TOM-1/Tomosyn is an inhibitor of growth cone protrusion and works with the UNC-6/Netrin receptor UNC-5

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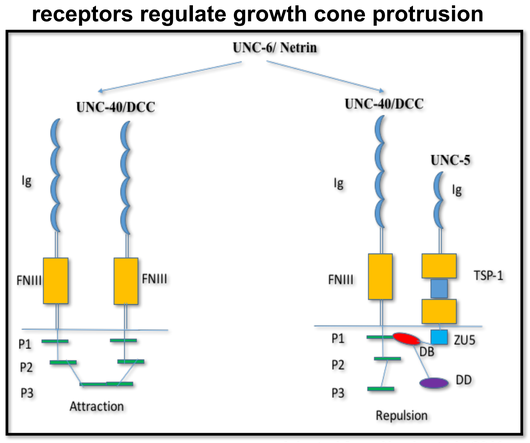
Introduction

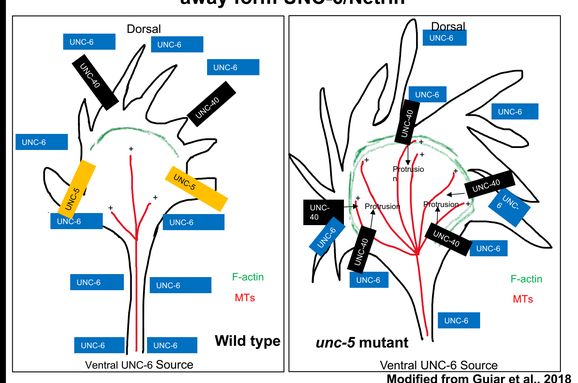
Previous work from the Lundquist lab showed that the UNC-6/Netrin receptors UNC-40 and UNC-5 regulate growth cone protrusion. UNC-40 stimulates protrusion whereas UNC-5 inhibits protrusion, and asymmetric distribution of protrusive activity across the growth cone results in directed growth cone migration away from UNC-6/Netrin (the Polarity/Protrusion model). UNC-5 inhibits protrusion using the FMO flavin monoxygenases, likely via actin inhibition, and by restricting growth cone microtubule entry via UNC-33/CRMP. MTs deliver vesicles into the growth cone, which fuse with the plasma membrane and drive protrusion. To explore the role of vesicle fusion in growth cone protrusion, we analyzed *tom-1/tomosyn* mutants. Tomosyn normally occludes formation of the SNARE complex by interacting with and inhibiting syntaxin-1. VD growth cones of *tom-1* mutants were similar to wild-type and showed no apparent protrusion defects. However, loss of *tom-1* suppressed the effects of constitutively-activated MYR::UNC-5, which alone causes small growth cones with little protrusion. *tom-1*; *MYR::UNC-5* displayed growth cones similar to the wild type, opposite to the small and non-protrusive growth cones of MYR::UNC-5. This suggests that TOM-1 is normally required for the inhibitory effects of MYR::UNC-5 on growth cone protrusion. Transgenic expression of wild-type *tom-1* resulted in small and non-protrusive growth cones, similar to activated MYR::UNC-5. Loss of *unc-5* results in large, overprotrusive growth cones. Transgenic expression of wild-type TOM-1 in *unc-5* loss-of-function mutants resulted in small, non-protrusive growth cones, consistent with a role of TOM-1 downstream of UNC-5. Future studies will involve analysis of actin and MTs in TOM-1 mutant growth cones, and an analysis of other regulators of vesicle fusion, including UNC-64/Syntaxin and UNC-13. In the polarity/protrusion model of growth cone outgrowth, UNC-6/Netrin inhibits growth cone protrusion via the UNC-5 receptor. These results suggest that UNC-5 employs TOM-1/tomosyn to prevent vesicle fusion and thus protrusion, in addition to destabilizing actin via the FMOs, and preventing growth cone MT entry via UNC-33/CRMP.

UNC-6/Netrin and the UNC-40 and UNC-5 receptors regulate growth cone protrusion



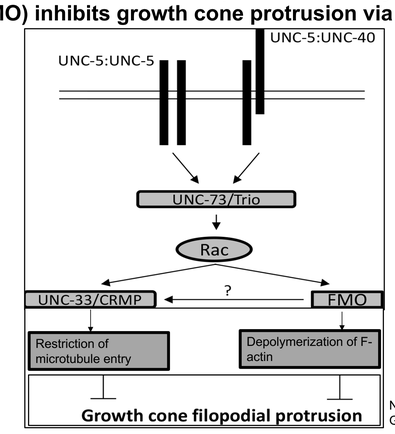
Adapted from Norris et al., 2011

Polarity/protrusion model of growth cone migration away form UNC-6/Netrin



Modified from Gujar et al., 2018

UNC-33/CRMP and Flavoprotein Monooxygenase (FMO) inhibits growth cone protrusion via UNC-5.



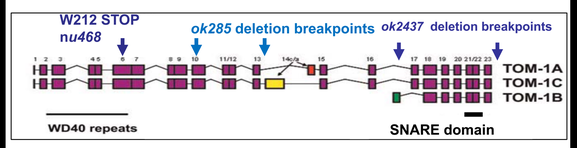
Norris et al., 2014
Gujar et al., 2017

Regulation of extension and retraction of growth cones by Tomosyn

Tomosyn is first identified as Syntaxin-binding partner from rat cerebral cytosol.

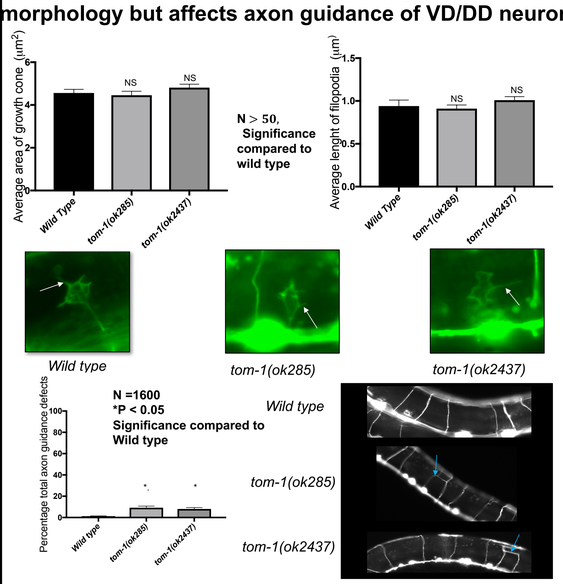
UNC-64/Syntaxin is a type of t-SNARE plays a crucial role in vesical fusion by formation of SNARE complex.

Tomosyn inhibits the formation of SNARE complex by interacting with Syntaxin and targets the vesicles to the leading edge of the growth cones.

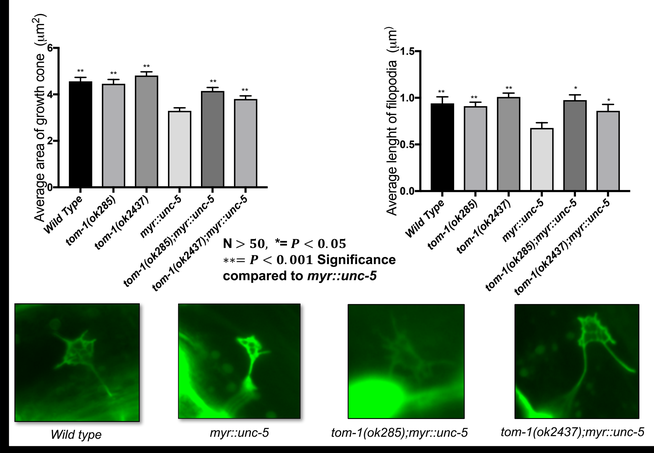


Toshiaki et.al.,2004
Modified from
Elena et .al., 2006

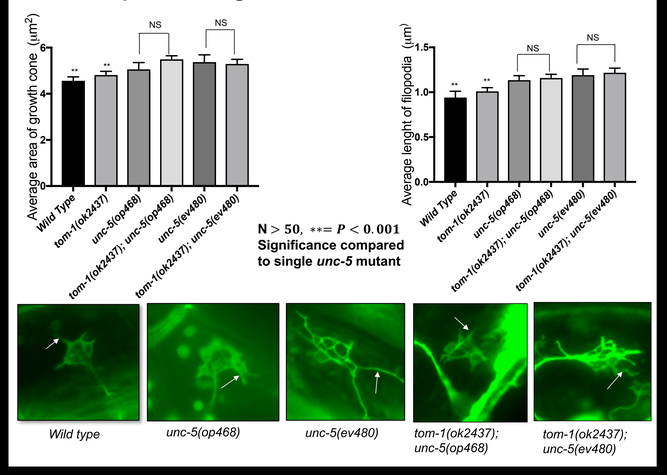
Loss of tomosyn-1 does not affect growth cone morphology but affects axon guidance of VD/DD neuron



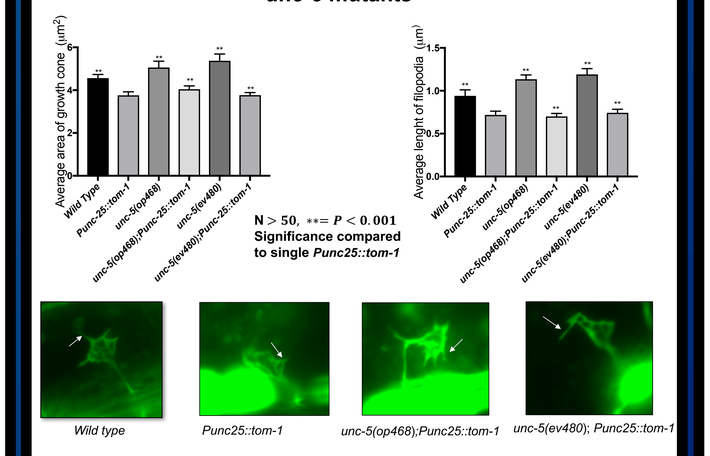
Activity of TOM-1 is required for the inhibitory effects of MYR::UNC-5 on growth cone protrusion



unc-5;*tom-1* growth cones resemble the large, protrusive growth cones of *unc-5* alone



Transgenic expression of Tomosyn-1 suppresses *unc-5* mutants



Conclusions and future directions

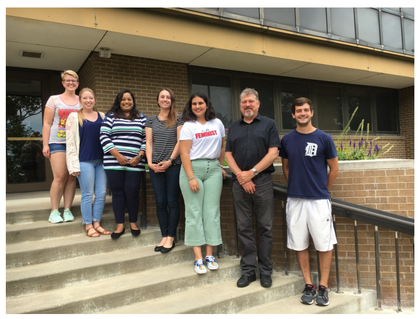
- The loss of function of *tom-1* does not suppress the VD growth cone area and filopodial length but affects the VD/DD axon guidance.
- Loss of *tom-1* rescues growth cone area and filopodial length of MYR::UNC-5, suggesting that Tomosyn-1 is required for the inhibitory effects of MYR::UNC-5.
- Double mutant analysis of *tom-1;unc-5* produces large and protrusive growth cones similar to the single *unc-5* mutant, suggesting that TOM-1 is a negative regulator of UNC-5.
- Transgenic expression of TOM-1 results in the small and non-protrusive growth cones even in the absence of UNC-5 activity, suggests that TOM-1 is likely acting downstream of UNC-5.
- Hence, Tomosyn-1 is playing a key role in regulating growth cone protrusion in UNC-6/Netrin signaling via receptor UNC-5 and it is likely via regulating vesical fusion in growth cones.
- To confirm the effects of vesicle fusion, analysis of other regulators of vesicle fusion like UNC-64/Syntaxin and UNC-13 is necessary.
- Also, looking at the polarization of F-actin and MTs distribution in *tom-1* mutants will be crucial in determining how polarity is regulating protrusion of growth cones and whether loss of *tom-1* has any effect of cytoskeleton of growth cones.
- Also, *in vivo* visualization of vesicle fusion in important to understand how Tomosyn is regulating vesicle fusion in different parts of growth cones to prevent the protrusion.
- To summarize, vesicle fusion is pro-protrusive in the growth cones and Tomosyn inhibits the growth cone protrusion by inhibiting vesicle fusion.

Acknowledgements

Lundquist lab members
Dr.Erik Lundquist
Dr. Vitoria Paolillo
Matt Ochs
Kelsey Ferguson
Angelica Lang
Isabel Pulido

Former Lab Members
Dr. Mahekta Gujar
Dr. Adam Norris
Aubrie Stricker
Eric Struckhoff

Dr. Brian Ackley and lab



K-INBRE
NIH R01 NS040945
NIH R21 NS070417
NIH P01
GM103418
NATIONAL INSTITUTES OF HEALTH
Funding:
CMADP COBRE:
NIH P01
GM103638