Scaffolding proteins are major contributors to the spatial and temporal orchestration of signaling cascades and hence cellular functions. RACK1 is a scaffolding protein that plays an important role in the regulation of, and cross-talk between, various signaling pathways. Here we report that RACK1 is a mediator of chromatin remodeling, resulting in an exon-specific expression of the brain-derived neurotrophic factor (BDNF) gene. Specifically, we found that following the activation of the cAMP pathway, nuclear RACK1 localizes at the promoter IV region of the BDNF gene by its association with histones H3 and H4, leading to the dissociation of the transcription repressor methyl-CpG-binding protein 2 (MeCP2) from the promoter, resulting in the acetylation of histone H4. These chromatin modifications lead to the activation of the promoter and to the subsequent promoter-controlled transcription of BDNF exon IV. Our findings expand our knowledge regarding the function of scaffolding proteins such as RACK1. Furthermore, this novel mechanism for the regulation of exon-specific expression of the BDNF gene by RACK1 could have implications on the neuronal functions of the growth factor including synaptic plasticity, learning, and memory.

Signal transduction cascades are tightly regulated events. Scaffolding proteins play an essential role in the spatial and temporal regulation of individual enzymes and provide the link between extracellular events and intracellular compartments including the nucleus (1). Scaffolding proteins, by means of protein-protein interactions, provide platforms for protein assembly. The scaffolding protein RACK1, which was originally identified as an anchoring protein of protein kinase C (2), belongs to the WD40 family of proteins characterized by seven WD40 repeats forming a seven-blade propeller structure (3, 4). This particular structure allows RACK1 to interact with various enzymes including Fyn kinase (5), focal adhesion kinase (6), receptor protein tyrosine phosphatase µ (7), the cyclic AMP-specific phosphodiesterase (PDE4) isoform PDE4D5 (8), as well as the intracellular tails of receptors such as the insulin-like growth factor 1 receptor (IGF-1R) (9, 10), the inositol 1,4,5-trisphosphate receptor (11), and ion channels such as the NR2B subunit of the N-methyl-D-aspartate receptors (5). These interactions, and others, put RACK1 at a focal point for spatial and temporal regulation of various signaling cascades. For example, in transformed cultured cancer cells, RACK1 was shown to form a scaffolding complex that includes the IGF-1R, β1 integrin, and focal adhesion kinase (6, 9, 10, 12). In addition, RACK1 is also found at the 40 S ribosomal subunit (3, 13, 14) and was shown to bridge protein kinase C-mediated signaling to the ribosomal translation machinery (15). RACK1 plays an important role in the central nervous system (16). For example, RACK1 links Fyn kinase to its substrate, the NR2B subunit of the N-methyl-D-aspartate receptor (5, 17), and contributes to the regulation of channel activity (18). Interestingly, the intracellular compartmentalization of RACK1 changes in response to stimuli. For example, cellular stress such as hypoxia and heat shock results in RACK1 sequestration into cytoplasmic stress granules, which prevents cell apoptosis by suppressing the stress-responsive mitogen-activated protein kinase (MAPK)2 pathway (19). In addition, we found that upon the activation of the protein kinase C pathway, RACK1 shuttles protein kinase C βII to its site of action (20), whereas activation of a cAMP-dependent pathway induces the translocation of RACK1 to the nucleus, resulting in alterations in gene expression including BDNF (18, 21, 22).

BDNF belongs to the nerve growth factor (NGF) family of neurotrophic factors (23). Through its receptor tyrosine kinase, TrkB, BDNF activates several signaling pathways such as the MAPK, phosphatidylinositol-3-OH kinase, and phospholipase Cγ cascades (24). BDNF plays an important role in neuronal proliferation, differentiation, and survival, as well as synaptic plasticity, learning, and memory (23, 25, 26). The genomic structure of the BDNF gene consists of eight 5’-non-coding exons and one 3’-coding exon (27, 28) and is very similar between human and rodents (27–30). The expression of each of the eight 5’ exons is separately controlled by an individual promoter, which is then spliced to the common 3’
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exon that encodes the BDNF protein (28, 31). Exon expression is differentially responsive to various types of stimulation (31–36). For example, exon IV is the major contributor to neuronal activity-dependent BDNF expression (33), and significant increases in the expression of the BDNF IV were observed in the amygdala and hippocampus of rats that had experienced a fear-conditioning paradigm (34, 35), whereas the level of BDNF exons I and VI are up-regulated in the hippocampus of rats 2 h after context exposure (34). Electroconvulsive seizures lead to increases in the expression of BDNF exon II, as well as exon VI, in rat hippocampus (37), and differential expression of BDNF exons was also found during different periods of prefrontal cortex development (38).

Here we aimed to identify the mechanism by which RACK1 acts as a transcription regulator of BDNF expression. We show that RACK1 specifically associates with BDNF promoter IV and regulates chromatin remodeling at the promoter.

EXPERIMENTAL PROCEDURES

Materials—pRNAT-H1.1/Shuttle vector was purchased from GenScript Corp. (Piscataway, NJ). Adeno-X vector and Adeno-X virus purification kit were purchased from Clontech. Chromatin immunoprecipitation (ChIP) assay kit, rabbit polyclonal anti-acetyl H3 (acetyl-Lys-9 and -14), rabbit polyclonal anti-acetyl H4 (acetyl-Lys-5, -8, -12, and -16), rabbit monoclonal anti-H3 (pan), and rabbit polyclonal anti-H4 (pan) antibodies were purchased from Millipore (Billerica, MA). Rabbit polyclonal anti-MeCP2 antibody was purchased from Abcam Inc. (Cambridge, MA). Mouse monoclonal anti-RACK1 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Lipofectamine 2000 was purchased from Invitrogen. For skolin and mouse monoclonal anti-

Cell Culture—SH-SY5Y human neuroblastoma cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum plus 1 X minimum Eagle’s medium non-essential amino acid solution. Cells were incubated in a low serum medium containing 1% fetal bovine serum for 2 days before experiments.

Preparation of Primary Hippocampal Neurons—A litter of Sprague-Dawley rats (Harlan, Indianapolis, IN) was obtained on either the day of birth or the first postnatal day (postnatal days 0–1) and were euthanized by rapid decapitation. The hippocampi were dissected out, pooled, and digested in a papain solution (Worthington Biochemical) for 30 min. Digestion was stopped by brief incubation with a trypsin inhibition solution (Sigma-Aldrich), and cells were mechanically dissociated by pipette trituration and plated on poly-d-lysine-treated plates (Nunc International, Rochester, NY). Neurons were maintained in culture for 3 weeks in Neurobasal-A medium (Invitrogen) supplemented with B27 and GlutaMax supplements (Invitrogen), as well as penicillin and streptomycin, with 50% of the media being changed every 3 days. Additionally, 10 μM cytosine arabinoside was added on Day 1 to inhibit growth of glial cells.

Reverse Transcription-PCR (RT-PCR)—Total RNA was isolated using TRIzol reagent and used for reverse transcription using the reverse transcription system at 42 °C for 30 min. RT products were used for PCR with 32 cycles run to measure total BDNF expression and 34 cycles to measure BDNF exon expression. Primers based on either the human or the rat BDNF genes were used for detection of BDNF expression in SH-SY5Y cells and hippocampal neurons, respectively (supplemental Table S1).

Immunoprecipitation—After treatment, SH-SY5Y cells were collected and homogenized in a radioimmune precipitation assay (RIPA) buffer (50 mM Tris-Cl, pH 7.6, 150 mM NaCl, 2
mm EDTA, 1% Nonidet P-40, 0.1% SDS, and 0.5% sodium deoxycholate) plus protease and phosphatase inhibitors as described previously (41, 42). Briefly, 500 μg of the homogenate was incubated with 4 μg of anti-RACK1 antibody in phosphate-buffered saline containing 0.1% Tween 20 overnight at 4 °C followed by a 2-h incubation with protein G-agarose beads. Immunoprecipitates were separated on 4–12% gradient SDS-PAGE gels followed by immunoblotting with anti-H3 or anti-H4 antibodies and then reprobed with anti-RACK1 antibody.

**ChIP**—ChIP was conducted using the chromatin immunoprecipitation assay kit following the manufacturer’s protocol. Immunoprecipitation of proteins, after ChIP with antibodies against MeCP2, acetyl H3, acetyl H4, or RACK1, was confirmed by Western blot analysis before the ChIP-PCR analysis of the fragments of BDNF promoters with the appropriate promoter primers (supplemental Table S2). Products of ChIP-PCR were separated on a 2% agarose gel with ethidium bromide, and the PCR signals were analyzed as described above.

**Quantification and Statistical Analysis**—PCR product bands were quantified to analyze the expression level of total BDNF or its exons. Results are expressed as ratios of total BDNF or its exons to the internal controls, actin or GAPDH. ChIP-PCR product bands indicated the DNA levels of BDNF promoters associated with the protein MeCP2, acetylated histones H3 or H4, or RACK1. For the analysis of BDNF promoter IV levels associated with acetylated histones H3 and H4, ChIP-PCR products were quantified and expressed as ratios of the acetylated histone (H3 or H4)-associated BDNF promoter IV amount (ChIP-PCR) to the total promoter amount (input-PCR). Results were obtained from at least three experiments, and the statistical significance was determined by Student’s t test or by one-way or two-way ANOVA test followed by the Student-Newman-Keuls test as indicated in the legends.

**RESULTS**

**RACK1 Is Required for the cAMP-mediated Increase in BDNF Expression**—We previously found that the activation of the cAMP pathway in hippocampal neurons leads to the translocation of RACK1 into the nucleus (18). We also obtained data suggesting that nuclear RACK1 contributes to BDNF expression in hippocampal and striatal neurons (18, 22). Specifically, we showed that a fragment of RACK1 expressed as a Tat fusion protein, acting as an inhibitory form of RACK1, prevented endogenous RACK1 from translocating to the nucleus (21) and also inhibited the increase in BDNF expression upon the activation of the cAMP pathway (18). To further explore the role of RACK1 in BDNF expression, we cloned two siRACK1 sequences (siRACK1-a and siRACK1-b) into an adenoviral vector and prepared the recombinant adenoviruses AdV-siRACK1-a and AdV-siRACK1-b to deliver the siRACK1s. Down-regulation of RACK1 expression was detected after infection of the dopaminergic-like SH-SY5Y cells with either virus (Fig. 1, A, B, and D). Similar down-regulation of RACK1 level was also observed in primary hippocampal neurons infected with AdV-siRACK1-a (Fig. 1C). BDNF mRNA levels were then measured in response to the activation of the cAMP pathway with the adenylate cyclase activator, forskolin (FSK), in both cell models. As shown in Fig. 2, A and B, FSK treatment induced BDNF expression in SH-SY5Y cells and hippocampal neurons. The increase in the expression of BDNF is specific because FSK did not induce the expression of NGF, a closely related neurotrophic factor (Fig. 2B). FSK treatment induced an increase in the expression of BDNF in cells infected with non-related sequence-recombinant adenovirus (control virus, AdV-siCT) (Fig. 2, C and D). However, down-regulation of RACK1 mRNA levels with AdV-siRACK1-a or AdV-RACK1-b significantly decreased BDNF expression induced by FSK in SH-SY5Y cells (Fig. 2C). Similarly, infection with AdV-siRACK1-a decreased BDNF expression in hippocampal neurons (Fig. 2D). Together, these results show that RACK1 is indeed required for cAMP-mediated induction of BDNF expression.

**RACK1 Displays a Specific Role in the Regulation of BDNF Exon IV Expression**—Because BDNF exons are differentially transcribed upon stimulation (31, 33–35), we tested whether RACK1 plays a selective role in the regulation of BDNF exon expression. To do so, we measured the mRNA levels of the four major exons of the BDNF gene: I, II, IV, and VI (supplemental Fig. S2) in primary hippocampal neurons in the presence and absence of FSK. As shown in Fig. 3, A and B, FSK treatment induced an increase in the expression of BDNF exons I, IV, and VI in neurons infected with AdV-siCT, whereas the FSK-induced increase in the mRNA of exon IV, but not exons I and VI, was blocked in neurons infected with AdV-siRACK1-a.
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Similar results were obtained in SH-SY5Y cells infected with AdV-siRACK1-a or AdV-siRACK1-b as compared with cells infected with AdV-siCT (Fig. 3C). We previously showed that treatment of cells with Tat-tagged full-length RACK1 (Tat-RACK1) results in the transduction of the recombinant protein into both the cytoplasm and the nucleus (21, 22). We further showed that treatment of hippocampal neurons with Tat-RACK1 results in up-regulation of the levels of BDNF (22). To confirm the selective role of RACK1 in regulating BDNF exon IV expression, we treated SH-SY5Y cells with Tat-RACK1 and measured the expression of BDNF and its exons IV and VI. As shown in Fig. 3D, transduction of Tat-RACK1 increases BDNF exon IV, but not exon VI. Together, these results strongly suggest a specific role for RACK1 in the regulation of BDNF exon IV expression.

RACK1 Associates with BDNF Promoter IV—The requirement of RACK1 for the transcription of BDNF exon IV raises a possibility that RACK1 participates in the regulation of the promoter IV activation. Thus, we hypothesized that upon activation of the cAMP pathway, RACK1 is translocated to the promoter region. Because RACK1 does not contain DNA-binding domains, a possible association of RACK1 with the promoter may occur with the chromatin complex and, specifically, with histones. To test this possibility, we examined whether histone H3 and or H4 can be co-immunoprecipitated with anti-RACK1 antibodies after FSK treatment. As shown in Fig. 4, FSK treatment of hippocampal neurons was infected with control adenovirus (siCT) or siRACK1-a recombinant adenovirus (siR1a) for 3 days before treatment with 10 μM FSK for 1 h followed by RT-PCR analysis of BDNF exon IV expression. The histogram depicts the mean ratios of BDNF exon IV to actin ± S.D. from six experiments. Two-way ANOVA test, **, p < 0.01; NS, no significant difference. C, SH-SH5Y cells were incubated without or with 10 μM FSK for 1 h followed by RT-PCR analysis of the expression of BDNF exon IV. The histogram depicts the mean ratios of BDNF exon IV to actin ± S.D. from three experiments. Student’s t test, **, p < 0.01.

FIGURE 2. Down-regulation of RACK1 expression attenuates FSK-mediated induction of BDNF expression. A, SH-SY5Y cells were treated without (Con) or with 10 μM FSK for the indicated times. BDNF expression was analyzed by RT-PCR. Actin was used as an internal control. The histogram depicts the mean ratio of BDNF to actin ± S.D. from three experiments. One-way ANOVA test, *, p < 0.05, as compared with control. B, hippocampal neurons were treated without (Con) or with 10 μM FSK for the indicated times, and BDNF and NGF expression was analyzed by RT-PCR. GAPDH was used as an internal control. The histogram depicts the mean ratios of BDNF or NGF to GAPDH ± S.D. from three experiments. One-way ANOVA test, *, p < 0.05; **, p < 0.01, as compared with control. C, SH-SY5Y cells were infected with control adenovirus (siCT) or siRACK1-a recombinant adenovirus (siR1a) or recombinant adenovirus siRACK1-b (siRACK1b). Three days after infection, cells were treated without or with 10 μM FSK for 1 h followed by RT-PCR analysis of total BDNF expression. The histogram depicts the mean ratios of BDNF to actin ± S.D. from six experiments. Two-way ANOVA test, *, p < 0.05; **, p < 0.01. D, hippocampal neurons were infected with the viruses AdV-siCT or AdV-siR1a for 3 days and then treated with 10 μM FSK for 1 or 3 h. BDNF expression was analyzed by RT-PCR. The histogram depicts the mean ratios of BDNF to GAPDH ± S.D. from four experiments (1 h treatment) or three experiments (3 h treatment). Two-way ANOVA test, *, p < 0.05; **, p < 0.01.

FIGURE 3. RACK1 is required for BDNF exon IV expression. A and B, hippocampal neurons were infected with control adenovirus (siCT) or siRACK1-a recombinant adenovirus (siR1a) for 3 days before treatment with 10 μM FSK for 1 h followed by RT-PCR analysis of BDNF exon IV expression. The histogram depicts the mean ratios of each BDNF exon to GAPDH ± S.D. from six experiments. Two-way ANOVA test, *, p < 0.05; **, p < 0.01. C, hippocampal neurons were infected with siCT or AdV-siR1a or siRACK1b for 3 days and treated without or with 10 μM FSK for 1 h followed by RT-PCR analysis of BDNF exon IV expression. The histogram depicts the mean ratios of BDNF exon IV to actin ± S.D. from six experiments. Two-way ANOVA test, *, p < 0.05; **, p < 0.01; NS, no significant difference. D, hippocampal neurons were incubated without (Con) or with 1 μM Tat-RACK1 for 2 h and then used for analysis of BDNF and its exon expression by RT-PCR. The histogram depicts the mean ratios of BDNF or its exon to actin ± S.D. from three experiments. Student’s t test, **, p < 0.01.
suggesting that RACK1 binds to the histone core within the chromatin structure. To provide a direct link between this RACK1-histone association and the RACK1-mediated BDNF exon IV expression, we applied the ChIP assay to specify the location of RACK1 at the promoters of the BDNF gene. After FSK treatment, SH-SY5Y cells were used for the ChIP assay to pull down RACK1 (Fig. 5A), and the association of RACK1 with BDNF promoter IV was detected by PCR (Fig. 5B). We found that FSK induced an association of RACK1 with BDNF promoter IV (Fig. 5B). This association was further detected in hippocampal neurons (Fig. 5C). However, no association of RACK1 with promoter VI in SH-SY5Y cells or hippocampal neurons (Fig. 5, D and E) or with promoter I in hippocampal neurons (Fig. 5E) was observed. These data indicate that RACK1 is specifically associated with the chromatin complex at BDNF promoter IV.

**RACK1 Mediates the Dissociation of MeCP2 from BDNF Promoter IV**—The activation of BDNF promoter IV can be achieved by chromatin remodeling via the release of the transcription repressor, MeCP2, from the promoter leading to DNA demethylation (34, 43). We therefore tested whether nuclear RACK1 up-regulates BDNF expression by promoting the dissociation of MeCP2 from BDNF promoter IV. SH-SY5Y cells were treated with or without FSK before ChIP using anti-MeCP2 antibody (Fig. 6A) to detect MeCP2 levels at BDNF promoter IV in inputs and was analyzed in parallel. Images are representative of multiple experiments.

**FIGURE 4.** Activation of the cAMP pathway induces an association of RACK1 with histones H3 and H4. SH-SY5Y cells were treated without (Con) or with 10 μM FSK for 30 min and then used for immunoprecipitation (IP) of RACK1 with anti-RACK1 antibody. Co-immunoprecipitated histone H3 and histone H4 were detected by immunoblotting with anti-H3 (A) or anti-H4 (B) antibodies followed by reprobing with anti-RACK1 antibody to detect the immunoprecipitated RACK1 protein. Images are representative of three experiments.

**FIGURE 5.** RACK1 associates with BDNF promoter IV. A, SH-SY5Y cells were treated without (Con) or with 10 μM FSK for 30 min before lysis for a ChIP procedure with normal IgG or anti-RACK1 antibody. Samples were subjected to immunoblotting with anti-RACK1 antibody. B, SH-SY5Y cells were treated without (Con) or with 10 μM FSK for the indicated times. Cells were then lysed for a ChIP assay with normal IgG or anti-RACK1 antibody. The RACK1-associated BDNF promoter IV (PIV) in the ChIP samples was detected by PCR with BDNF PIV primers. The level of promoter IV in input was analyzed in parallel. The histogram depicts the mean ratios of RACK1-associated PIV to input PIV ± S.D. from three experiments. One-way ANOVA test, *p < 0.05, as compared with control. C, hippocampal neurons were treated without (Con) or with 10 μM FSK for 30 min before lysis for a ChIP assay with normal IgG or anti-RACK1 antibody. The RACK1-associated BDNF promoter IV in the ChIP samples was detected by PCR with BDNF PIV primers. Promoter IV in the input sample was analyzed in parallel. Images are representative of multiple experiments. D, SH-SY5Y cells were treated without (Con) or with 10 μM FSK and used for a ChIP assay as described above. The RACK1-associated BDNF promoter VI (PIV) in the ChIP samples was detected by PCR with BDNF promoter VI primers. The promoter VI in inputs was analyzed in parallel. Images are representative of three experiments. E, hippocampal neurons were treated without (Con) or with 10 μM FSK for 30 min before conducting the ChIP assay with anti-RACK1 antibody followed by PCR with the primers of BDNF promoter I and promoter VI. The promoters in inputs were analyzed in parallel. Images are representative of multiple experiments.
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**FIGURE 7.** The cAMP signaling pathway mediates RACK1-dependent acetylation of histone H4 at promoter IV of BDNF. A, SH-SYSY cells were treated without (Con) or with 10 μM FSK for the indicated times before a ChIP assay with anti-acetyl H3 or anti-acetyl H4 antibodies followed by immunoblotting with the antibodies. B, SH-SYSY cells were treated without (Con) or with 10 μM FSK for the indicated times. Cells were then lysed for a ChIP assay with normal IgG or anti-acetyl H4 antibodies. The acetylated H4-associated BDNF promoter IV was detected by PCR with BDNF PIV primers. Promoter IV in inputs was analyzed in parallel. The histogram depicts the mean ratios of acetylated H3-associated PIV to input PIV con 25% 50% 75% 100% FSK (min).

**DISCUSSION**

Here we show that following the activation of the cAMP pathway, nuclear RACK1 localizes at the promoter IV region of the BDNF gene, resulting in the dissociation of the BDNF transcription repressor MeCP2 from the promoter and histone H4 acetylation. These chromatin modifications lead to the activation of the promoter and to the consequent promoter-controlled BDNF exon IV transcription.

The function of RACK1 has been mainly related to the regulation of signaling cascades via its association with enzymes such as kinases and phosphatases, although recent studies have expanded the role of the protein. Specifically, Liu et al. (47) showed that RACK1 interacts with the transcription factor hypoxia-inducible factor (HIF-1), resulting in HIF-1 ubiquitination, and Arimoto et al. (19) showed that RACK1 coordinates between two stress responses via association with, or dissociation from, cytoplasmic stress granules. Our results reveal another new and important role for RACK1 as a mediator of chromatin remodeling that controls an exon-specific expression of the BDNF gene.

We show that nuclear RACK1 is linked to the chromatin complex by its interaction with histones including H3 and H4. Histones within chromatin provide a platform for epigenetic regulation of gene transcription through modifications on their N-terminal tails that are related to chromatin remodeling and transcriptional activity (48). The specific localization of RACK1 within the promoter IV region of the BDNF gene, together with its selective role in BDNF exon IV expression, suggests that RACK1 plays an important role in chromatin remodeling that results in the induction of the transcriptional activity of BDNF promoter IV. However, the mechanism underlying the specific targeting of RACK1 to promoter IV remains unclear. RACK1 can form homodimers and heterodimers with WD40 repeat-containing proteins, such as the β subunit of G protein (17, 49). Several WD40 proteins are localized in the nucleus. For example, the MS11-like WD40 repeat-containing proteins are found in the nucleus and are associated with the chromatin complex (50). Therefore, it is plausible that RACK1 is spatially restricted to BDNF promoter IV via a protein network. In addition, this promoter-specific binding of RACK1 may also be directed by interchromosomal reorganization (51), which allows gene promoters access to transcriptional activators or repressors (52).

Chromatin remodeling is an intricate response regulated through multiple modifications of DNA and histones. A major contributor to the control of chromatin remodeling is MeCP2. MeCP2 is the major methylated DNA-binding protein that associates with methylated DNA at the gene promoter to form a repression complex that includes histone deacetylases and DNA methyltransferase 1 (Dnmt1) (45, 46, 53, 54), which maintains DNA methylation. Dissociation of MeCP2 from a gene promoter leads to DNA demethylation and to histone acetylation through relocation of histone deacetylases (45, 46), result-
ing in transcription (55). MeCP2 dissociation from BDNF promoter IV was previously reported (43, 55), and our results imply that the dissociation of MeCP2 from BDNF promoter IV requires RACK1, indicating that RACK1 participates in regulation of chromatin remodeling at BDNF promoter IV via the release of MeCP2 from the promoter. Interestingly, in MeCP2 mutant mice, a reduction, rather than an increase, of BDNF protein levels was observed (56), suggesting that MeCP2 can also act as a transcription activator. MeCP2 can be modified by phosphorylation, and this modification is considered to direct the function of this DNA-binding protein as a transcriptional repressor or activator (57). RACK1 association with various kinases and phosphatases has been well established (16, 58). It is therefore plausible that nuclear RACK1 also contributes to the regulation of the phosphorylation state of MeCP2 at the BDNF promoter. In addition, our results imply that nuclear RACK1 coordinates DNA demethylation and histone H4 acetylation. The mechanisms controlling such chromatin modifications at a specific gene promoter are not well understood, and to our knowledge, this is the first report of a multifunctional scaffolding protein that translocates to the nucleus to mediate the activation of a particular promoter via chromatin remodeling. Our results suggest that scaffolding proteins such as RACK1 may provide a link between an extracellular stimulus and chromatin modifications at a particular gene promoter.

Up-regulation of BDNF mRNA levels have been mainly linked to neuronal depolarization resulting in influx of calcium, which, in turn, leads to the activation of the cAMP-response element-binding protein (33, 59). We report here a unique additional mechanism by which the cAMP-mediated pathway controls the level of BDNF via RACK1. BDNF is a major contributor to synaptic plasticity, learning, and memory, as well as neuronal morphology (23, 25, 26), and the cAMP pathway has been linked to various forms of learning (60). BDNF and the cAMP pathways are also tightly associated with various psychiatric disorders and addiction (61–63). Our results suggest that RACK1 serves as a converging point linking the cAMP pathway and the BDNF signaling cascade and thus may be an important mediator of the physiological and pathological phenotypes that are associated with these pathways.

In summary, our results suggest a new role for RACK1 as a regulator of chromatin remodeling and demonstrate a specific role for RACK1 in the epigenetic regulation of BDNF expression. It would be of great interest to determine whether RACK1 controls the expression of other genes via similar mechanisms. Furthermore, this novel mechanism for the regulation of exonspecific expression of the BDNF gene by RACK1 could have implications for the mechanism underlying BDNF-mediated functions in the central nervous system.

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