop?
function?
control behavior?



**Robert
Horvitz**

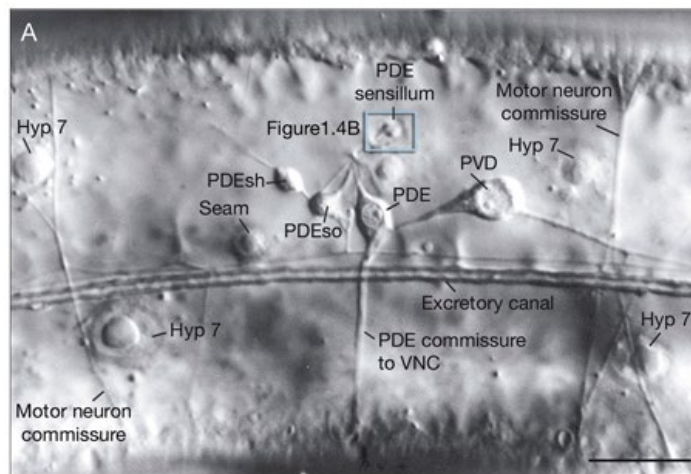
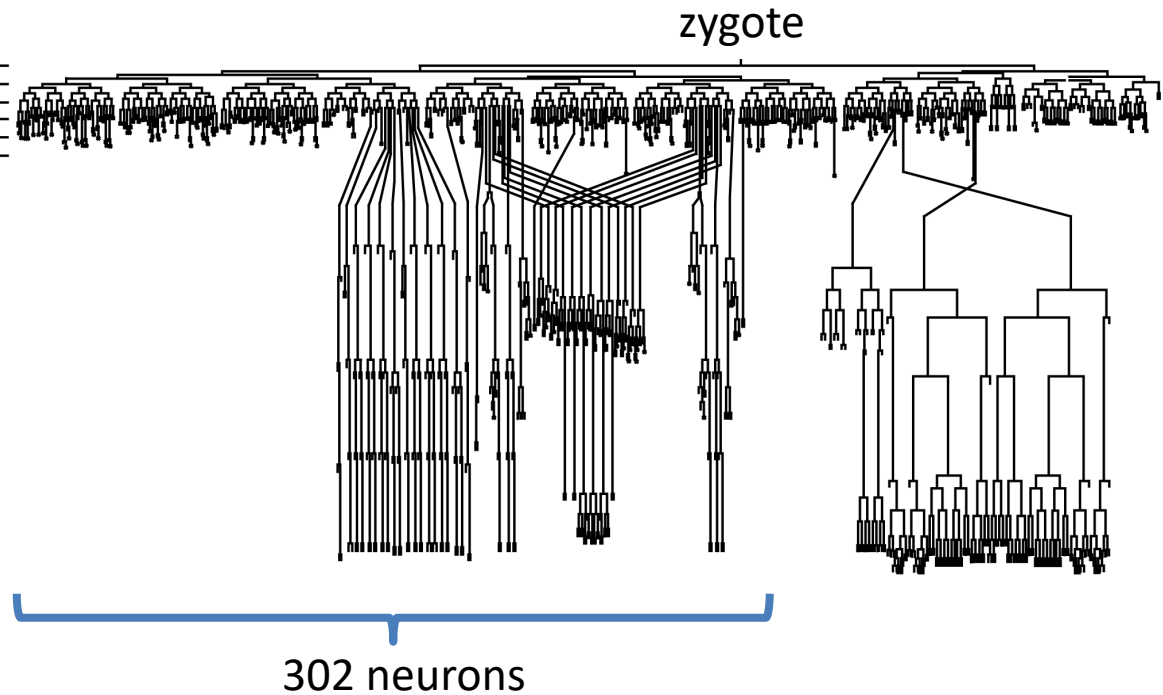


**John
Sulston**



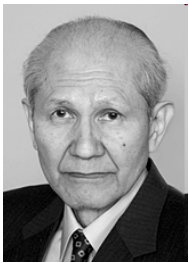
Identified all cells

Invariant cell lineage of 959 somatic cells

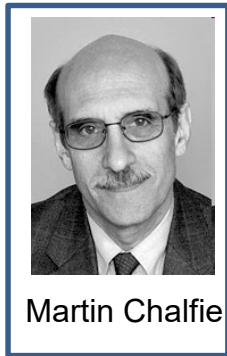


1994: 1st showing of a GFP reporter in a multicellular organism

2008 recipients of the Nobel Prize in Chemistry
for the discovery and development of GFP



Osamu
Shimomura



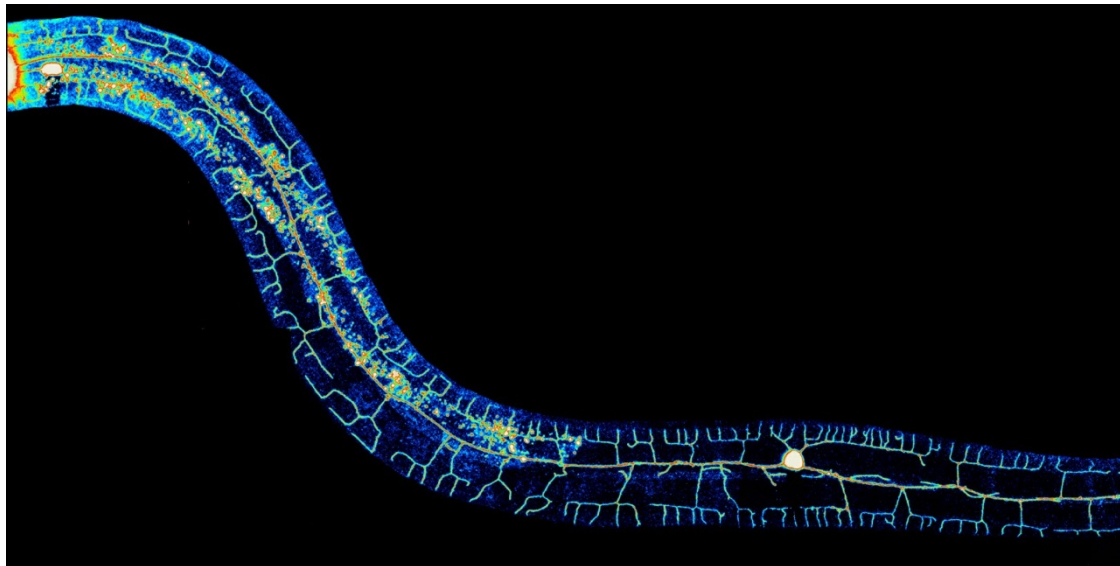
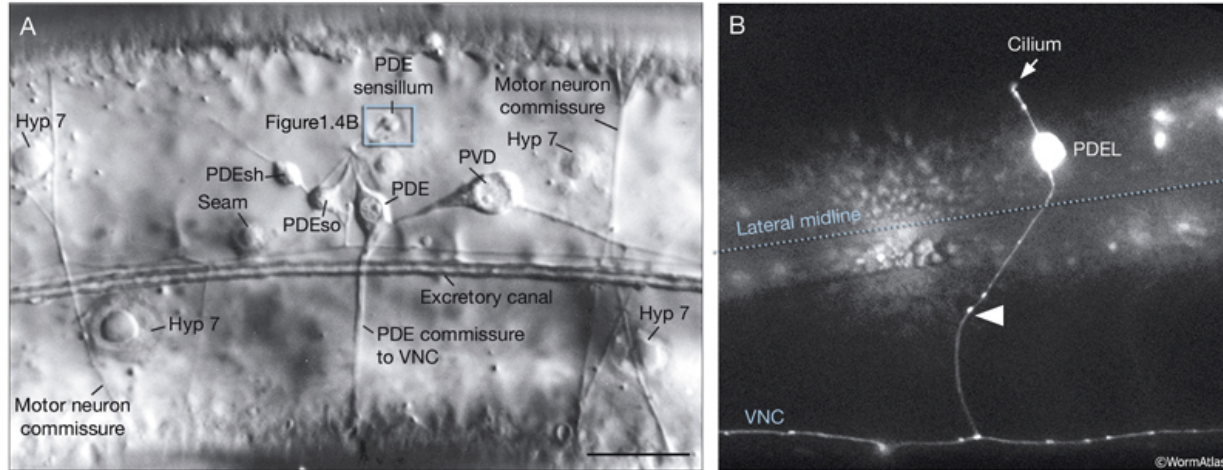
Martin Chalfie



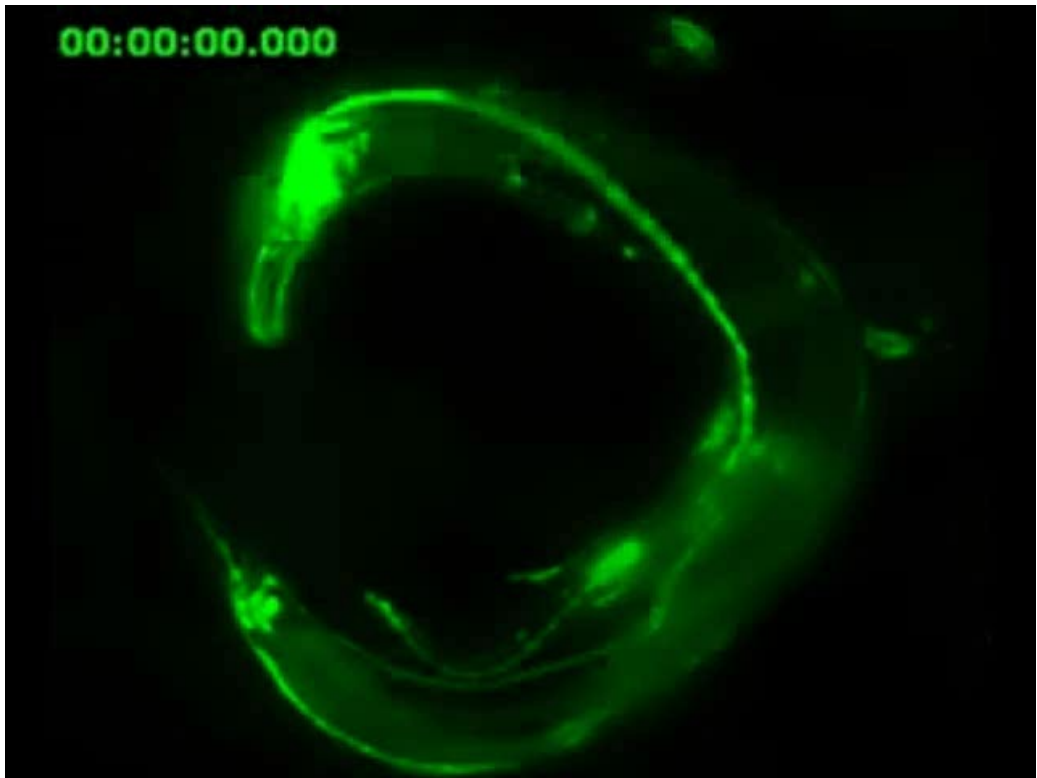
Roger Y. Tsien



Importance of fluorescence proteins in *C. elegans* research



00:00:00.000

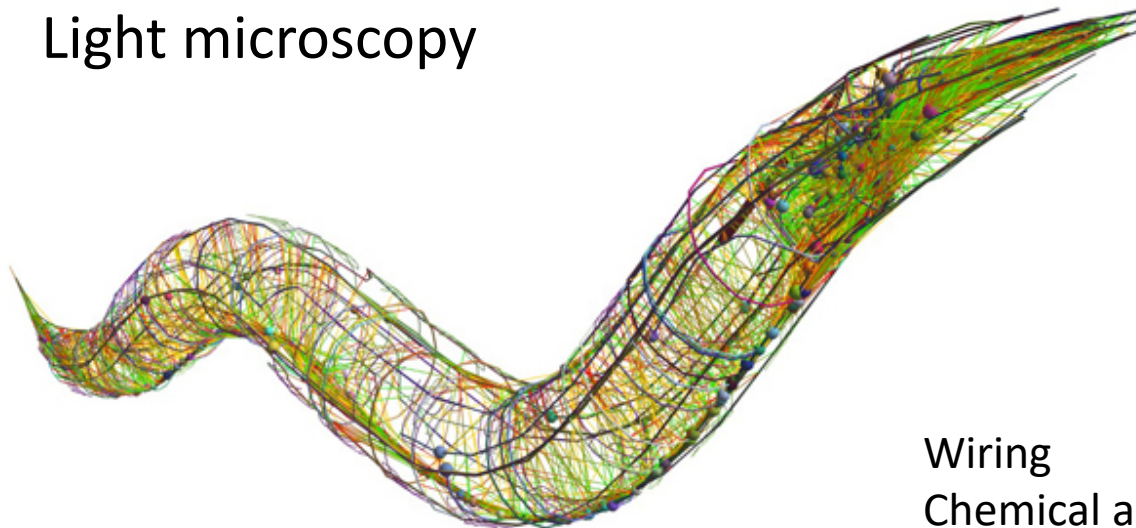


Advantages of *C. elegans* as model to study nervous system development & function

- Invariant cell lineage 302 neurons
- Transparent
- Neural circuitry completely mapped:

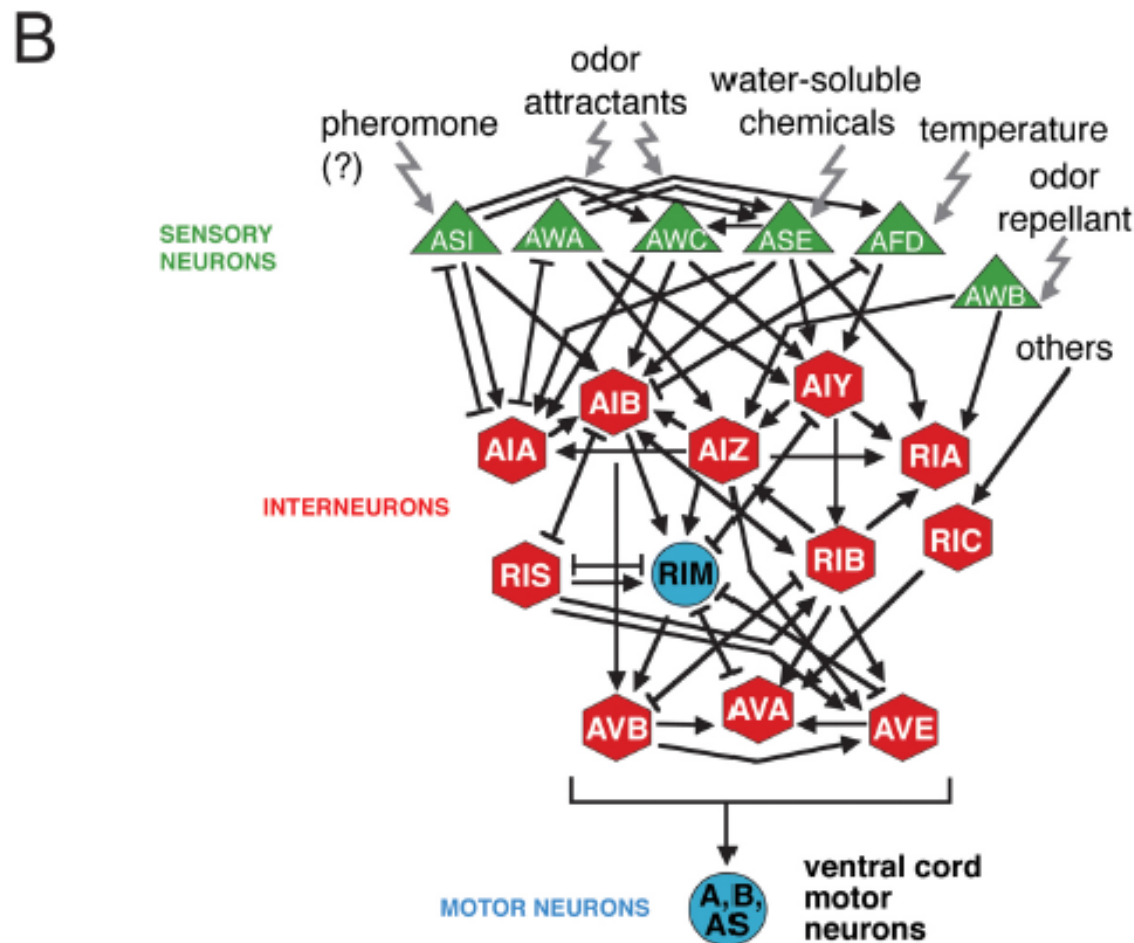
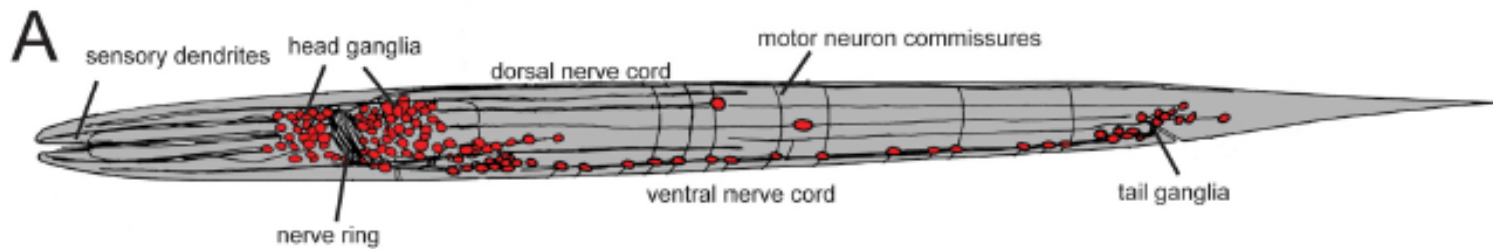
serial electron micrographs

Light microscopy



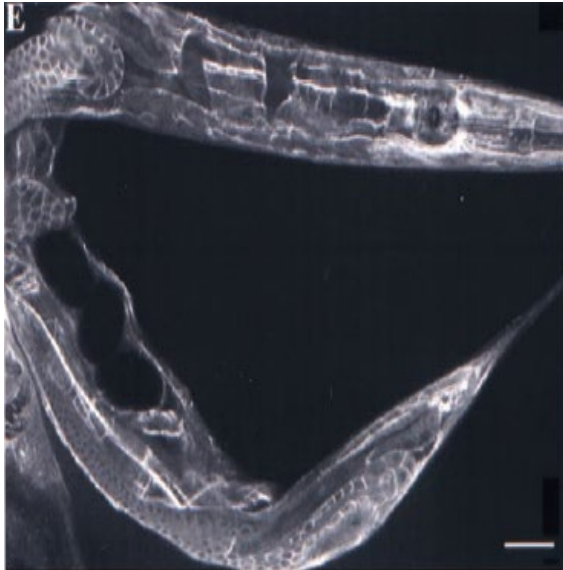
Wiring

Chemical and electrical synapses

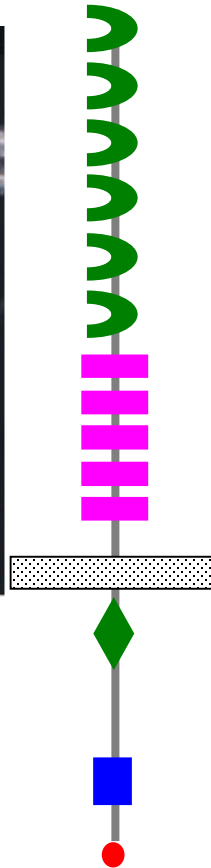


C. elegans L1CAMs

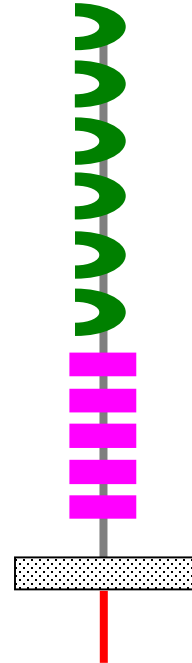
expressed ubiquitously



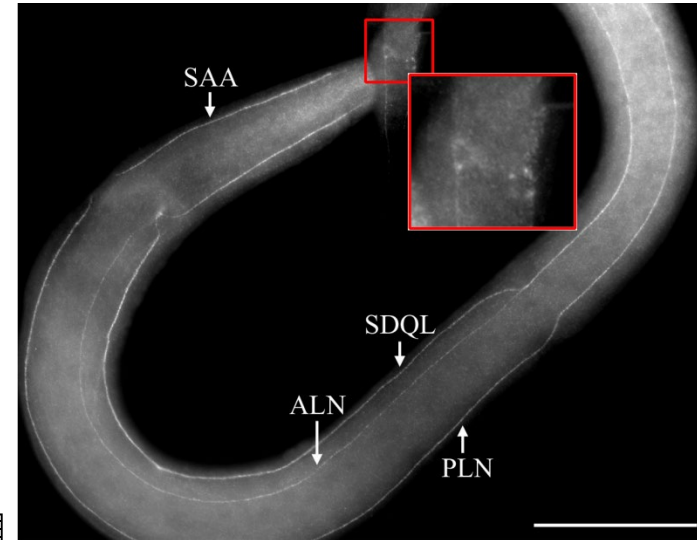
SAX-7



LAD-2



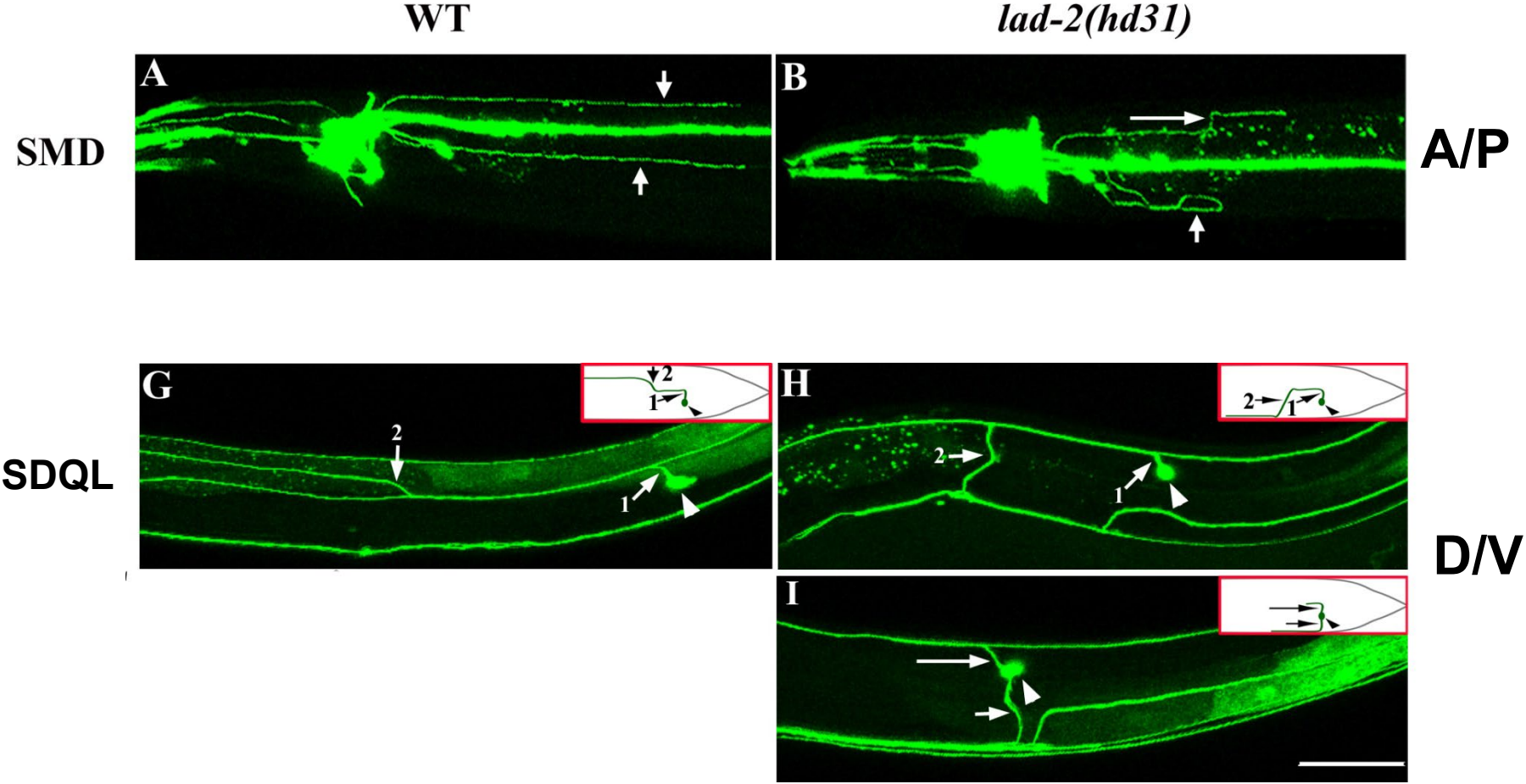
expressed in 14 neurons



Chen et al., 2001. J. Cell Biol.

Wang et al., 2008. J. Cell Biol.

lad-2 animals show axon guidance defects



Which axon guidance pathway does
LAD-2 mediate?

Netrins

Slits

Semaphorins

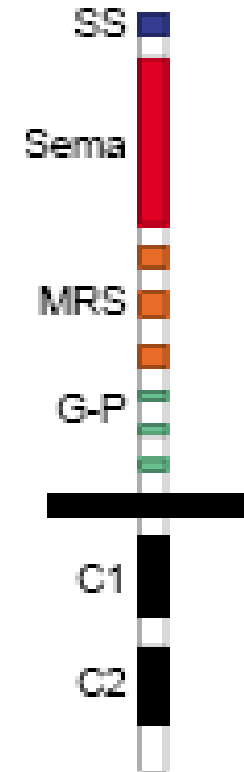
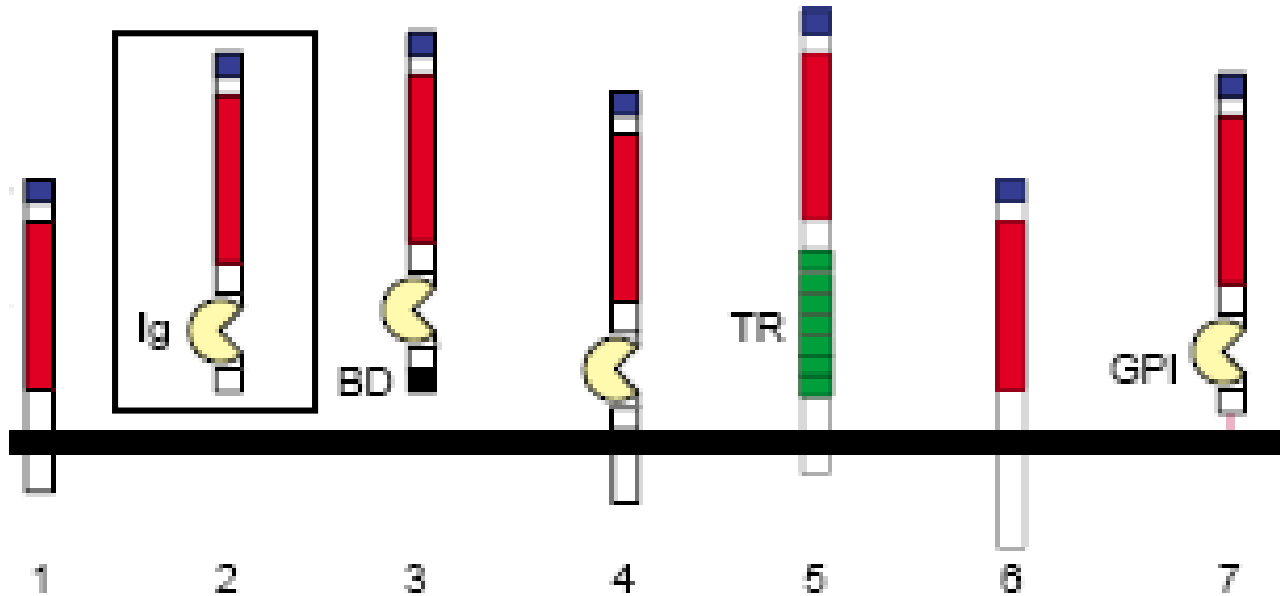
Ephrins

Semaphorin family and Receptor

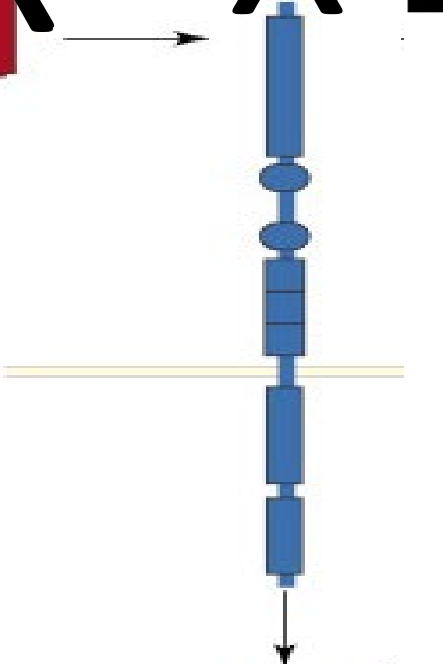
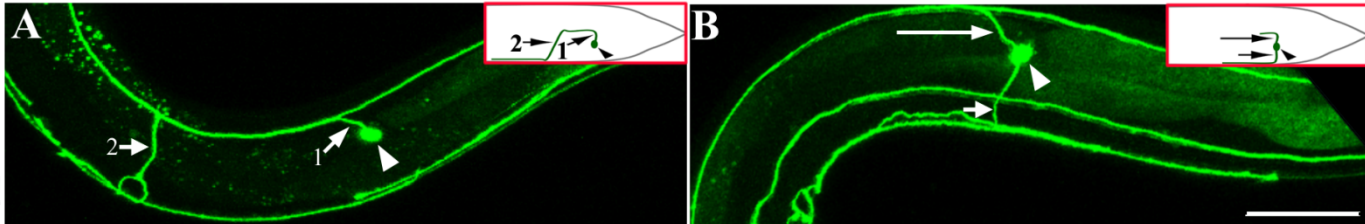
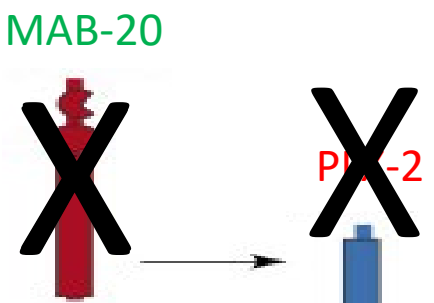
PLX-2

PLEXIN RECEPTOR

MAB-20

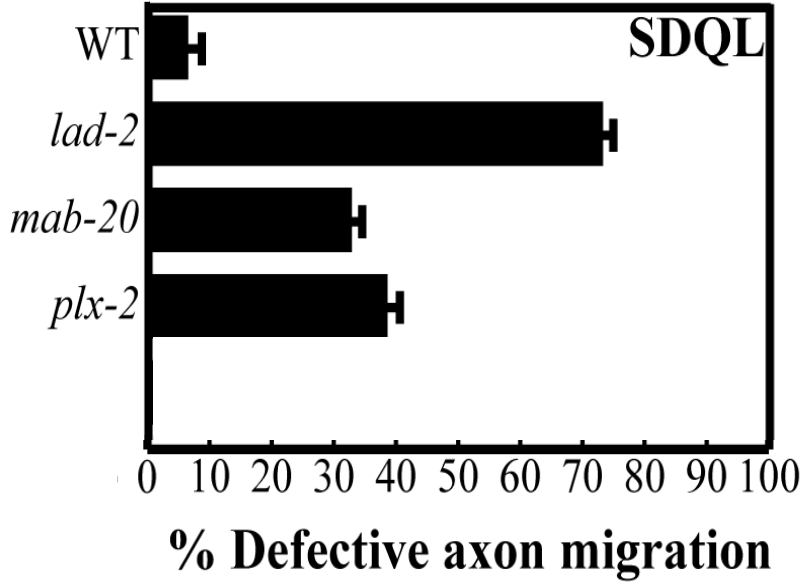


mab-20/Sema2 & *plx-2* have axon guidance roles

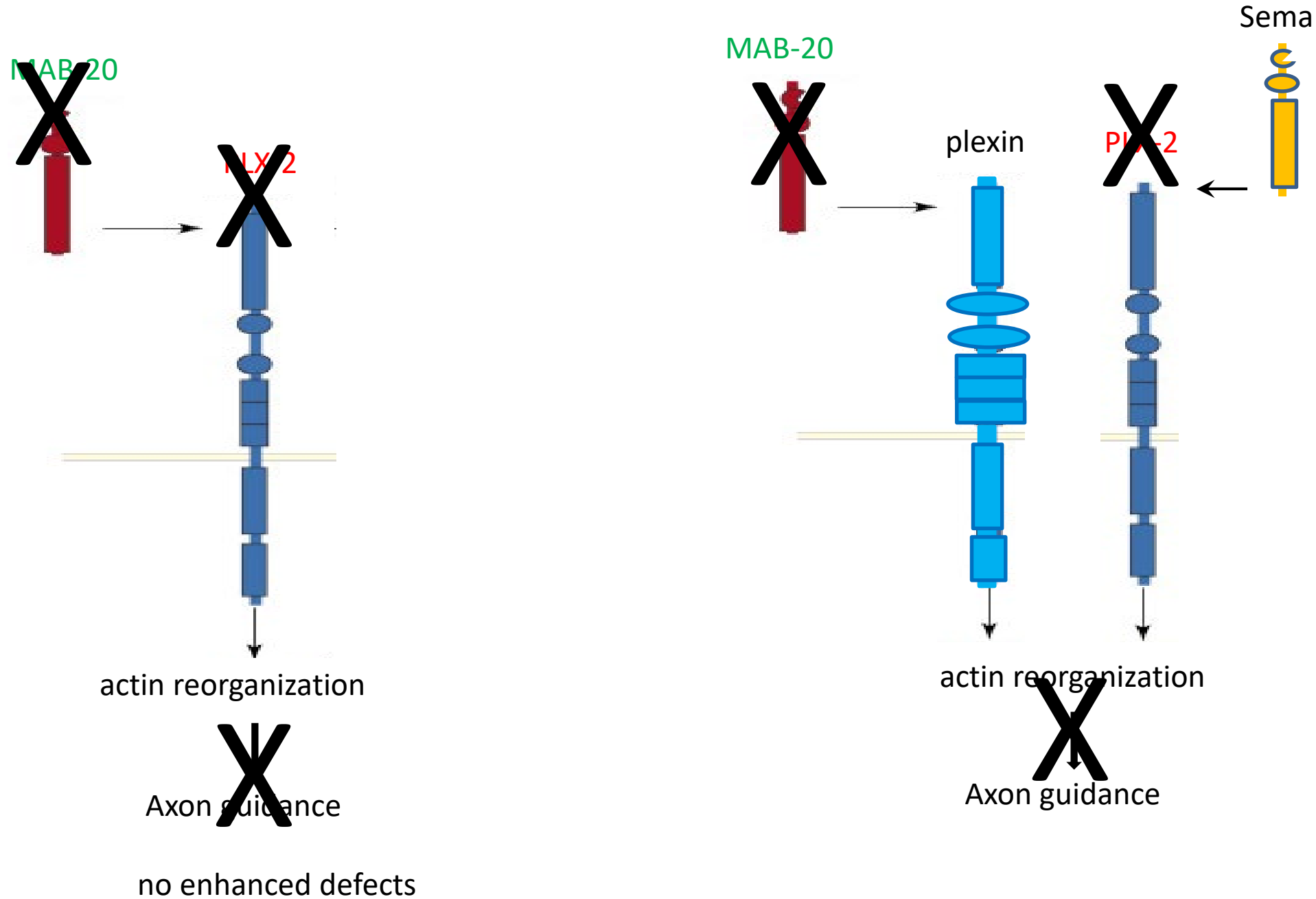


actin reorganization

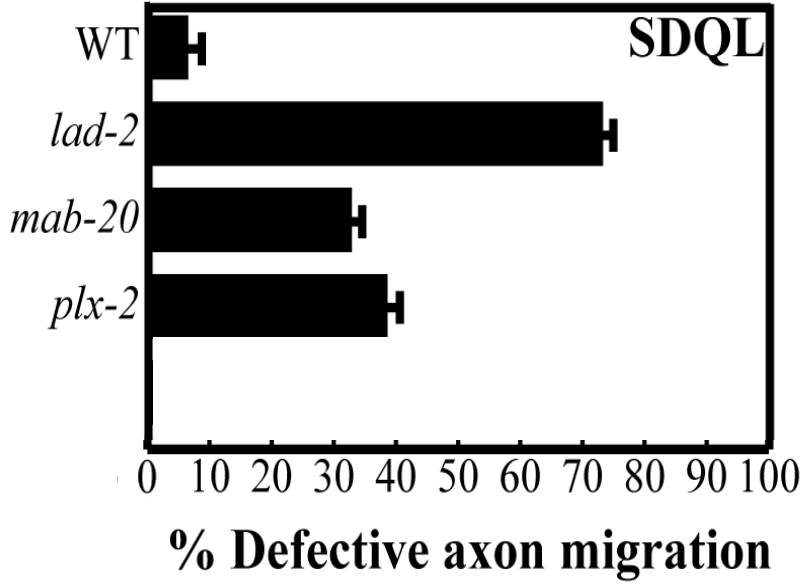
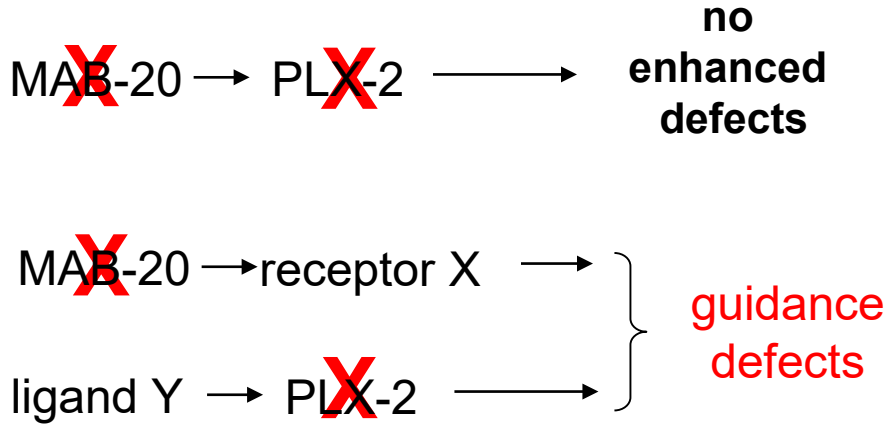
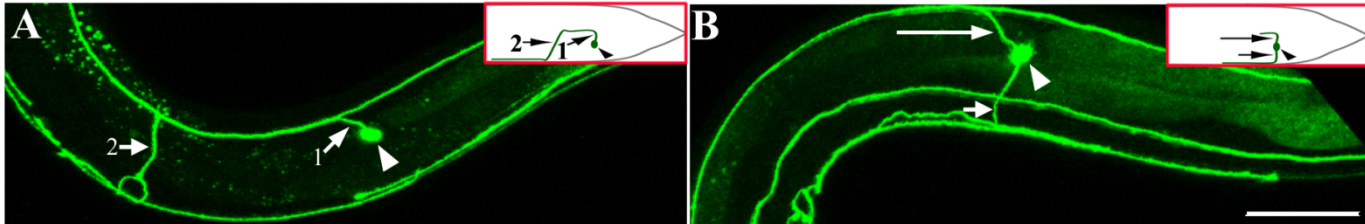
~~Axon guidance~~



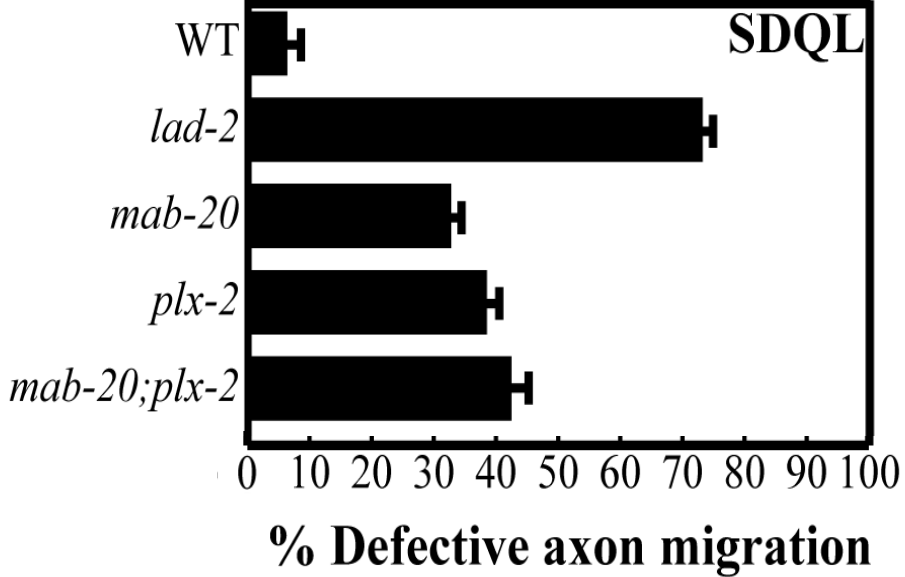
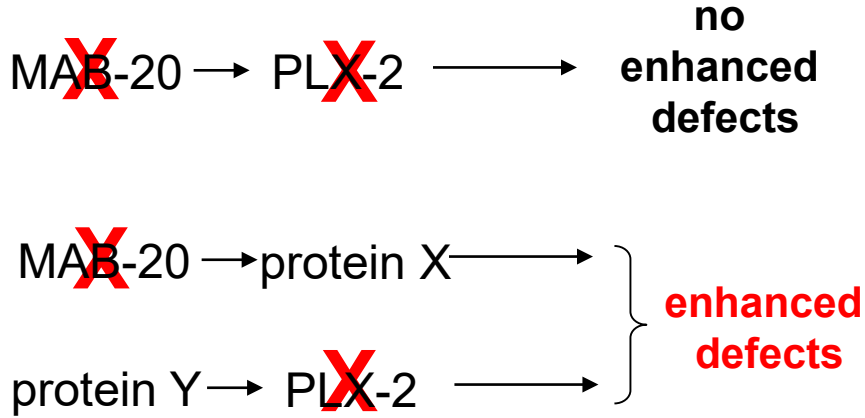
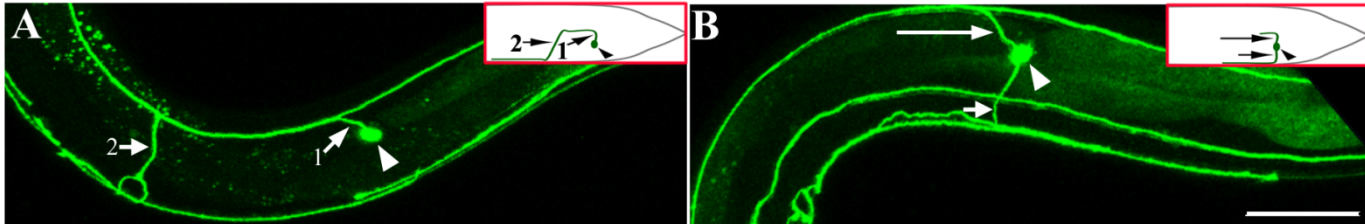
Possible semaphorin pathways



mab-20/Sema2 & *plx-2* have axon guidance roles

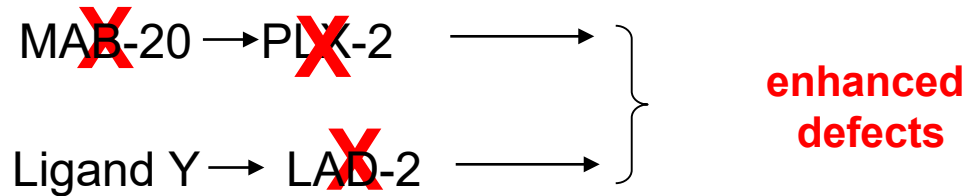


mab-20/Sema2 & *plx-2* have axon guidance roles

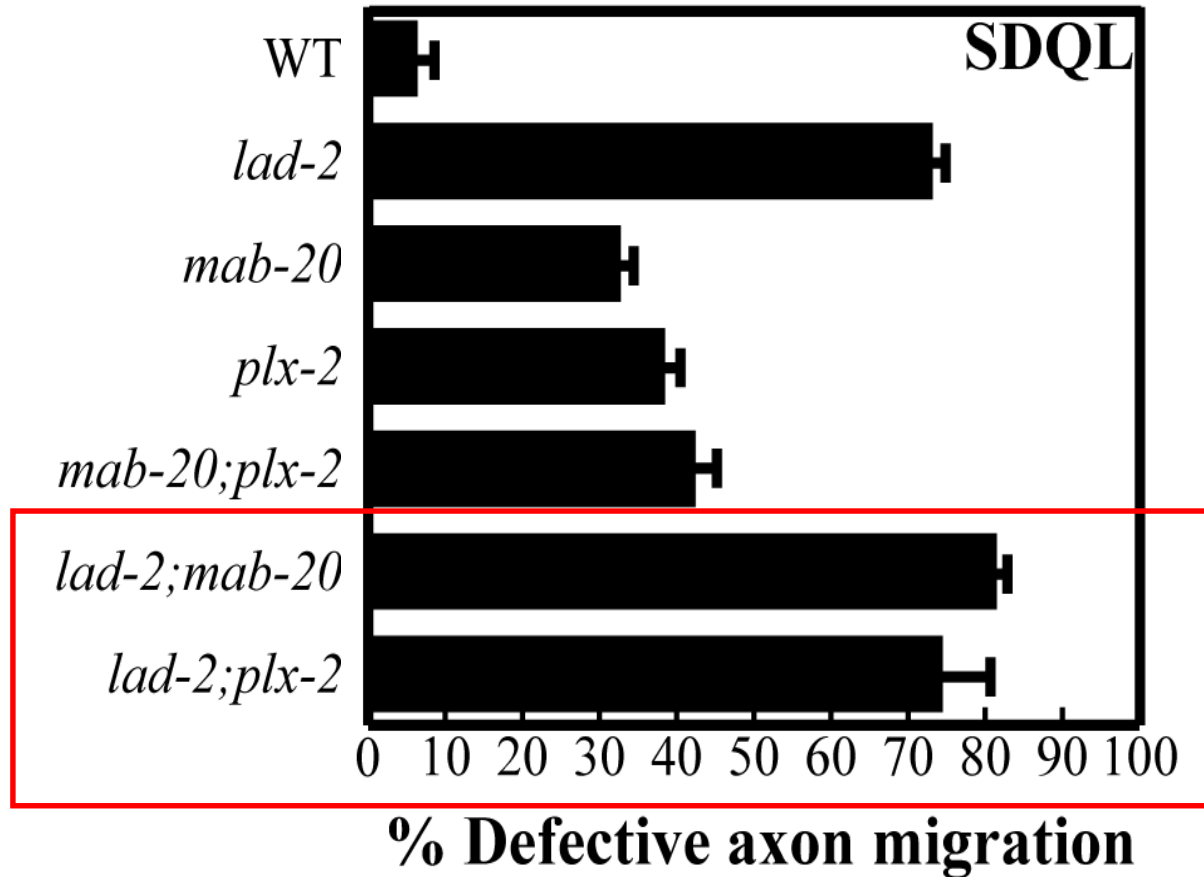


Does LAD-2 mediate axon guidance via MAB-20/Sema2?

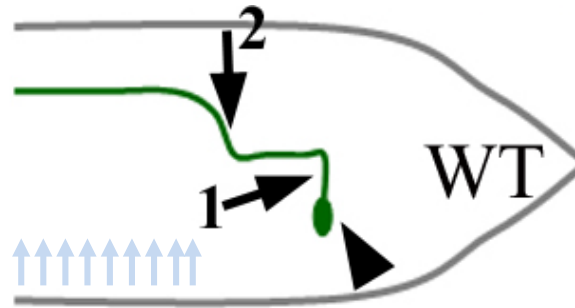
Does LAD-2 mediate axon guidance via MAB-20/Sema2?



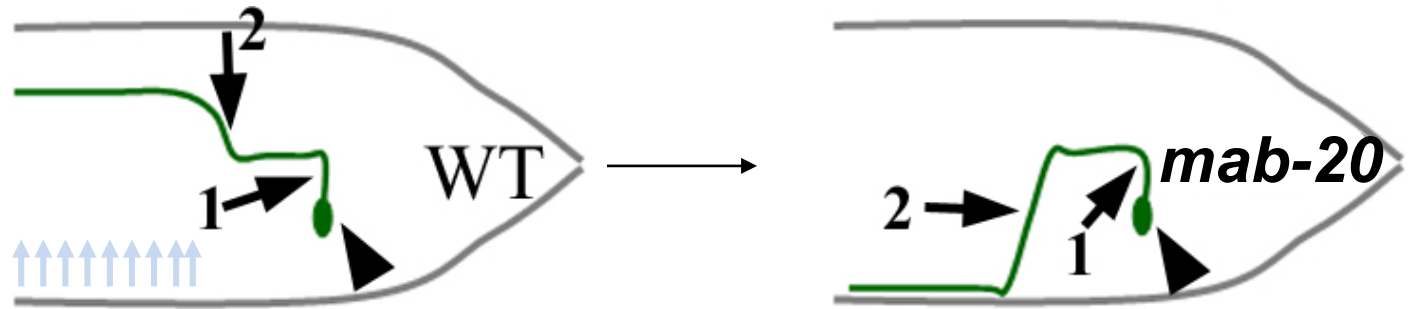
LAD-2 functions in MAB-20/Sema2-mediated axon guidance



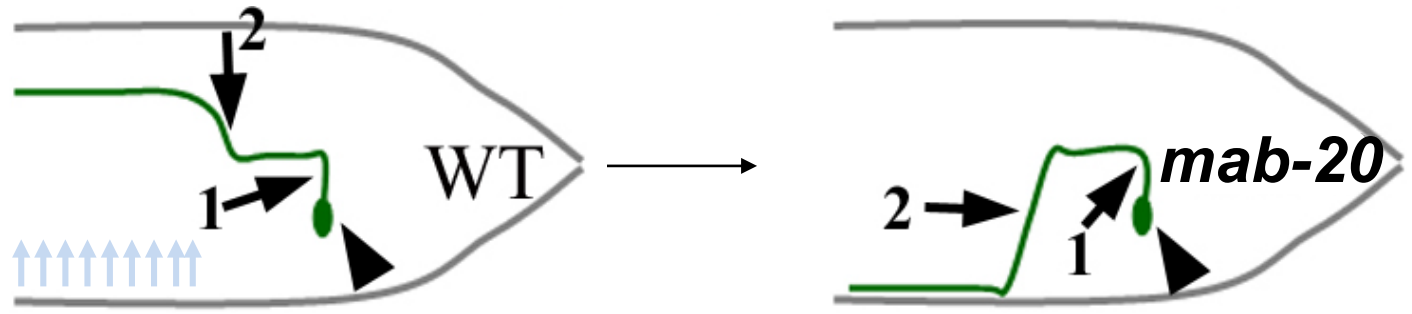
Does MAB-20/Sema2 act as a repellent to SDQL?



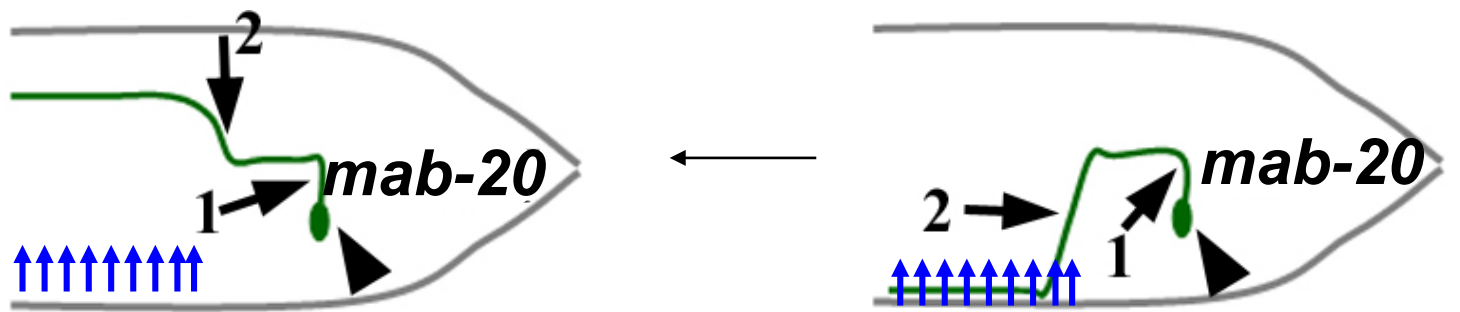
Does MAB-20/Sema2 act as a repellent to SDQL?



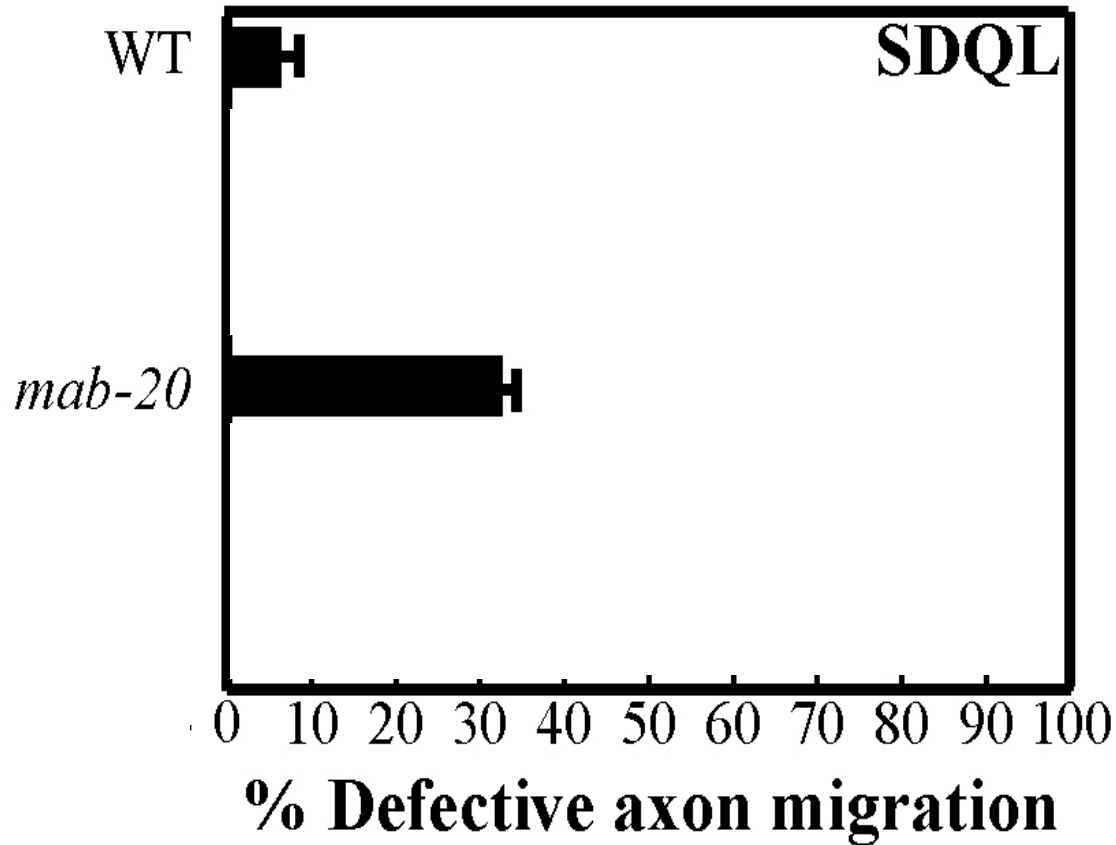
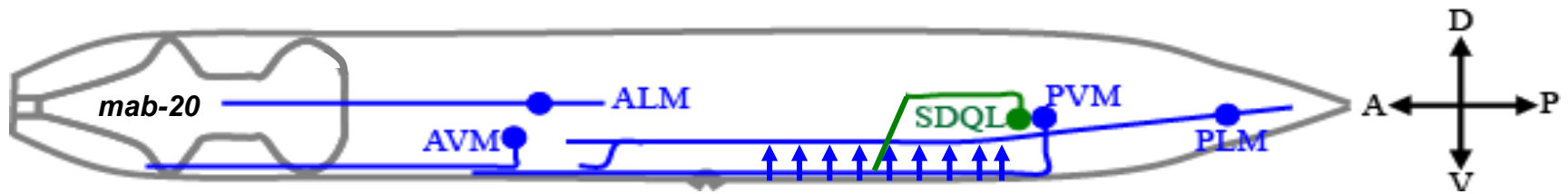
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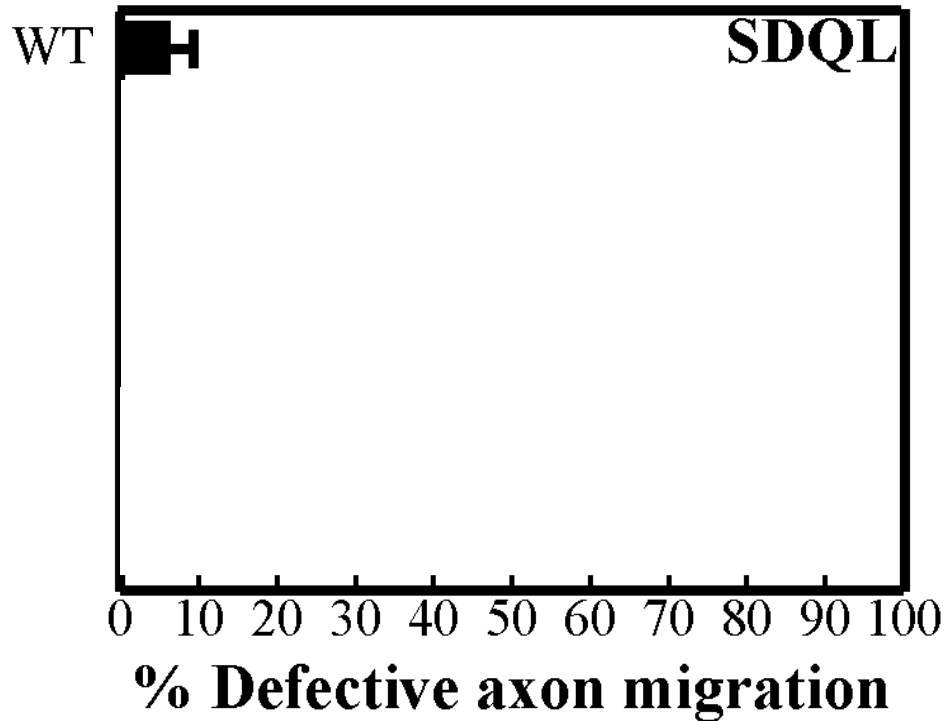
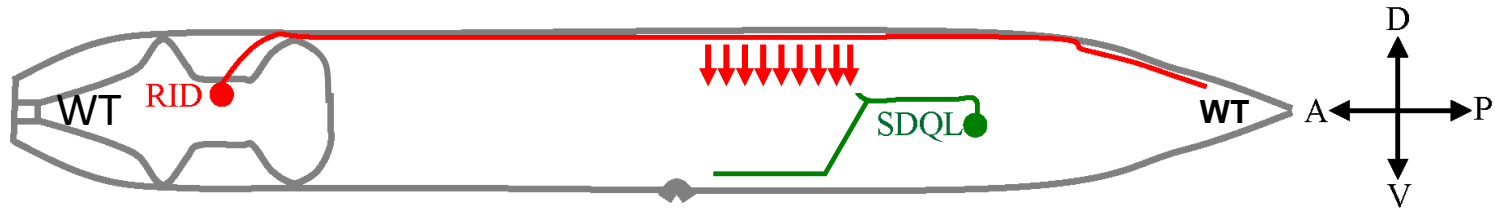
Ectopic ventral MAB-20 expression



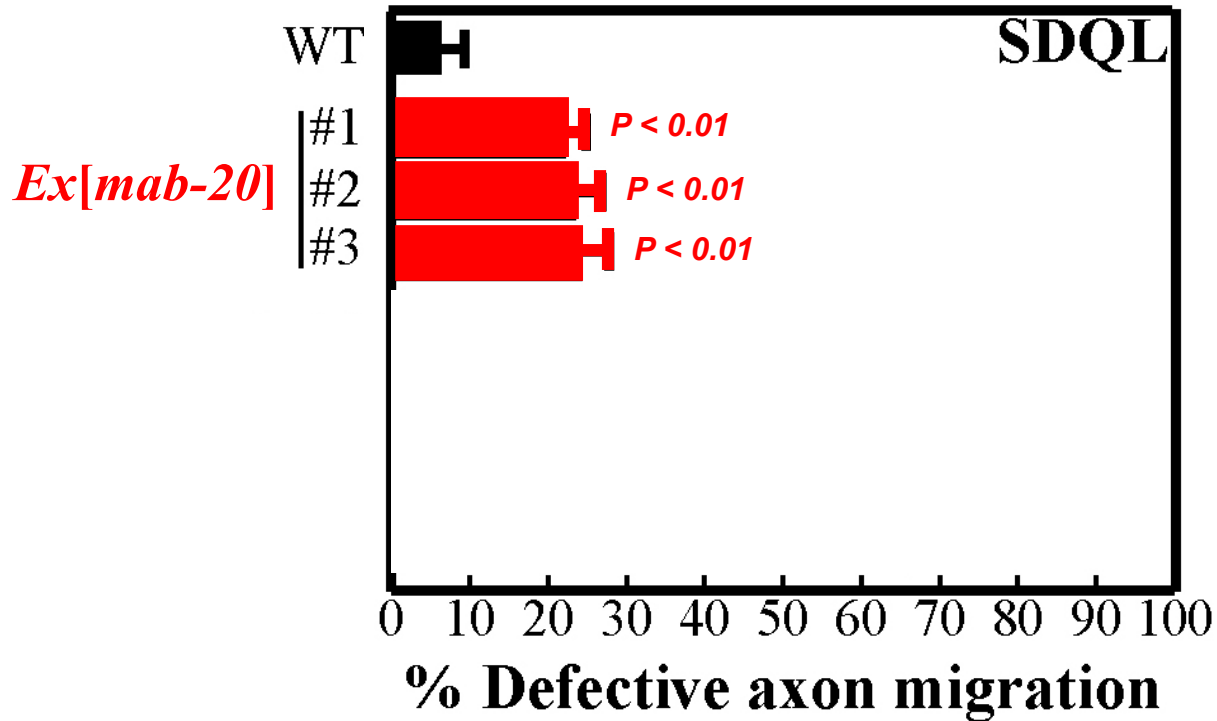
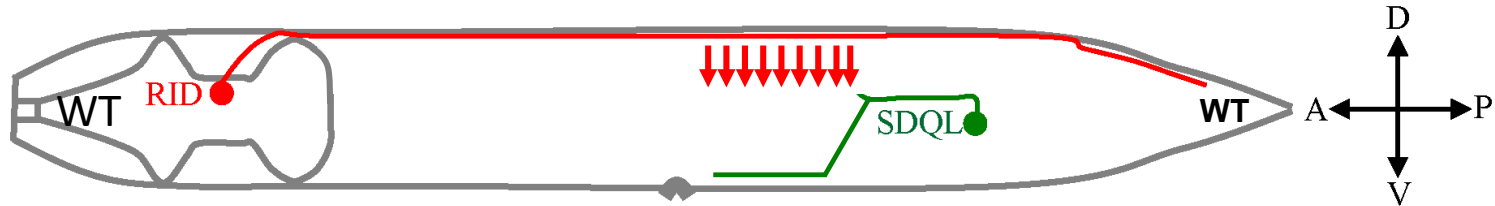
MAB-20/Sema2 acts as a repellent



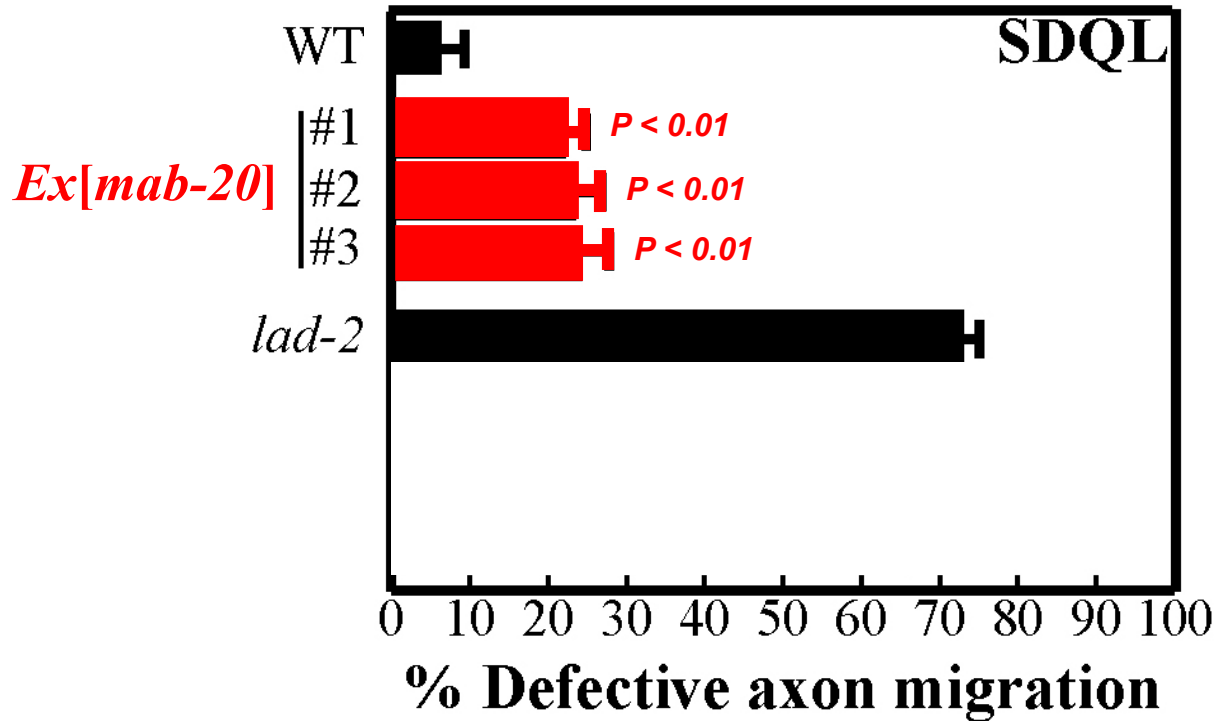
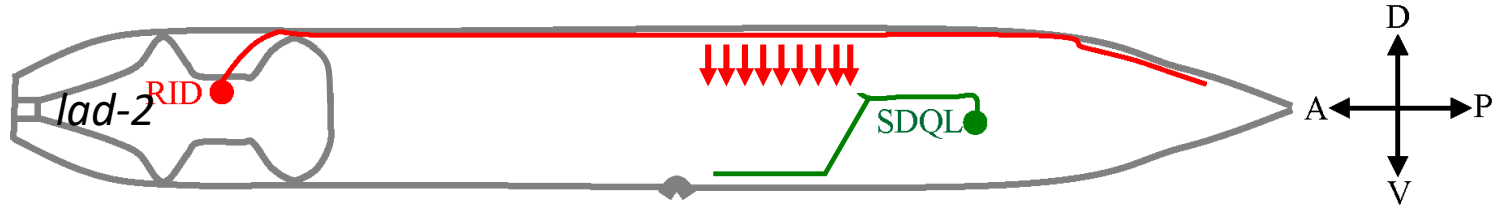
Can dorsal MAB-20/Sema2 induce ventral migration of SDQL axon?



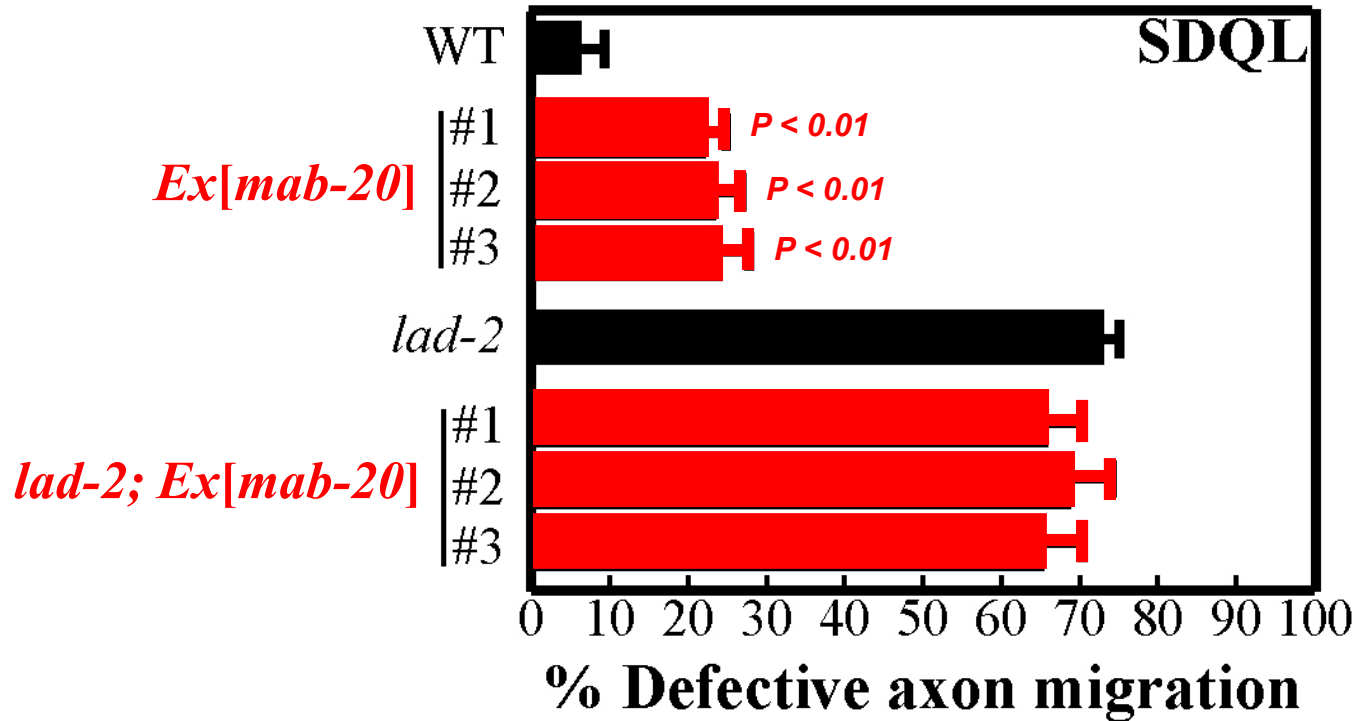
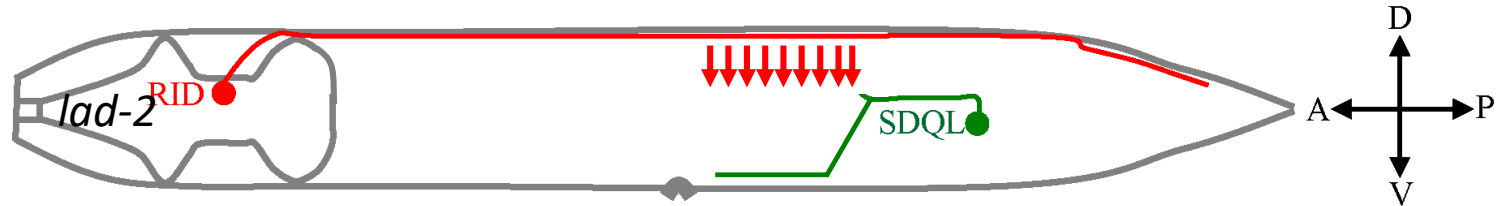
MAB-20/Sema2 acts as a repellent



Is LAD-2 required to mediate MAB-20/Sema2 signal?



LAD-2 is required to mediate MAB-20/Sema2 signal!



Can MAB-20/Sema2 interact with PLX-2?

MAB-20/Sema2 interacts with PLX-2

Myc::PLX-2

+ -

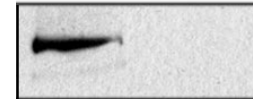
+ -

FLAG::MAB-20

+ +

+ +

Myc::PLX-2



FLAG::MAB-20



3 4

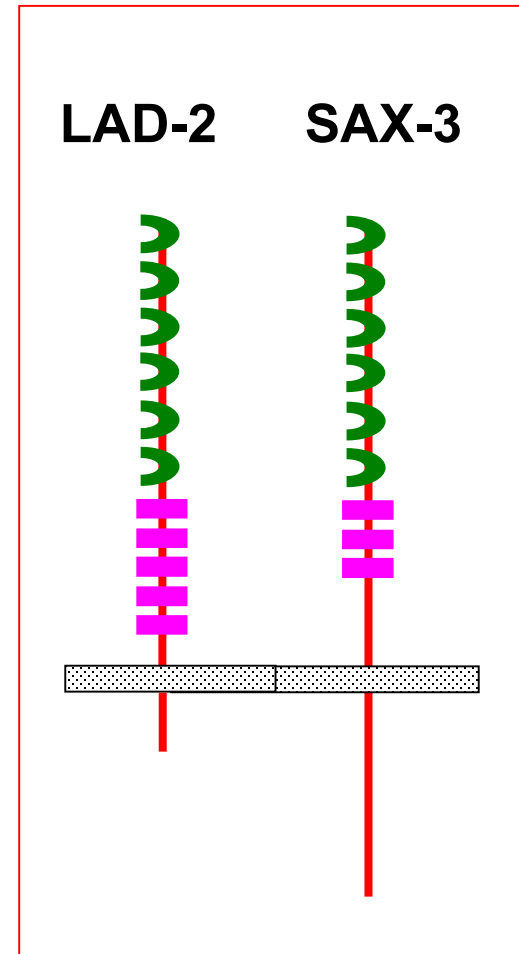
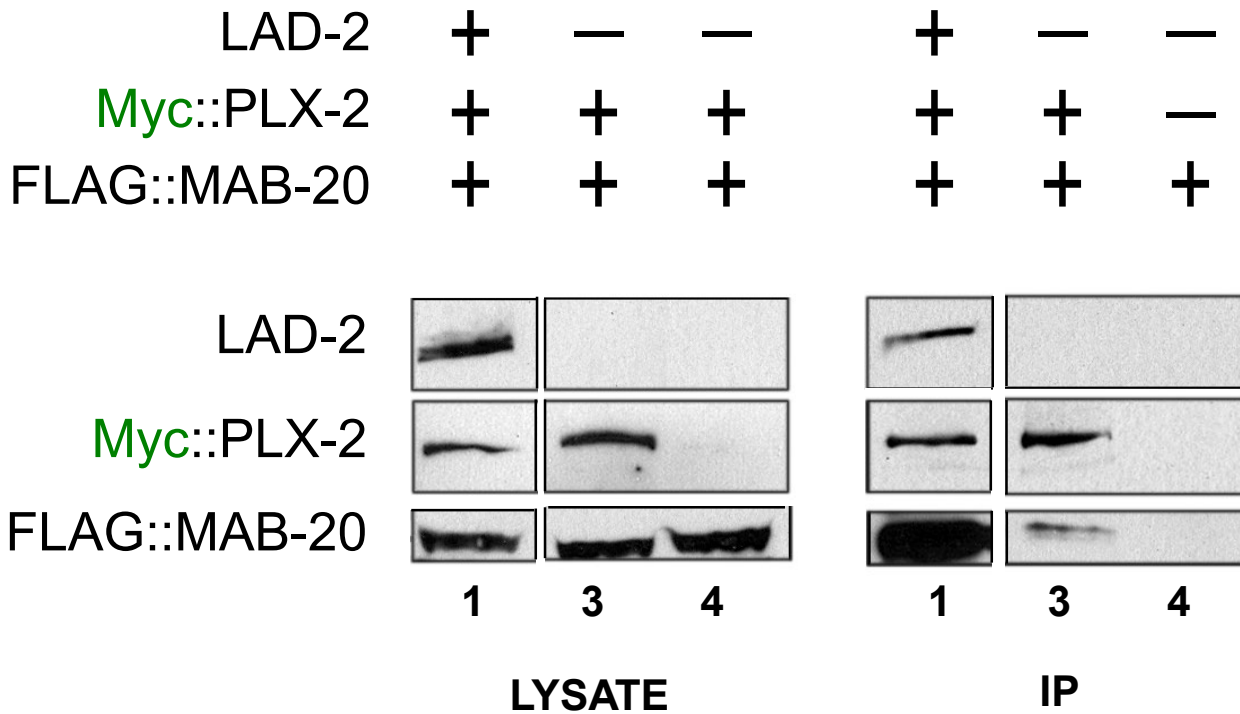
3 4

LYSATE

IP

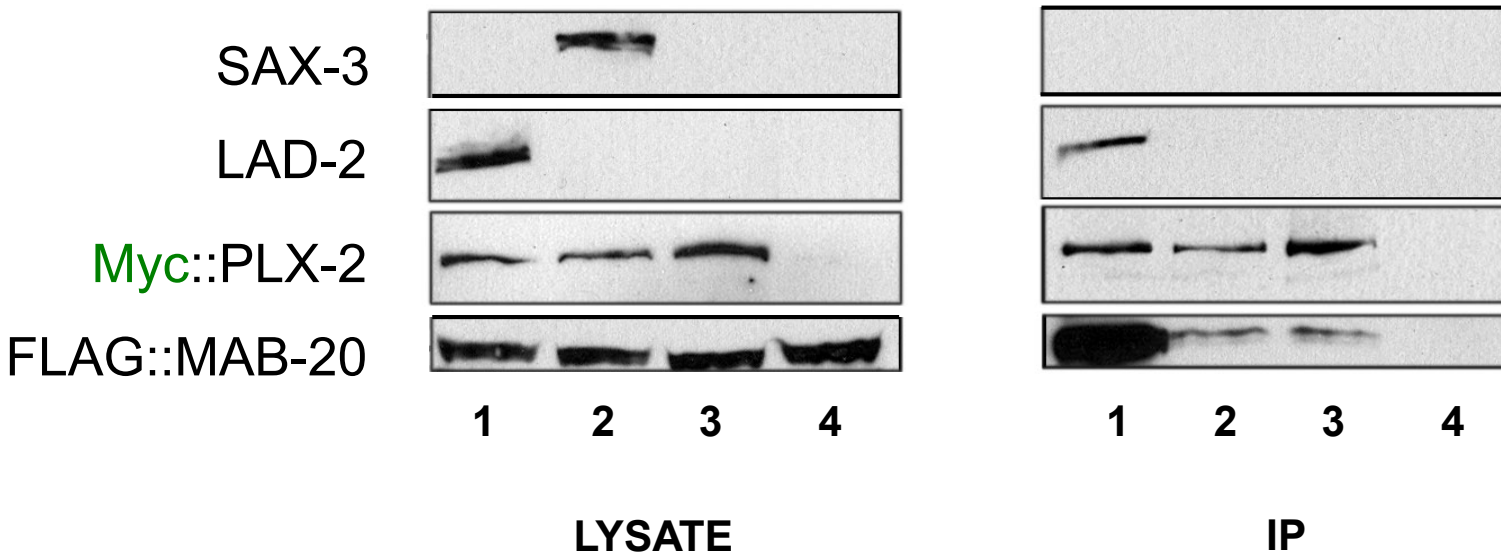
Can LAD-2 interact with MAB-20 & PLX-2?

LAD-2 interacts with MAB-20/Sema2 & PLX-2

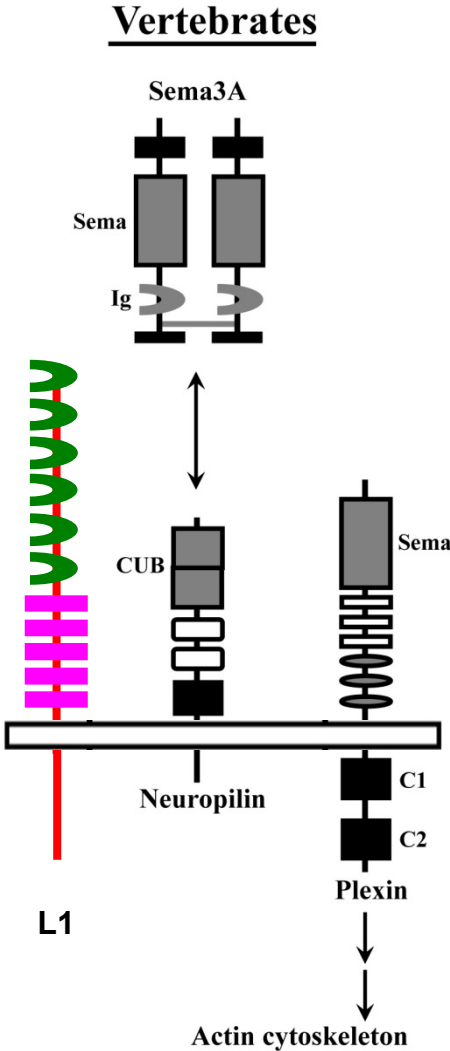
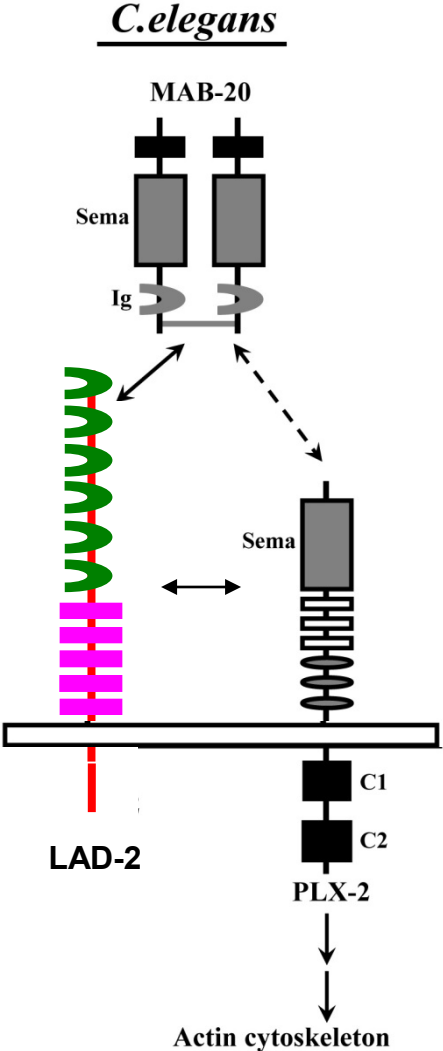


LAD-2 secures the MAB-20/Sema2 - PLX-2 interaction

SAX-3	-	+	-	-	-	+	-	-
LAD-2	+	-	-	-	+	-	-	-
Myc::PLX-2	+	+	+	+	+	+	+	-
FLAG::MAB-20	+	+	+	+	+	+	+	+



Model for how LAD-2 mediates MAB-20/Sema2 function



Neuropilin present (Putnam et al., 2007)
 No L1CAMs (C. Magie, Pers. comm)