Activity-dependent regulation of dendritic complexity by Semaphorin3A through Farp1

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ABSTRACT

Dendritic arbors are complex neuronal structures that receive and process synaptic inputs. One mechanism regulating dendrite differentiation is Semaphorin/Plexin signaling, specifically through binding of soluble Sema3A to Neuropilin/PlexinA co-receptors. Here, we show that the protein Farp1 [FERM, RhoGEF (ARHGEF), and pleckstrin domain protein 1], a Rac1 activator previously identified as a synaptogenic signaling protein, contributes to establishing dendrite tip number and total dendritic branch length in maturing rat neurons and is sufficient to promote dendrite complexity. Aiming to define its upstream partners, our results support that Farp1 interacts with the Neuropilin-1/PlexinA1 complex and colocalizes with PlexinA1 along dendritic shafts. Functionally, Farp1 is required by Sema3A to promote dendritic arborization of hippocampal neurons, and Sema3A regulates dendritic F-actin distribution via Farp1. Unexpectedly, Sema3A also requires neuronal activity to promote dendritic complexity, presumably because silencing neurons leads to a proteasome-dependent reduction of PlexinA1 in dendrites. These results provide new insights into how activity and soluble cues cooperate to refine dendritic morphology through intracellular signaling pathways.

INTRODUCTION

The architecture of dendritic arbors determines how synaptic inputs are received and integrated. Mechanisms that regulate the development of dendritic trees include soluble cues, adhesive interactions, and neuronal activity. The secreted chemotropic protein Sema3A regulates neuronal polarity and aspects of dendritic development by binding Neuropilin-1/PlexinA1 receptor complexes on immature dendrites (Polleux *et al*, 2000; Tran *et al*, 2009; Shelly *et al*, 2011). Sema3A remodels the neuronal cytoskeleton through the Rho family GTPase Rac1 as well as the actin-depolymerizing factor cofilin (Jin and Strittmatter, 1997; Aizawa *et al*, 2001), and additional signaling mechanisms are still being elucidated.

Studies in multiple systems have demonstrated that neuronal activity plays key roles in dendritic development. However, It is unclear whether activity and soluble cues like Sema3A may engage distinct pathways to selectively control different aspects of outgrowth and branching, or converge on the same regulators to shape dendritic development in concert. Among suspected Semaphorin signaling components is Farp1, a dendritically localized cytoskeletal signaling molecule which we recently identified as a robust regulator of excitatory synapse formation downstream of the adhesion protein SynCAM (Cheadle and Biederer, 2012). Here, we show that Farp1 and the Sema3A receptor PlexinA1 colocalize and interact in dendrites, and characterize a mechanism whereby Sema3A and Farp1 coordinate to drive dendritic complexity in an activity-dependent manner (Cheadle and Biederer, 2014).

METHODS

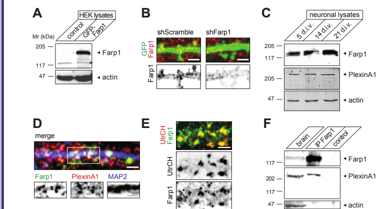
Cell culture and Immunocytochemistry. Hippocampal neurons were dissected from Sprague-Dawley rats at P1, and dissociated and plated on matrigel-treated coverslips. Neurons were transfected at 5 DIV. Treatment with TTX or PTX was carried out from 14 DIV to 21 DIV, with Sema3A for 20 hrs at 20 DIV, and with protease inhibitors for 2 hrs at 21 DIV. All cultures were fixed in 4% PFA and 4% sucrose at 21 DIV, permeabilized and blocked in PBS adjusted to 3% FBS and .1% TritonX-100, then stained with primary antibody at 4° C O/N followed by secondary AlexaDyes for 1 hr at RT.

Biochemistry. Immunoprecipitation was performed from P21 rat brains homogenized in 25 mM HEPES and 320 mM sucrose + protease inhibitors, cleared by centrifugation, and extracted by 1% TritonX-100 and 50 mM NaCl. The homogenate was pre-cleared with protein-G beads before IP with purified Farp1 antibody for 2.5 hrs at 4° C. Beads were washed with extraction buffer and protein eluted with 2% SDS. Western blotting procedures were standard.

Microscopy and Analysis. Data were acquired and analyzed blind to condition. Imaging was performed on a PerkinElmer UltraView VoX spinning disk confocal microscope equipped with a Hamamatsu C9100-50 camera and a Nikon perfect focus autofocus system. Dendritic morphology was analyzed manually in ImageJ. Fluorescence intensity measurements were made in Volocity (PerkinElmer). Data analysis was performed using Graphpad Prism 5. Statistical analyses included ANOVA or Student's *t* test with errors corresponding to the SEM.

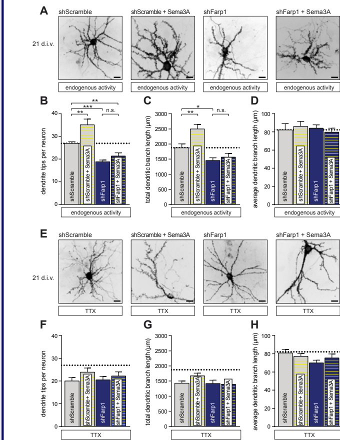
RESULTS

1. Farp1 interacts with PlexinA1 in the brain.



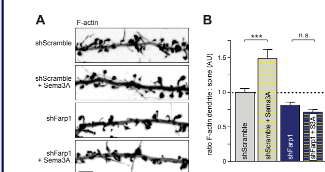
A, B, Characterization of our specific Farp1 antibody in western blotting and immunostaining. **C.** Lysates of cultured neurons contain Farp1 and PlexinA1 across development. **D.** Farp1 and PlexinA1 co-localize in dendritic shafts. **E.** Farp1 is also present in spines, where it co-localizes with a marker of F-actin. **F.** PlexinA1 co-immunoprecipitates with Farp1 from brain. All scale bars, 2 μm.

2. Activity-dependent regulation of dendrites by Farp1 and Sema3A.



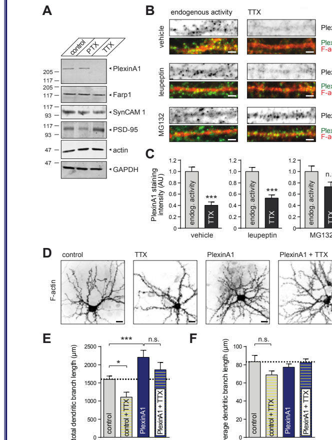
A. Representative grayscale images of cultured neurons +/- Farp1 knockdown and Sema3A treatment. Scale bar, 15 μm. **B-D.** Quantification of dendritic tips, total branch length, and average branch length per neuron. **E.** Same as ***A***, but after silencing for 20 hr with TTX. Scale bar, 15 μm. **F-H.** Quantification of tips and total and average branch lengths.

3. Farp1 and Sema3A control F-actin distribution.



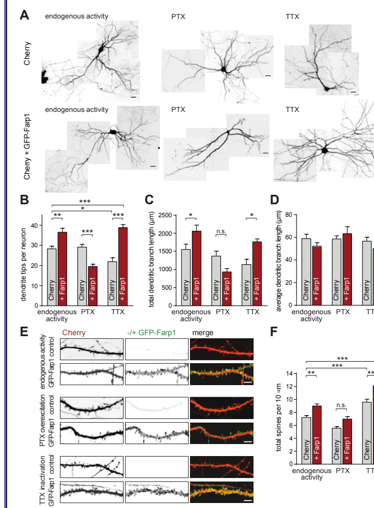
A. Representative grayscale images of the F-actin marker UtrCH-Cherry demonstrating the regulation of dendritic actin by Sema3A and Farp1. Scale bar, 5 μm. **B.** Quantification of the ratio of F-actin in the dendrite versus proximal spines.

4. Activity blockade leads to proteasomal degradation of PlexinA1.



A. Western blot of neuronal lysates shows activity-dependent proteasomal mechanism of PlexinA1 degradation. Scale bars, 5 μm. **B,C.** Quantitative immunostaining of TTX-treated neurons. **D.** Grayscale images of neurons treated with TTX and overexpressing PlexinA1. Scale bars, 15 μm. **E, F.** Quantification of total and average dendritic branch length. Elevated expression of PlexinA1 rescues the TTX-dependent simplification of dendritic arbors.

5. Farp1 is sufficient to promote dendritic complexity in the absence of activity.



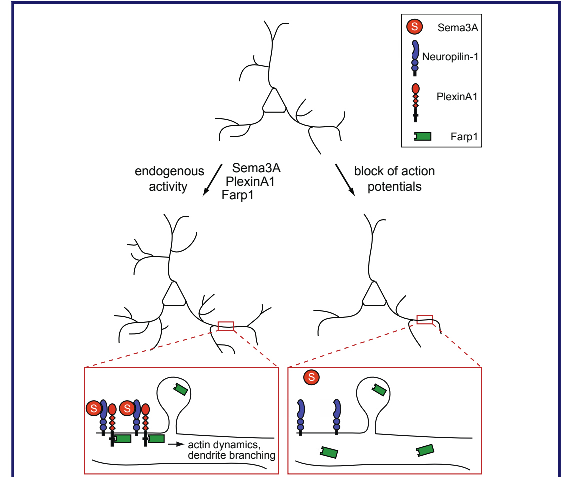
A. Tiled grayscale images of neurons overexpressing Farp1. Scale bars, 15 μm. **B-D.** Quantification of dendritic tips and total and average branch length demonstrate activity-independent ability of Farp1 to drive dendritic complexity. **E, F.** Magnified images of dendrites and quantification of increased spine densities after Farp1 overexpression. Scale bars, 5 μm.

CONCLUSIONS

- Farp1 and PlexinA1 are expressed in developing neurons, where they colocalize in dendritic shafts and form a complex.
- The soluble cue Sema3A requires Farp1 to increase parameters of dendritic complexity including tip number and total dendritic branch length. TTX treatment blocks dendritic regulation by Sema3A.
- Sema3A and Farp1 coordinately regulate F-actin distribution in dendrites.
- Surprisingly, activity blockade leads to proteasomal degradation of the Sema3A receptor PlexinA1. Consistent with a necessity for PlexinA1 in Sema3A-driven dendrite maturation, elevated levels of PlexinA1 can rescue total dendritic branch length after TTX treatment.
- Although Farp1 participates with Sema3A in activity-dependent dendrite development, Farp1 is sufficient to drive complexity even in the presence of TTX, consistent with Farp1 acting downstream of Sema3A.

Sema3A promotes dendritic complexity through a Neuropilin-1/PlexinA1/Farp1 signaling axis.

MODEL



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