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Small GTPase Rnd1 is involved in neuronal activity-dependent dendritic development in hippocampal neurons

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Abstract

Rho family small GTPases are key regulators for neuronal morphogenesis including dendritogenesis. We recently have shown that Rnd1, a member of the Rho family, is highly expressed in brain during the synaptogenic stage and is involved in dendritic spine formation. However, the mechanism by which Rnd1 regulates dendritic development including spine morphogenesis remains unknown. Here we report that Rnd1, a member of the Rho family, plays a critical role in neuronal activity-dependent dendritic development in hippocampal neurons. Overexpression of Rnd1 promoted dendritic growth and branching in cultured hippocampal neurons. On the other hand, suppression of endogenous Rnd1 expression by RNA interference significantly inhibited neuronal activity-dependent dendritic development and this inhibitory effect was canceled by inhibition of RhoA effector ROCK. In addition, knockdown of Rnd1 also abolished dendritic development promoted by treatment with brain-derived neurotrophic factor in hippocampal neurons. Our findings demonstrate that Rnd1 is involved in signaling pathways of neuronal activity-dependent dendritic development.

Keywords: Rnd; Rho; Dendrite; Branching; Neuronal activity; BDNF

In the developing nervous system, neurons extend and retract axons and dendrites toward proper targets and induce synaptic connections with appropriate partners. Morphological structures of dendrites are critical in that dendrites are the primary sites of synaptic contacts [9]. Multiple environmental cues, including neuronal activity and neurotrophins, regulate dendritic morphogenesis through changes in the organization of cytoskeleton [24,29].

The Rho family of small GTPases is implicated in morphological changes of various cells by reorganizing cytoskeleton [4]. Rho family GTPases serve as molecular switches by cycling between GDP-bound inactive state and GTP-bound active state, and once activated, they transduce signals to a variety of specific downstream effectors, leading to morphological changes [3]. Among them, the functions of Rho, Rac, and Cdc42 have been characterized extensively, and they have been shown to regulate distinct aspects of dendritic development [19]. Recent reports have also revealed that environmental cues, such as neuronal activity and extracellular factors, modulate activity of the Rho family to regulate dendritic morphogenesis [6,27]. However, precise signaling pathways by which such cues regulate dendritic development are not well known.

The Rnd subfamily, consisting of Rnd1, Rnd2, and Rnd3/RhoE, is a new branch of the Rho family, whose function is little known [20]. Unlike other Rho family GTPases, the Rnd subfamily possesses very low intrinsic GTPase activity and constitutively binds to GTP, indicating that they are constitutively active. Rnd1 and Rnd3 antagonize RhoA-signaling pathways by activating RhoA inactivator, p190 RhoGAP [28], or binding to its effector, Socius [12], and then they lead to loss of stress fibers, retraction and rounding of the cell body, and protrusion of extensively branching processes in fibroblasts. Recently, we have shown that Rnd1 is highly expressed in cortical and hippocampal neurons during early postnatal stage, and is essential for dendritic spine formation in hippocampal neurons [8]. But so far, the mechanism by which Rnd1 regulates dendritic development including spine morphogenesis remains unknown.

In the present study, we examined the role of Rnd1 in dendritic morphogenesis mediated by neuronal activity in cultured hippocampal neurons. Our findings demonstrate a key function of Rnd1 in the activity-dependent dendritic development.

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Myc-tagged Rnd1 (Myc-Rnd1) and hemagglutinin A (HA)-tagged $RhoA^{V14}$ (HA-$RhoA^{V14}$) were obtained as described previously [2,12]. A short interfering RNA (siRNA) for Rnd1 [7,21] was designed to target 19 nucleotides after the two adenine residues at nucleotides 145 and 146 (5′-ttacacagcctgtttggag-3′) of the rat Rnd1 transcript, and was expressed by using an siRNA expression vector pSilencer (Ambion, Austin, TX, USA). We used a pSilencer plasmid encoding a hairpin siRNA whose sequence is not found in the mouse, human, or rat genome databases supplied with the kit as a control. Enhanced yellow fluorescent protein (EYFP) expression plasmid with the CAG promoter was a generous gift from Dr. J. Miyazaki of Osaka University.

Hippocampal cultures were prepared from the hippocampi of embryonic day 18–19 rats as previously described [5], and cultured in astroglia-conditioned culture medium [Neurobasal medium (Invitrogen, Carlsbad, CA, USA) with 2% B27 supplement (Invitrogen), 0.5 mM L-glutamine, and 100 U/ml penicillin] under the humidified conditions in 95% air and 5% CO_2 at 37 °C. Hippocampal neurons were transfected with test plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. For high K^+ treatment, 50 mM KCl or 50 mM NaCl (as an osmotic control) was added to culture medium at 3 days in vitro (3 DIV), and the culture was incubated for 24 h. At the same time, 10 μM Y-27632 (a generous gift from Mitsubishi Pharma Corporation, Saitama, Japan) was

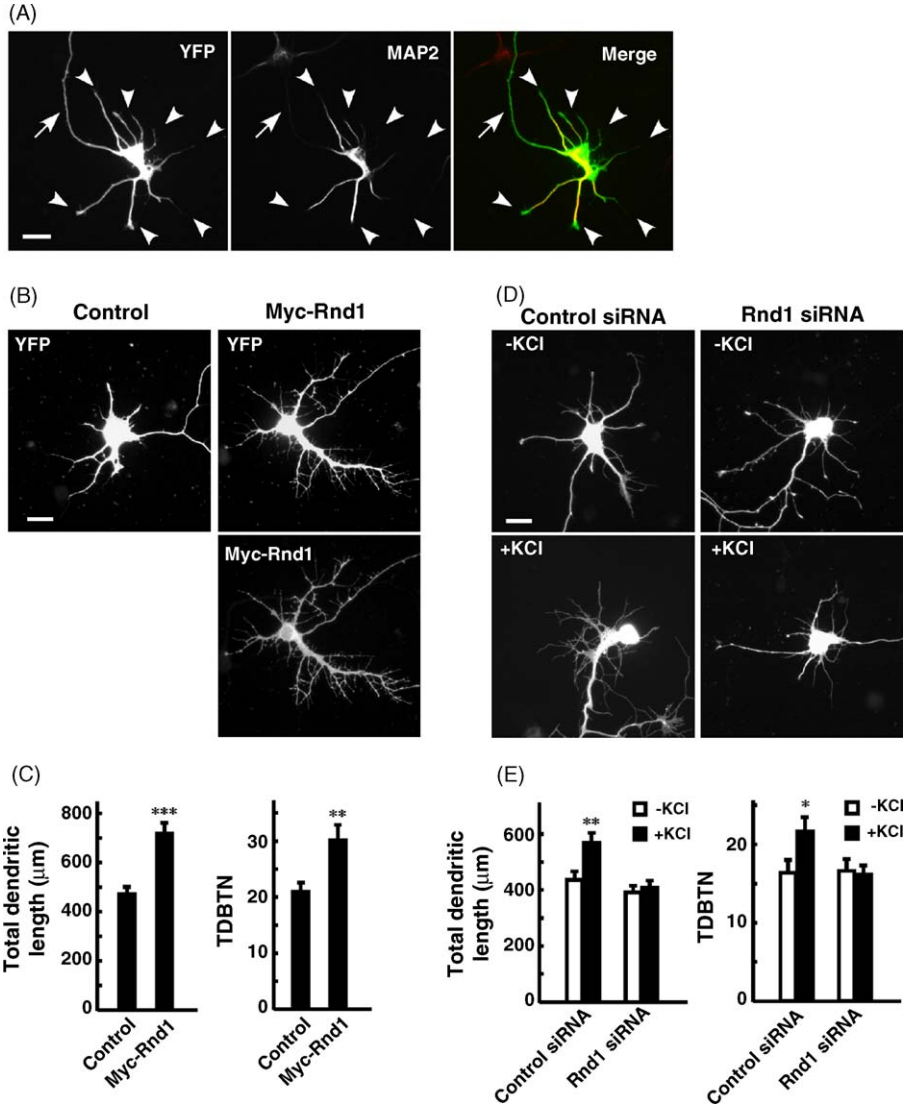

Fig. 1. Effects of Rnd1 overexpression and Rnd1 RNAi on dendritic development in cultured hippocampal neurons. (A and B) Cultured hippocampal neurons from rat embryos were transfected at 3 DIV with a plasmid encoding YFP together with or without a plasmid encoding Myc-Rnd1. Cells at 4 DIV were fixed and immunostained with anti-MAP2 or anti-Myc antibody. Immunofluorescence microscopy for a representative example of neurons expressing YFP alone (A and B, left) or YFP and Myc-Rnd1 (B, right) was shown. (A) YFP and MAP2 labeling were shown in left and middle panels, respectively. A right panel (Merge) showed the superposition of left and middle images. An arrow and arrowheads indicate an axon and dendrites, respectively. (B) YFP and Myc-Rnd1 labeling were shown in top and bottom panels, respectively. (D) After 2-DIV hippocampal neurons were transfected with control (left) or Rnd1 siRNA (right) expression plasmid together with a plasmid encoding YFP, cells at 3 DIV were stimulated with 50 mM KCl for 24 h. At 4 DIV, cells were fixed. Representative YFP images of transfected neurons treated with (bottom) or without (top) KCl were shown. Scale bars, 20 μm. (C and E) Total dendritic length and TDBTN of transfected neurons were measured. The data are the mean ± S.E.M. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.