Function of Coro1A in Netrin-Mediated Axon Branching and TRIM67 Binding

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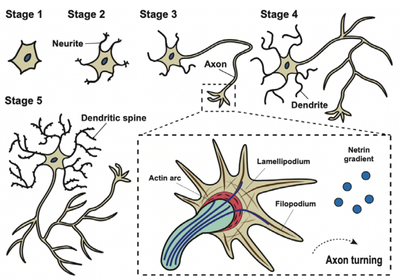
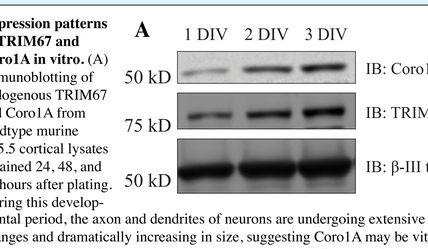
Introduction

Neuronal morphogenesis



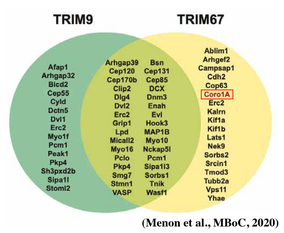
(Ho and Gupton, Encycl. Biol. Chem. III., 2021)

TRIM67 is required for netrin-dependent axon turning



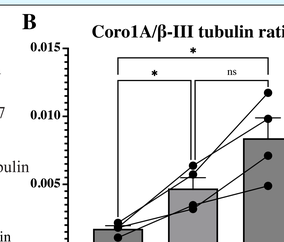
(Boyer et al., JCB, 2020)

Mass spectrometry to identify potential binding partners of TRIM9 and TRIM67



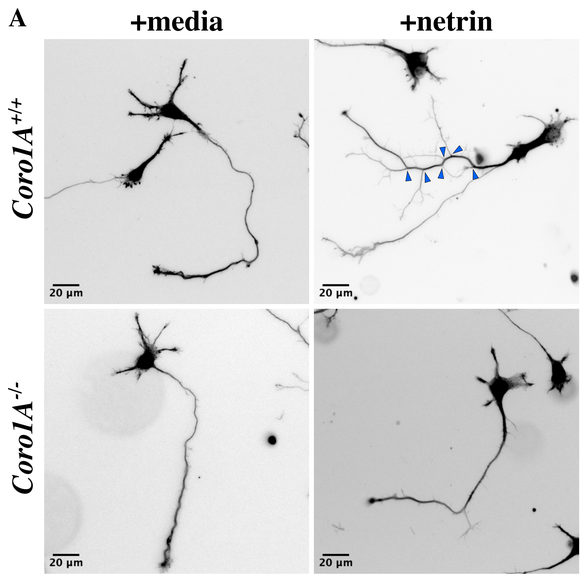
(Menon et al., MBoC, 2020)

Loss of TRIM67 impairs netrin-mediated axon branching



(Boyer et al., JCB, 2020)

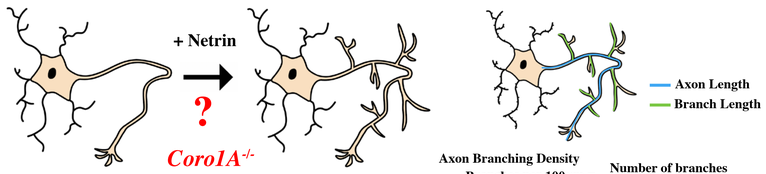
Loss of Coro1A inhibits netrin-mediated increases in axon branching density, axon length, and branch length



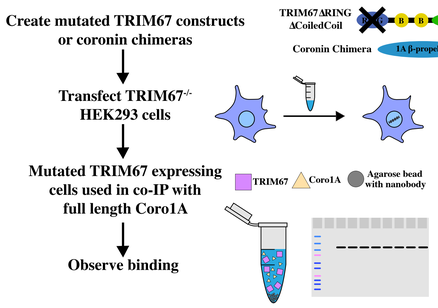
Axon branching density and axonal outgrowth depend on Coronin1A. (A) Inverted LUT fluorescent images of *Coro1A*$^{+/+}$ and *Coro1A*$^{-/-}$ neurons treated with either netrin or media. The arrow heads indicate axon branches. (B) Quantification of axon branching density per 100 μm for *Coro1A*$^{+/+}$ and *Coro1A*$^{-/-}$ neurons with and without netrin treatments. A Kruskal-Wallis nonparametric ANOVA test with Dunn's correction was used for statistical analysis. (C) Quantification of total axon length (μm) for *Coro1A*$^{+/+}$ and *Coro1A*$^{-/-}$ neurons with and without netrin treatments. An ANOVA with Bonferroni's post hoc correction was used for statistical analysis. (D) Quanitification of individual branch lengths (>20 μm) for *Coro1A*$^{+/+}$ and *Coro1A*$^{-/-}$ neurons with and without netrin treatments that contained branches. A Kruskal-Wallis nonparametric ANOVA test with Dunn's correction was used for statistical analysis. For (B,C) n (cells) = 289 +/+ with media, 339 +/+ with netrin, 257 -/- with media, 273 -/- with netrin. For (D) n (cells) = 205 +/+ with media, 304 +/+ with netrin, 166 -/- with media, 165 -/- with netrin. *, P < 0.05; **, P < 0.01.

Graphical abstract

Axonal responses to guidance cues in wildtype vs *Coro1A*$^{-/-}$ neurons

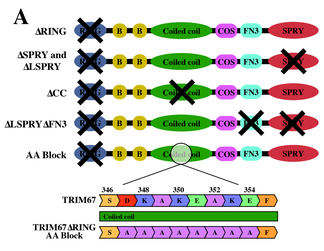


Mapping the binding domains of TRIM67 and Coro1A



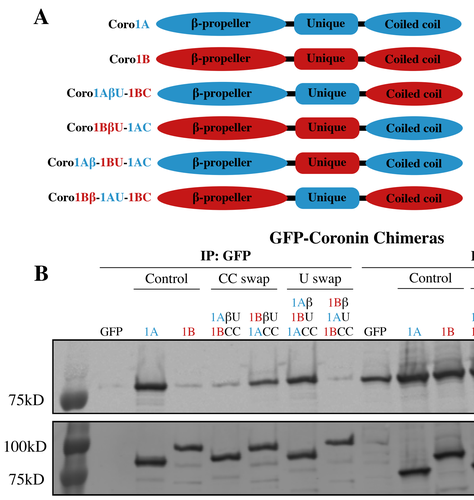
Coronin1A is a *trimer* whereas TRIM67 is a *dimer*. Previous work from the lab has verified the interaction between the two proteins, yet the binding sites are undetermined.

TRIM67 binding domains of Coro1A



Mapping the binding domain of TRIM67 required for the interaction with Coronin1A. (A) Graphical depictions of the TRIM67ΔRING mutants used in co-immunoprecipitation assays with full length Coronin1A. (B, C, and D) Co-immunoprecipitation assays using TRIM67$^{-/-}$ HEK Cells transfected with various TRIM67ΔRING mutants. Whole cell lysates were separated by SDS-PAGE electrophoresis and analyzed through immunoblotting. FL: Full-length TRIM67ΔRING, CC: coiled coil, AA Block: Alanine block mutation.

Coro1A binding domains of TRIM67



Mapping the binding domains of Coro1A required for the interaction with TRIM67 (A) Graphical depictions of full-length Coro1A, full-length Coro1B, and the Coro1A/Coro1B chimeras used in co-immunoprecipitation assays with TRIM67ΔRING. (B) Co-immunoprecipitation assays using TRIM67$^{-/-}$ HEK Cells transfected with full length and chimera coronins. Whole cell lysates were separated by SDS-PAGE electrophoresis and analyzed through immunoblotting. (C) Individual data points and violin plots of coprecipitated myc-TRIM67 relative to GFP-Coronins. 1A: Coronin1A, 1B: Coronin1B, β: β propeller, U: unique, CC: coiled coil.

Expression of Coro1A *in vitro*

Expression patterns of TRIM67 and Coro1A in vitro. (A) Immunoblotting of endogenous TRIM67 and Coro1A from wildtype murine E15.5 cortical lysates obtained 24, 48, and 72 hours after plating. During this developmental period, the axon and dendrites of neurons are undergoing extensive changes and dramatically increasing in size, suggesting Coro1A may be vital in these early stages of neuronal morphogenesis. (B) Quantification of the *in vitro* expression of Coro1A normalized to β-III tubulin 24, 48, and 72 hours after neuronal dissociation. *, P < 0.05.

Conclusions

- Netrin-dependent axon branching responses and axon outgrowth were lost in Coronin1A knockouts
  - The loss of Coronin1A inhibited netrin-mediated increases in axon branching density, total axon length, and branch length
- The coiled coil domains of both Coronin1A and TRIM67 are required for their interaction
  - TRIM67ΔRINGΔCoiledCoil mutants could not effectively bind to Coro1A
  - Coro1A chimeras lacking the 1A Coiled Coil domain could not efficiently interact with TRIM67ΔRING
  - Coro1B chimeras containing the 1A Coiled Coil domain significantly increased their interaction with TRIM67ΔRING compared to full-length Coro1B

Future Directions

- Determine if conserved residues in the coronin beta-propeller contribute to the low residual binding of Coro1A chimeras lacking the 1A Coiled Coil domain
- Rescue experiments using *Coro1A*$^{-/-}$ neurons with full length and mutated Coro1A constructs
- Experiments on additional netrin-dependent axonal responses – such as axon turning, axon regeneration, and synaptogenesis – that may be dependent on Coro1A
- Investigations on if Coro1A is netrin-specific using different guidance cues known to increase axon branching density in *Trim67*$^{+/+}$ cortical neurons

Acknowledgements

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