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Dapper Antagonist of Catenin-1 (Dact1) contributes to dendrite arborization in forebrain cortical interneurons

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Abbreviations: Dact1, Dapper antagonist of catenin 1; DIV, day in vitro; Dvl1, Dishevelled 1; GABA, gamma-aminobutyric acid; GE, ganglionic eminence(s); PCP, planar cell polarity

In mice, genetically engineered knockout of the Dapper Antagonist of Catenin-1 (*Dact1*) locus, which encodes a scaffold protein involved in Wnt signaling, leads to decreased excitatory input formation on dendrites of developing forebrain neurons. We have previously demonstrated this in both (excitatory, glutamatergic) pyramidal neurons of the hippocampus and in (inhibitory GABAergic) interneurons of the cortex. We have also demonstrated that knockout of the *Dact1* locus leads to decreased dendrite complexity in cultured hippocampal pyramidal neurons, and to decreased spine formation on dendrites of forebrain pyramidal neurons in vitro and in vivo. Synapse phenotypes resulting from Dact1 loss in cultured cortical interneurons can be rescued by recombinant overexpression of the Dact1 binding partner, Dishevelled-1 (Dvl1), but not by recombinant expression of a constitutively active form of the small GTPase Rac1. This contrasts with dendrite spine phenotypes resulting from Dact1 loss in cultured hippocampal pyramidal neurons, which can be fully rescued by recombinant expression of activated Rac1. Taken together, these data suggest that in maturing forebrain neurons there are molecularly separate requirements for Dact1 in dendrite arborization/spine formation vs. synaptogenesis. Here, we show that the developmental requirement for Dact1 during dendrite arborization, which we previously demonstrated only in hippocampal pyramidal neurons, is also present in cortical interneurons, and we discuss mechanistic implications of this finding.

Neurons are among the most polarized and morphologically complex cells in metazoans. One manifestation of polarization in neurons is their spatially discrete presynaptic sites that release neurotransmitters and other factors from an axon terminus, often located at great distances from the neuronal soma. One manifestation of morphological complexity in neurons is the highly ramified extensions of their soma, the dendrite arbor, upon which they receive manifold postsynaptic inputs from apposed presynaptic release sites of other neurons. The dendrite arbor greatly expands the surface area of each neuron and thereby increases the number of inputs it can receive. At the same time the shape and distribution of the dendrite arbor determines the number, type, and ratio of inputs received from other neurons, including from those located nearby and from those located far away and communicating via projecting axons. Regions of the brain with distinct emotional, cognitive, and behavioral functions have correspondingly distinct neural architecture; this includes composition by distinct neuron classes and subclasses, many of which can be distinguished by dendrite arborization and connectivity patterns, as well as by expression of cell type-specific molecular markers.

In the mammalian cortex, excitatory neurons that synthesize and release the neurotransmitter glutamate generally have axons that project beyond their own cortical layer or brain area, and in most cortical regions and layers, they have a characteristic pyramid-shaped dendrite arbor. These are therefore referred to somewhat interchangeably as "excitatory," "glutamatergic," "projection," or "pyramidal" neurons. In contrast, cortical inhibitory neurons that synthesize and release the neurotransmitter GABA generally have axons that project locally or within only a few cortical layers. They commonly communicate within a spatially restricted region and so are referred to generically as "interneurons." Unlike the typically pyramid-shaped projection neurons in most regions of the cortex, interneurons in different cortical regions and layers have diverse dendrite arbor morphologies depending on their local circuit functions. Another morphological difference between pyramidal neurons and interneurons is that whereas nearly all pyramidal neurons

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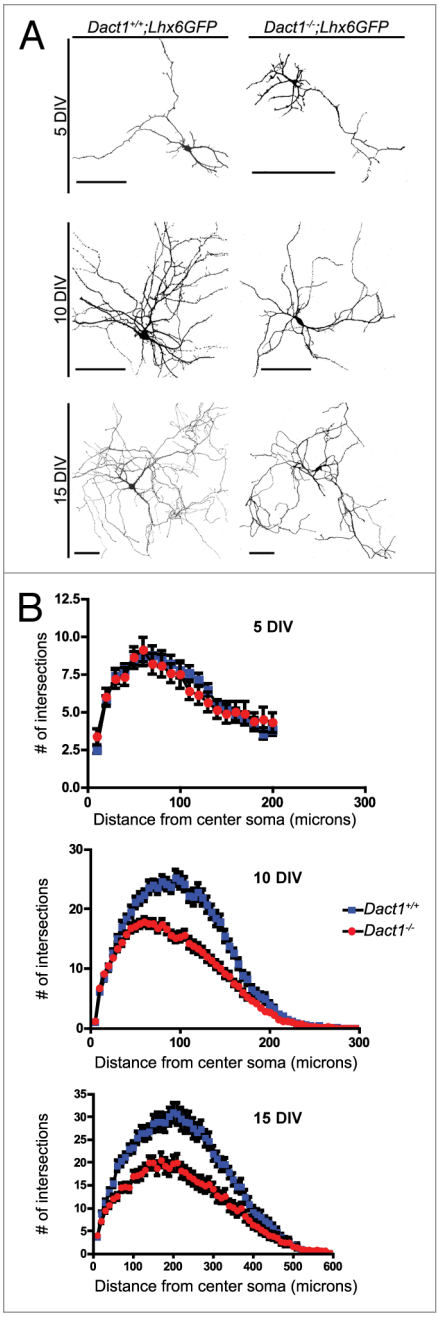

Figure 1. *Dact1 null* cortical interneurons have reduced dendrite complexity. (**A**) Representative neurons from primary cortical cultures prepared from neonatal *Dact1*$^{-/-}$*;Lhx6GFP* (right) and littermate control (left) brains, fixed at 5, 10, and 15 DIV. (**B**) Sholl analysis performed at the indicated DIV. Scale bars = 100 μm.

have protrusions called "spines" on their dendrites corresponding to excitatory inputs from other neurons, cortical interneurons have mostly smooth (aspiny) dendrites, even at sites where they receive such excitatory input.[1]

Healthy mental activity and behavior requires that the morphology of these different cortical neuron types, including their dendrite arborization pattern and synaptic complement, is assembled properly during development and continues to be regulated throughout life while allowing for appropriate plastic changes.[2] In mammals, the mechanisms that govern dendrite arborization, spine formation, and synaptogenesis have been studied predominantly in pyramidal neurons; relatively less scientific attention has focused on these same neurodevelopmental events in interneurons.[3-5]

Wnt signaling is a major form of intercellular communication that regulates diverse neurodevelopmental events: from brain regionalization, neural proliferation and cell fate determination, to neuronal migration, dendritogenesis, synaptogenesis, and plasiticity.[6,7] For conceptual purposes, Wnt signaling can be divided into 2 major biochemical branches: "canonical" Wnt/β-catenin signaling and "non-canonical" β-catenin-independent Wnt signaling.[8,9] While Wnt/β-catenin signaling characteristically involves the nuclear translocation of β-catenin and subsequent transcriptional regulation of target genes, diverse β-catenin-independent Wnt signaling pathways have been described in different biological contexts. These include the planar cell polarity (PCP) pathway and the Wnt/Ca^{2+} pathway, both of which can regulate Rho GTPases as one of their downstream effectors to affect cell polarity, shape, and movement.[10-12]

The Dapper Antagonist of Catenin-1 (Dact1; aka "Dapper," "Frodo") scaffold protein functions in both Wnt/β-catenin and β-catenin-independent Wnt signaling.[13-17] The *Dact1* gene is expressed in the embryonic forebrain of the mouse, including in the separate progenitor zones of cortical excitatory neurons and inhibitory interneurons.[18-21] We have previously shown that Dact1 is required during forebrain pyramidal neuron development for the elaboration of fully complex dendrite arbors as well as for spine and excitatory synapse formation. We have further shown that dendrite spine phenotypes resulting from Dact1 loss in cultured hippocampal pyramidal neurons can be rescued by a constitutively active form of the Rho GTPase family member Rac1 (RacCA), and that this phenotype correlates with levels of activated Rac, but not activated β-catenin, in the developing murine hippocampus. These data support an endogenous role for Dact1 in a β-catenin-independent biochemical pathway during neuronal maturation events such as dendrite spine formation in forebrain pyramidal neurons.[22] Separately, we have shown that deletion of Dact1 in cortical interneuron precursors leads to a reduction in synapse numbers along their dendrites. Unlike the dendrite spine phenotype in *Dact1* mutant pyramidal neurons, the synapse phenotype in *Dact1* mutant interneurons is *not* rescued by RacCA, but can be rescued instead by recombinant overexpression of Dishevelled-1 (Dvl1), a direct binding partner of the Dact1 protein.[20] This suggests that these 2 phenotypes—in dendrite spine formation and in synapse formation—may result from different molecular requirements for Dact1 during neuronal maturation in the forebrain. Here we show that, as in cultured hippocampal pyramidal neurons, Dact1 is required for elaboration of fully complex dendrite arbors in cultured forebrain cortical interneurons. Our data support a conserved role across neuron subtypes for this scaffold protein in β-catenin-independent signaling contributing to multiple neuronal maturation events in the postnatal mammalian forebrain.

Dendrites of *Dact1* mutant cortical interneurons are less complex

We and others have previously reported that *Dact1* is expressed in the embryonic ganglionic eminences (GE) in pre-migratory interneuron precursors and in the migratory immature cortical interneurons derived from them.[18-21] Nonetheless, Dact1 loss during embryonic stages has no substantial effects on interneuron migration during development or on interneuron distribution in the postnatal cortex. Instead, there is a cell-autonomous requirement for Dact1 in the production of postsynaptic contacts, including both excitatory and inhibitory postsynaptic contacts, in forebrain cortical interneuron dendrites.[20] There is a similar requirement for Dact1 during synaptogenesis in hippocampal pyramidal neurons, in which it is also required for dendrite spine formation and for the elaboration of fully complex dendrite arbors.[22] In the present study, we asked whether Dact1 is important for the elaboration of complex dendrite arbors in cortical interneurons of the postnatal mouse forebrain.

To test this hypothesis, we genetically crossed a bacterial artificial chromosome (BAC) transgenic *Lhx6GFP* mouse line that labels GE-derived interneurons[23] into the constitutive *null Dact1* knockout mouse line,[14] thereby facilitating the specific identification of cortical interneurons in mice mutant for *Dact1* and in littermate control mice not mutant for *Dact1*.[20] Cortical neurons taken from neonatal *Dact1*$^{-/-}$*;Lhx6GFP* (*Dact1* mutant) and *Dact1*$^{+/+}$*;Lhx6GFP* (littermate control) mice were cultured for 5, 10, and 15 d prior to microscopic analysis. Randomly selected *Lhx6GFP* expressing neurons from *Dact1* mutant mice appeared by inspection to have normal dendrite arborization at 5 d in vitro (DIV) but less complex dendrite arborization at 10 and 15 DIV, compared with those cultured simultaneously under identical conditions from littermate control mice (**Fig. 1A**). Systematic quantification by Sholl analysis confirmed this observation: *Lhx6GFP* expressing interneurons from *Dact1* mutant mice had significantly fewer numbers of dendrite branch intersections at 10 and 15 DIV compared with those from control mice, whereas no significant differences were observed at 5 DIV (**Fig. 1B**).

Our results support a requirement for Dact1 in the postnatal arborization of dendrites in forebrain cortical interneurons, in addition to the prior reported role for this protein during synapse formation in these cells. Sholl analysis convincingly demonstrates that the differences in complexity between *Dact1* mutant and control interneuron dendrite arbors at 10 DIV and 15 DIV are robust; nevertheless the conclusion that Dact1 is *directly* involved in dendritogenesis remains provisional: We cannot exclude the possibility that the emergence of reduced dendrite complexity in mutant interneurons cultured beyond 5 DIV occurs secondary to the requirement for this protein in synapse formation, either in the interneurons themselves, or in their co-cultured mutant excitatory synaptic partners.[22] This is an important consideration given our prior finding that this protein is important for excitatory synapse formation,[20,22] and given that synaptic activity can promote dendritogenesis.[24-27] Further experimental work in which we avail ourselves of electrophysiology combined with more sophisticated genetic strategies (i.e., interneuron-specific[20] and/or temporally-controlled knockout and rescue) can help resolve this remaining issue.

Methods

Regulatory approval

All methods complied with a protocol approved by the Institutional Animal Care and Use Committee at the University of California San Francisco.

Animals

The constitutive *null Dact1* allele was derived from *Dact1*$^{tm1.Bnrc}$ as previously described.[14] The *Lhx6-GFP* BAC transgenic mouse line was provided by the Gene Expression Nervous System Atlas (GENSAT) Project (Rockefeller University, New York, NY). Mutant and control animals were littermate offspring from a *Dact1*$^{-/+}$*;Lhx6*-GFP$^{/+}$ intercross.

Primary neuronal cultures

Cortical neuron cultures were prepared as described previously[23] and plated at low density (6.25 x 10^4 cells per cm^2) so that all dendrites from each GFP$^+$ interneuron analyzed could be readily distinguished from neurites of any neighboring GFP$^+$ cells.

Immunofluorescence staining (anti-GFP)

Cultures at the indicated day in vitro were fixed, blocked, immunostained, and mounted for imaging as previously described.[20] The primary antibody was rabbit anti-GFP (Invitrogen) diluted 1:400 in blocking solution and incubated overnight at 4°C. The secondary antibody was Alexa-Fluor 488-conjugated goat anti-rabbit (Invitrogen) diluted 1:400 in blocking solution and incubated for 2 h at room temperature.

Image Analysis

A Nikon Spectral C1si confocal microscope was employed for all image acquisition. A 40 × oil objective was used for cultures at 5 and 10 DIV; a 20 × objective for cultures at 15 DIV.[23] A z stack consecutive image series was assembled for each GFP$^+$ target interneuron to ensure that processes were fully visualized in 3 dimensions. The Sholl Analysis plugin (Anirvan Ghosh, University of California, San Diego, La Jolla, CA) for the NIH ImageJ software suite was used for dendrite arbor complexity analysis.

Sample Size

Results represent data from at least 2 independent experiments, each experiment involving at least 2 mice per genotype with 15-20 interneurons analyzed per animal.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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