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PTPRT regulates the interaction of Syntaxin-binding protein 1 with Syntaxin 1 through dephosphorylation of specific tyrosine residue

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ABSTRACT

PTPRT (protein tyrosine phosphatase receptor T), a brain-specific tyrosine phosphatase, has been found to regulate synaptic formation and development of hippocampal neurons, but its regulation mechanism is not yet fully understood. Here, Syntaxin-binding protein 1, a key component of synaptic vesicle fusion machinery, was identified as a possible interaction partner and an endogenous substrate of PTPRT. PTPRT interacted with Syntaxin-binding protein 1 in rat synaptosome, and co-localized with Syntaxin-binding protein 1 in cultured hippocampal neurons. PTPRT dephosphorylated tyrosine 145 located around the linker between domain 1 and 2 of Syntaxin-binding protein 1. Syntaxin-binding protein 1 directly binds to Syntaxin 1, a t-SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) protein, and plays a role as catalysts of SNARE complex formation. Syntaxin-binding protein 1 mutant mimicking non-phosphorylation (Y145F) enhanced the interaction with Syntaxin 1 compared to wild type, and therefore, dephosphorylation of Syntaxin-binding protein 1 appeared to be important for SNARE-complex formation. In conclusion, PTPRT could regulate the interaction of Syntaxin-binding protein 1 with Syntaxin 1, and as a result, the synaptic vesicle fusion appeared to be controlled through dephosphorylation of Syntaxin-binding protein 1.

1. Introduction

Syntaxin-binding protein 1 (Sec/Munc18-like protein) has not been known to be a member of SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) protein complexes, but it is linked to synaptic vesicle fusion by virtue of its tight binding to Syntaxin 1 [1–4]. Membrane fusion in eukaryotic cells is mediated by assemblies of SNAREs and Sec1/Munc18-like proteins [5]. SNAREs are characterized by SNARE motifs, stretches of 60–70 amino acids which contain heptad repeats, and assemble into specific four-helix bundles. SNAREpins were composed of v-SNAREs on transport vesicles and t-SNAREs on the target membrane [6]. The Sec1/Munc18-like proteins bind to SNAREs and control their activity. Originally, Sec1/Munc18 proteins, mammalian homologue of *unc-18*, were determined to be a brain protein which binds stably to Syntaxin [7–9]. When bound to Syntaxin-binding protein 1, Syntaxin 1 appeared to adopt a 'closed' conformation, rendering it inaccessible to its partner SNAREs [10]. It has been thought that Syntaxin 1 must dissociate from Syntaxin-binding protein 1, and switch to an open conformation, to assemble into a SNARE complex [11,12]. However, the open Syntaxin 1 mutation (L165A, E166A) has been shown to have no effect on the interaction with Syntaxin-binding protein 1 [13]. Instead, when the N-terminus motif of Syntaxin 1 was mutated (L8A), the interaction with Syntaxin-binding protein 1 was attenuated and the stimulatory function of Syntaxin-binding protein 1 in SNARE-catalyzed membrane fusion was dramatically reduced. Recently, the primary target of Syntaxin-binding protein 1 during membrane fusion has been thought to be the tertiary SNARE complex, which forms multiple contact sites with Syntaxin-binding protein 1, including core regions from both t- and v-SNARE subunits as well as the N-terminus of Syntaxin 1 [13–15].

It has been reported that Syntaxin-binding protein 3 (Munc18c) is regulated through tyrosine phosphorylation, and tyrosine phosphorylation of Syntaxin-binding protein 3 can dissociate it from Syntaxin 4 [16,17]. Syntaxin-binding protein 3 mutant mimicking dephosphorylation of tyrosine 219 (Y219F) inhibited glucose-stimulated SNARE complex formation and insulin granule exocytosis. Previously, the activity of Syntaxin-binding protein was reported to be regulated through phosphorylation of serine/threonine residues by cyclin-dependent kinase 5 or protein kinase C [18,19]. However, tyrosine kinase (or tyrosine phosphatase) has not yet been assigned a role in regulation of Syntaxin-binding protein, and the regulation mechanism of phosphorylation is not well understood.

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In this new work, PTPRT was found to interact with Syntaxin-binding protein 1 in the synaptosome of rat brain, and to regulate the dephosphorylation of Syntaxin-binding protein 1. Tyrosine 145 of Syntaxin-binding protein 1 was the major target of PTPRT tyrosine phosphatase. PTPRT regulated the interaction of Syntaxin-binding protein 1 with Syntaxin 1 through dephosphorylation of tyrosine 145 of Syntaxin-binding protein 1. Conclusively, PTPRT appears to control the activity of Syntaxin-binding protein 1 in formation of SNARE complexes during fusion of synaptic vesicles.

2. Materials and methods

2.1. Ethics statement

This study was performed in accordance with the regulations outlined by the Korean law. The animal experiment protocols were approved by the Animal Use and Care Committee of Korea Research Institute of Bioscience and Biotechnology (Permit Number: KRIBB-AEC-11022). Animals were sacrificed using CO_2 gas, and all efforts were made to minimize suffering.

2.2. DNA constructs

Full-length human PTPRT (NM_133170, aa 1-1460) was subcloned into GW1-CMV as described previously [20]. For the construction of RT-D, a catalytic domain of PTPRT, DNA encoding aa 921–1173 of PTPRT was subcloned into p3xFLAG-CMV-7.1 (Sigma, USA). Full-length human Syntaxin-binding protein 1 isoform b (NM_001032221.3, aa 1-594) and full-length mouse Syntaxin 1 (NM_016801, aa 1-288) were subcloned into GW1-CMV. The mutant constructs were generated by site-directed mutagenesis using the Quick-Change system (Stratagene, USA).

2.3. Antibodies

Anti-PTPRT monoclonal antibody was prepared as described previously [20]. Anti-Syntaxin-binding protein 1 polyclonal antibody was raised against the C-terminus epitope (aa 580–594) as an antigen in rabbit and purified from rabbit serum using antigen peptide covalently linked with Sulfolink coupling gel (Pierce, USA). Other antibodies were purchased from commercial sources: α-tubulin, MAP2, and FLAG (Sigma, USA), Synaptophysin and Syntaxin 1 (Santa Cruz, USA), NF-L (Cell signaling, USA), PSD-95 and phosphotyrosine 4G10 (Upstate, Germany), Cy3- and FITC-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, USA).

2.4. Cell culture and transfection

HEK293T cells, maintained in DMEM containing 10% fetal bovine serum, were transfected by the calcium phosphate method. After 2 days, cells were lysed with ice-cold 1% (v/v) Triton X-100 in Dulbecco's phosphate-buffered saline (pH 7.4) (Invitrogen, USA) containing inhibitors of proteases and phophatases. After incubation on ice for 30 min, the cell lysates were centrifuged, and the cleared extracts were immunoprecipitated with antibodies.

2.5. Culturing hippocampal neurons, immunohistofluorescence, and image acquisition

Primary hippocampal neurons were prepared from embryonic day 18 (E18) rat, grown on glass coverslip as described previously [21]. At days *in vitro* 15 (DIV 15), hippocampal neurons were fixed, permeabilized, incubated with primary antibodies, and finally incubated with fluorophore-conjugated secondary antibodies as described previously [21]. Individual neurons were imaged using a 63x objective, employing confocal microscopy (LSM 510 Meta, Zeiss, Germany).

3. Results

3.1. Syntaxin-binding protein 1 interacted with PTPRT

Syntaxin-binding protein 1 was identified in this work as a possible interaction partner of PTPRT through mass spectrometry-based proteomics that was used to analyze immunoprecipitated synaptosome proteins of rat brain, employing an anti-PTPRT monoclonal antibody [20] (Fig. 1A and B). Using the Mascot database search program, the amino acid sequences of the combined MS/MS spectra of peptide ions were identified as those of Syntaxin-binding protein 1. Syntaxin-binding protein 1 was efficiently pulled down by anti-PTPRT antibody and conversely PTPRT also was pulled down by anti-Syntaxin-binding protein 1 antibody in deoxycholate-extracted rat synaptosome (Fig. 1C and D). Additionally, when expressed in heterologous cells, PTPRT was shown to interact with Syntaxin-binding protein 1 (Fig. 1E and F). The catalytic domain of PTPRT (RT-D) as well as full-length PTPRT (RT-FL) was pulled down by anti-Syntaxin-binding protein 1 antibody, and these results suggested that Syntaxin-binding protein 1 appeared to interact with the intracellular catalytic domain of PTPRT. During rat brain development, the expression level of Syntaxin-binding protein 1 increased similarly to PTPRT, of which expression gradually increased up to adult stage [20] (Fig. 1G). These data suggested that Syntaxin-binding protein 1 could interact with PTPRT in the neuronal synapses.

The Syntaxin-binding protein1 polyclonal antibody was raised against the C-terminus epitope (aa 580–594) as an antigen, and used for immunohistofluorescence. In rat hippocampal neurons, Syntaxin-binding protein 1 was present in dendrites and axons of neurons marked by staining for MAP2 and NF-L, respectively (Fig. 2A and B). Syntaxin-binding protein 1 co-localized with the representative post- and pre-synaptic markers, PSD-95 and synaptophysin, respectively (Fig. 2C and D). Syntaxin-binding protein 1 was also co-localized with PTPRT in neuronal synapses (Fig. 2E). Thus, Syntaxin-binding protein 1 was found as a new interaction partner of PTPRT; a brain-specific, expressed, tyrosine phosphatase.

3.2. Syntaxin-binding protein 1 is a new substrate of PTPRT

When phospho-tyrosine level of Syntaxin-binding protein 1 was examined in cultured hippocampal neurons treated with pervanadate, a potent phospho-tyrosine phosphatase inhibitor, Syntaxin-binding protein 1 was shown to be significantly phosphorylated at the tyrosine residue (Fig. 3A). Previously, the neuronal tyrosine kinase Fyn, was shown to interact with PTPRT and to phosphorylate the endogenous substrate of PTPRT [20,21]. In the present work, the catalytic domain of PTPRT was shown to interact with Syntaxin-binding protein 1 (Fig. 1E), and therefore it was determined whether Syntaxin-binding protein 1 could be a substrate of PTPRT. Interestingly, Fyn phosphorylated Syntaxin-binding protein 1, and PTPRT significantly decreased the phosphorylation of Syntaxin-binding protein 1 when they were co-expressed in heterologous cells (Fig. 3B). In order to identify the main target of tyrosine phosphorylation, several Syntaxin-binding protein 1 mutants mimicking non-phosphorylation of tyrosine residues, were produced (Fig. 3C and Fig. S1). When the phosphorylation level was examined, tyrosine 145 was shown to be the

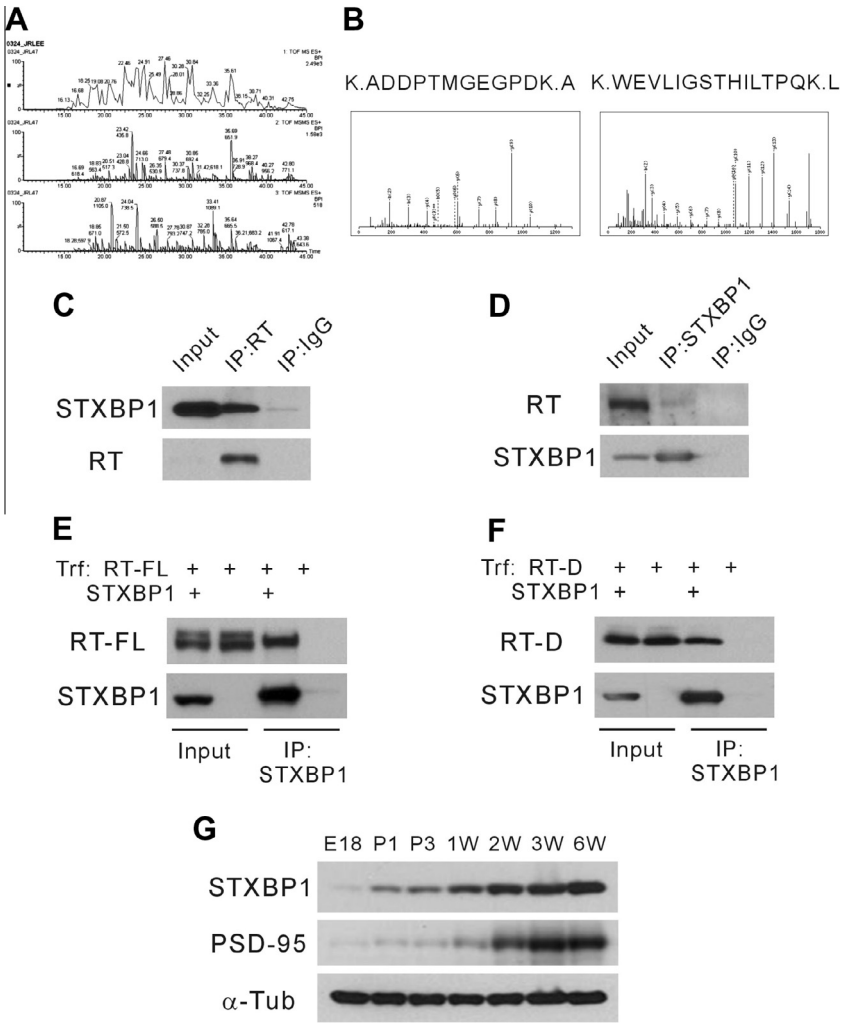

Fig. 1. Syntaxin-binding protein 1 identified as an interaction partner of PTPRT. (A) Representative base peak chromatograms generated in MS and MS/MS modes. The top panel shows the base peak chromatogram of the MS survey scan, and the lower two panels are the base peak chromatograms from the MS/MS channels. (B) Combined MS/MS spectra of Syntaxin-binding protein 1 peptide ions. The amino acid sequences were identified using the Mascot database search program. The matched fragment ion peaks for the most probable sequence are labeled on the spectra. (C) Co-immunoprecipitation of rat brain synaptosome proteins. Syntaxin-binding protein 1 was co-precipitated by an anti-PTPRT monoclonal antibody. *Input: 5%.* (D) Co-immunoprecipitation of rat brain synaptosome proteins. PTPRT was co-precipitated by an anti-Syntaxin-binding protein 1 polyclonal antibody. *Input: 5%.* (E) Co-immunoprecipitation was conducted using Syntaxin-binding protein 1 and full-length PTPRT in HEK cells. Full-length PTPRT was pulled down by Syntaxin-binding protein 1. *Input: 5%.* (F) Co-immunoprecipitation was conducted using Syntaxin-binding protein 1 and deletion mutant PTPRT in HEK cells. The catalytic domain of PTPRT was pulled down by Syntaxin-binding protein 1. *Input: 5%.* (G) Developmental pattern of Syntaxin-binding protein 1 in the rat brain. E, embryonic; P, postnatal days. *STXBP1*, Syntaxin-binding protein 1; *RT*, PTPRT; *RT-FL*, PTPRT full length; *RT-D*, PTPRT catalytic domain; *α-Tub*, α-tubulin; *IP*, immunoprecipitation.

strongest candidate for phosphorylation. A Syntaxin-binding protein 1 mutant mimicking non-phosphorylation of tyrosine 145 (Y145F) showed a significantly reduced level of phosphorylation when co-expressed with Fyn tyrosine kinase. Thus, Tyr145 appeared to be the principal target of Fyn tyrosine kinase and of PTPRT tyrosine phosphatase.

3.3. PTPRT regulated the interaction of Syntaxin-binding protein 1 with Syntaxin 1

Although Syntaxin-binding protein 1 is not a component of SNARE proteins, it is known to control the spatial and temporal activity of SNARE proteins through direct binding to SNAREs as an accessory protein [2,10]. Syntaxin-binding protein 1 is known to be an arch-shaped molecule with a central cavity formed by Domains 1 and 3a that provides the binding surfaces for Syntaxin 1 [22]. The interaction between Domains 1 and 2 appeared to be very important for the packing arrangement to form an arch-shape and a central cavity for Syntaxin 1. Because Tyr145 is located around the linker between Domains 1 and 2, it was determined whether the phosphorylation and dephosphorylation of Tyr145 could regulate the interaction of Syntaxin-binding protein 1 with Syntaxin 1.

The N-terminus of Syntaxin 1 is known as a main domain for the interaction with Syntaxin-binding protein 1, and it has been reported that the interaction was inhibited by the mutation of leucine 8 into alanine (L8A) [13]. Here, Syntaxin 1 mutant (L8A) was shown to decrease the interaction of Syntaxin 1 with Syntaxin-binding protein 1, compared with wild type Syntaxin 1 (*Immunoprecipitation/Input:* Syntaxin 1-WT pulled down by Syntaxin-binding protein 1-WT, 0.30; Syntaxin 1-L8A pulled down by Syntaxin-binding protein 1-WT, 0.10, *Quantification* in the figure legends) (Fig. 4). On the other hand, the open form mutant (L165A, E166A) of Syntaxin 1 did not decrease interaction with Syntaxin-binding protein 1, as described previously [13]. Interestingly, when mutation mimicking tyrosine non-phosphorylation (Y145F) was applied to Syntaxin-binding protein 1, it did not decrease the interaction with the L8A mutant of Syntaxin, differentially from wild type Syntaxin-binding protein 1 (Syntaxin 1-WT pulled down

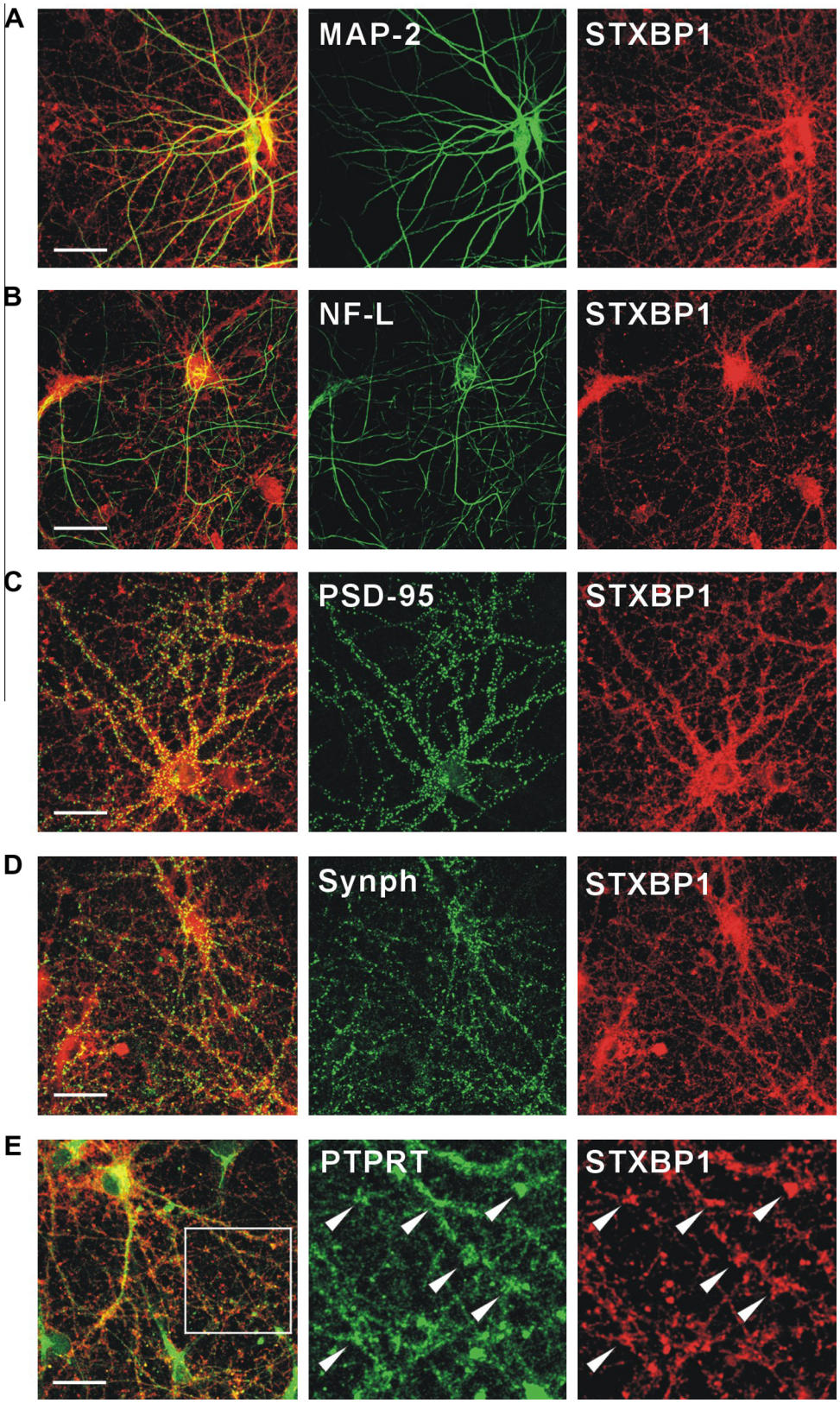

Fig. 2. Syntaxin-binding protein 1 co-localized with PTPRT in hippocampal neurons. (A,B) Syntaxin-binding protein 1 localized to dendritic spine and axon of cultured hippocampal neurons marked by staining for MAP2 and Neurofilament L (NF-L), respectively. Scale bar, 20 μm. (C,D) Syntaxin-binding protein 1 localized to neuronal synapses stained with PSD-95 and Synaptophysin, the representative post- and pre-synaptic marker, respectively. Scale bar, 20 μm. (E) Syntaxin-binding protein 1 co-localized with PTPRT stained with its monoclonal antibody. Scale bar, 20 μm. *STXBP1*, Syntaxin-binding protein 1; *Synph*, Synaptophysin.

by Syntaxin-binding protein 1-Y145F, 0.41; Syntaxin 1-L8A pulled down by Syntaxin-binding protein 1-Y145F, 0.47). Furthermore, Syntaxin-binding protein 1-Y145F appeared to interact with wild type Syntaxin 1 more strongly than wild type Syntaxin-binding protein 1 (Syntaxin 1-WT pulled down by Syntaxin-binding protein 1-WT, 0.30; Syntaxin 1-WT pulled down by Syntaxin-binding

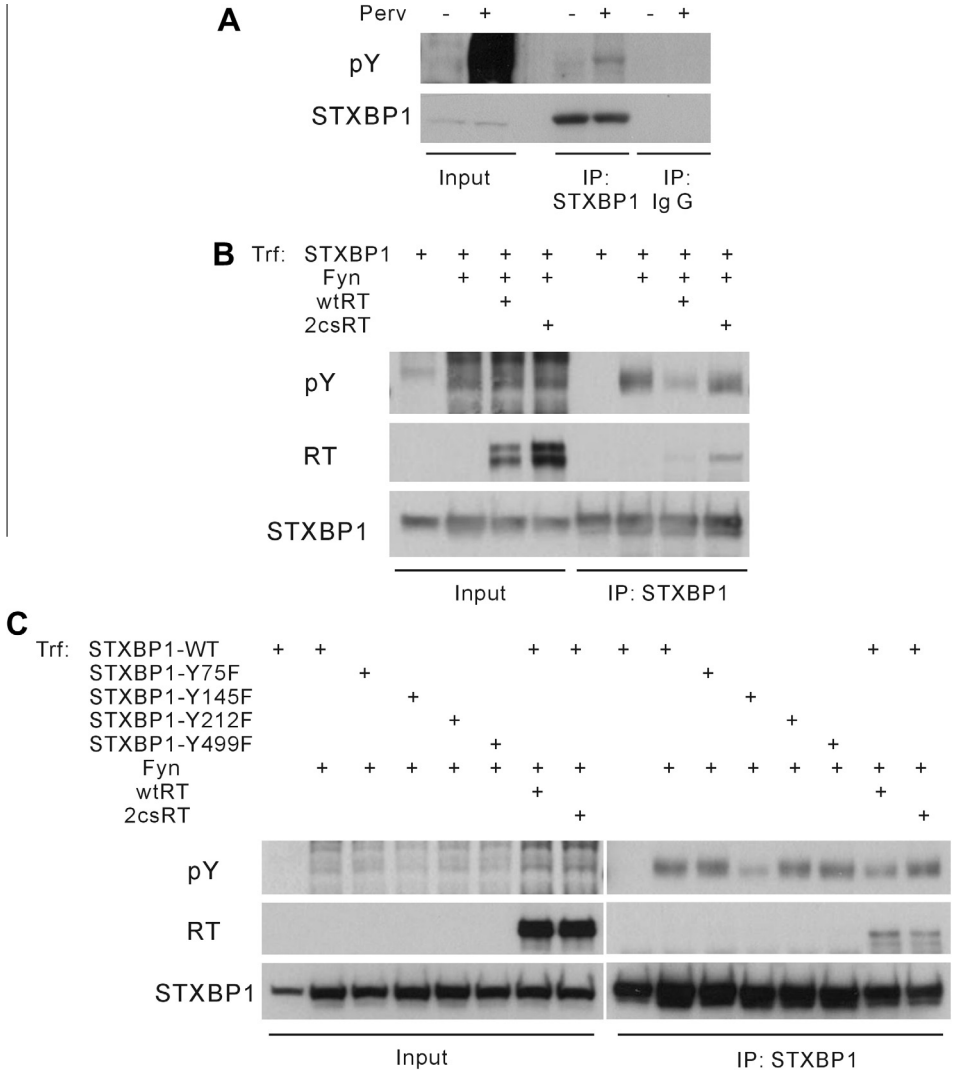

Fig. 3. Syntaxin-binding protein 1 dephosphorylated by PTPRT. (A) Tyrosine phosphorylation of Syntaxin-binding protein 1 in cultured hippocampal neurons. DIV 15 rat hippocampal neurons were solubilized and endogenous Syntaxin-binding protein 1 was immunoprecipitated. When cultured neurons were treated with pervanadate, the tyrosine residue of Syntaxin-binding protein 1 was phosphorylated. Input, 5%. (B) When Syntaxin-binding protein 1, Fyn, and PTPRT were expressed in HEK cells, Syntaxin-binding protein 1 was phosphorylated by Fyn and dephosphorylated by PTPRT. The level of the phosphorylated Syntaxin-binding protein 1 increased when Fyn was co-expressed with Syntaxin-binding protein 1. Significant Syntaxin-binding protein 1 was dephosphorylated upon expression of active PTPRT (wt, wild type), but the level of phophorylation did not decreased upon expression of inactive PTPRT (2cs mutant). Quantification (compared with co-expression of Fyn): with Fyn and wt-PTPRT, 0.30; with Fyn and 2cs-PTPRT, 0.82. (C) Tyrosine 145 of Syntaxin-binding protein 1 is a major target of Fyn and PTRPT. The level of the phosphorylated Syntaxin-binding protein 1 was decreased by mutation of tyrosine 145 to phenylalanine by more than when tyrosine residues 75, 212, and 499 were mutated. The decreased level of phosphorylation by mutation of tyrosine 145 was comparable to the decreased level of phosphorylation upon expression of active PTPRT. Quantification (compared with Syntaxin-binding protein 1-WT co-expressed with Fyn): Syntaxin-binding protein 1-Y75F, 0.94; Syntaxin-binding protein 1-Y145F, 0.22; Syntaxin-binding protein 1-Y212F, 0.90; Syntaxin-binding protein 1-Y499F, 0.99; Syntaxin-binding protein 1-WT with wt-PTPRT, 0.48; Syntaxin-binding protein 1-WT with 2cs-PTPRT, 0.96. *STXBP1*, Syntaxin-binding protein 1; *Perv*, pervanadate treated; *pY*, phosphor-tyrosine; *wtRT*, active PTPRT; *2csRT*, inactive PTPRT, *IP*, immunoprecipitation.

protein 1-Y145F, 0.41). These results suggested that dephosphorylation of Syntaxin-binding protein 1 by PTPRT increased interaction with Syntaxin 1, and that PTPRT could regulate the interaction of Syntaxin-binding protein 1 with Syntaxin 1 through tyrosine dephosphorylation.

4. Discussion

In this study, Syntaxin-binding protein 1 was found for the first time to interact with PTPRT, and dephosphorylation of its tyrosine residue by PTPRT was attributed to its interaction with Syntaxin 1. PTPRT has been shown to regulate synaptic formation and neuronal development of hippocampal neurons, but its regulation mechanism is not yet fully understood [20,21]. This time, PTPRT appeared to control the interaction of Syntaxin-binding protein 1 with Syntaxin 1 through dephosphorylation, and resulted in the regulation of SNARE-complex formation. It was reported previously, that the tyrosine phosphorylation of Syntaxin-binding protein 3, regulated SNARE complex formation and insulin granule exocytosis; although information about specific tyrosine kinase or phosphatase is not known [16,17]. In the central nervous system, the regulation of synaptic vesicle fusion is known to be very important for synaptic functions as well as for neuronal development. Therefore, the result that the interaction of Syntaxin-binding protein 1 with SNARE proteins could be regulated through tyrosine dephosphorylation, suggested a role for PTPRT in synaptic vesicle fusion.

De novo mutations of Syntaxin-binding protein 1 were found in patients with Ohtahara syndrome, an early infantile epileptic encephalopathy [23]. The attenuated binding of Syntaxin-binding

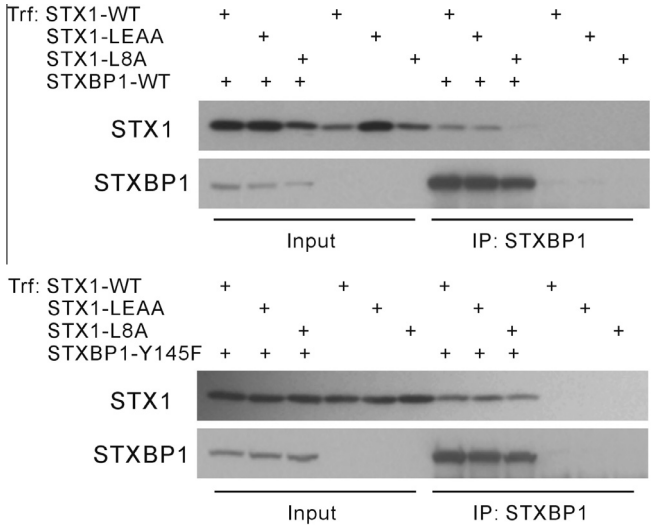

Fig. 4. Interaction between Syntaxin-binding protein 1 and Syntaxin 1 regulated by PTPRT. The interaction of Syntaxin-binding protein 1 with Syntaxin 1 was increased by tyrosine dephosphorylation. When Syntaxin-binding protein 1 and Syntaxin 1 were expressed in HEK cells, Syntaxin-binding protein 1 mutant mimicking tyrosine non-phosphorylation (Y145F) pulled down much more Syntaxin 1 (L8A) compared with wild type Syntaxin-binding protein 1. Quantification (IP/Input): Syntaxin 1-WT pulled down by Syntaxin-binding protein 1-WT, 0.30; Syntaxin 1-L8A pulled down by Syntaxin-binding protein 1-WT, 0.10; Syntaxin 1-WT pulled down by Syntaxin-binding protein 1-Y145F, 0.41; Syntaxin 1-L8A pulled down by Syntaxin-binding protein 1-Y145F, 0.47. *STX1*, Syntaxin 1; *STXBP1*, Syntaxin-binding protein 1; *IP*, immunoprecipitation.

protein 1 mutant form to Syntaxin 1, inhibited SNARE complex formation and resulted in severe epilepsy. The interaction between Syntaxin-binding protein 1 and Syntaxin 1, therefore, appears very important for normal brain functions. It has been reported that the Arg50 → Cys mutant of Rop, the Drosophila orthologue of Syntaxin-binding protein 1, increased neurotransmitter release [8]. In the Syntaxin-binding protein 1–Syntaxin 1 complex, Arg39, located in Domain 1 of Syntaxin-binding protein 1 (Arg50 of Rop); directly contacts Glu234 of Syntaxin 1 and also interacts with Asp148, located in Domain 2 of Syntaxin-binding protein 1. The linker between Domains 1 and 2 appeared to be important for the packing arrangement of Syntaxin-binding protein 1 and the interaction of Syntaxin-binding protein 1 with Syntaxin 1 [22]. In this study, tyrosine 145 located around the linker between Domains 1 and 2 of Syntaxin-binding protein 1, was found to be dephosphorylated by PTPRT, and to play roles in the interaction with Syntaxin 1. In the Rop, the Drosophila orthologue of Syntaxin-binding protein 1, tyrosine 145 is substituted into phenylalanine. When tyrosine 145 of Syntaxin-binding protein 1 was changed to phenylalanine to make the mutant mimicking non-phosphorylation, the interaction between Syntaxin-binding protein 1 and Syntaxin 1 was significantly enhanced (Fig. 4). The mutation of Y145F enhanced the interaction of Syntaxin-binding protein 1 with wild type Syntaxin 1 as well as mutant Syntaxin 1 (L8A). Although N-terminal peptide of Syntaxin 1 was critical for the interaction with Syntaxin-binding protein, it was shown that Syntaxin-binding protein 1 still associated with SNARE liposome when N-peptide motif was mutated (L8A) by Shen et al. [13]. They suggested that the primary target of Syntaxin-binding protein 1 during membrane fusion is the 'tertiary' SNARE complex, and Syntaxin-binding protein 1 appeared to interact with both t- and v-SNARE subunits as well as the N-terminal peptide of Syntaxin 1. However, there could be a possibility that Syntaxin-binding protein 1 was transformed so as to interact with even mutant Syntaxin 1 when associated with SNARE liposome. Because Tyr145 of Syntaxin-binding protein 1 is located around the linker between Domains 1 and 2, the dephosphorylation of Tyr145 could transform the arch-shaped structure of Syntaxin-binding protein 1, and as a result, the interaction of Syntaxin-binding protein 1 with Syntaxin 1 appeared to be enhanced [22]. Previously, PTPRT was shown to localize to synaptic vesicle in the sub-cellular fractionation of rat brain [20]. It could be suggested that PTPRT regulates the synaptic vesicle fusion machinery through dephosphorylation of Syntaxin-binding protein 1. Tyrosine 212 of Syntaxin-binding protein 1 (tyrosine 219 of Syntaxin-binding protein 3) appeared not to be a major target of PTPRT (Fig. 3C) although it has been reported that mutation of Y219F significantly increased the affinity of Syntaxin-binding protein 3 for Syntaxin 4, and inhibited SNARE complex formation in insulin granule exocytosis [16]. The result that dephosphorylation of Syntaxin-binding protein 1 increased the interaction with Syntaxin 1, could suggest the role of PTPRT in neurotransmitter release as well as in SNARE complex formation in central nervous systems [20,24,25].

PTPRT is known to regulate the development of hippocampal neurons, in addition to the formation of neuronal synapses [21]. When PTPRT was knocked down, dendritic arborization was severely attenuated and the number of dendritic spines was significantly reduced. It has been reported that the speed of neurite outgrowth decreased during early development in the Syntaxin-binding protein 1-null brains [26]. It is interesting that PTPRT knock-down and the Syntaxin-binding protein 1 null-mutant had the same phenotype, and these results suggest that the roles of Syntaxin-binding protein 1 in neuronal development could be regulated through dephosphorylation by PTPRT.

In conclusion, PTPRT interacted with Syntaxin-binding protein 1, and appeared to regulate the formation of SNARE protein complex. Tyrosine residue located around the linker of Syntaxin-binding protein 1 could be a major target of PTPRT tyrosine phosphatase, and the dephosphorylation of specific tyrosine residue, enhanced interaction with Syntaxin 1 by changing the binding surface of Syntaxin-binding protein 1.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.08.033.

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