Cdk5/p35 Phosphorylates mSds3 and Regulates mSds3-mediated Repression of Transcription*

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Cyclin-dependent kinase 5 (Cdk5), a serine/threonine kinase that displays kinase activity predominantly in neurons, is activated by two non-cyclin activators, p35 or p39. Here, we report a physical and functional interaction between the Cdk5/p35 complex and mouse Sds3 (mSds3), an essential component of mSin3-histone deacetylase (HDAC) co-repressor complex. mSds3 binds to p35 both in vitro and in vivo, enabling active Cdk5 to phosphorylate mSds3 at serine 228. A mSds3 S228A mutant retained mSin3 binding activity, but its dimerization was not greatly enhanced by p35 when compared with wild type. Notably, p35 overexpression augmented mSds3-mediated transcriptional repression in vitro. Interestingly, mutational studies revealed that the ability of exogenous mSds3 to rescue cell growth and viability in mSds3 null cells correlates with its ability to be phosphorylated by Cdk5. The identification of mSds3 as a substrate of the Cdk5/p35 complex reveals a new regulatory mechanism in controlling the mSin3-HDAC transcriptional repressor activity and provides a new potential therapeutic means to inhibit specific HDAC activities in disease.

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¶¶ The abbreviations used are: Cdk5, cyclin-dependent kinase 5; HDAC, histone deacetylase; STAT, signal transducers and activators of transcription; mSds3, mouse Sds3; cDNA, complementary DNA; HA, hemagglutinin; MEF, mouse embryonic fibroblast; GST, glutathione S-transferase; MOPS, 4-morpholinepropanesulfonic acid; DBD, DNA binding domain; S1D, mSin3 interaction domain.

expression is ubiquitous in mammalian tissues, the expression of its activators is largely restricted to neurons. Therefore, the temporal and spatial specification of Cdk5 kinase activity is due to the neuronal expression of p35 and p39 (3–6).

During the last decade, significant advances have been made in understanding the functions of Cdk5 partly due to the identification and characterization of the substrates for Cdk5. To date, more than 30 substrates have been identified for Cdk5 and the functional roles of their interactions with Cdk5 have been explored (2, 7–13). By phosphorylating these diverse cellular targets, Cdk5 plays a multifunctional role in neurons including neurite outgrowth, axonal guidance, cytoskeleton assembly, membrane transport, synaptic function, dopamine signaling, and drug addiction (2, 7, 14, 15). Recent studies have revealed an important role of Cdk5 in regulating gene expression in various cell types such as the expression of acetylcholine receptors in skeletal muscle and the expression of insulin in pancreatic β-islet cells (16–18). Both Cdk5 and p35 could be detected in the nucleus, albeit at a much lower level (19, 20). Although it is not clear whether Cdk5/p35 acts on the nuclear machinery to regulate gene transcription, accumulating evidence indicates that a number of transcription factors can serve as the substrates of Cdk5. For example, Cdk5-directed phosphorylation of retinoblastoma and p53 can modulate their activities and influence apoptotic processes (21, 22). Similarly, Cdk5-dependent phosphorylation of the transcription factor myocyte enhancer factor-2 leads to the inhibition of its pro-survival functions and also engenders apoptosis (23). The Cdk5 complex can also phosphorylate STAT3 and modulate its transcriptional activity (20). Finally, SET protein, a chromatin-remodeling factor, serves as a substrate for Cdk5 and binds the core histones, protecting it from acetylation by different histone acetyltransferases (19, 24, 25). Taken together, these studies support the view that Cdk5/p35-directed phosphorylation plays a pivotal regulatory role capable of modulating the activity of numerous signaling and transcriptional molecules governing diverse cellular processes.

In this study, a yeast two-hybrid screen revealed an unanticipated interaction between p35 and mouse Sds3 (mSds3), an essential component of the mSin3-histone deacetylase (HDAC) complex that is conserved from yeast to human (26). mSds3 associates with mSin3A co-repressor with high stoichiometry (27). mSds3 represses transcription in part through its capacity to recruit HDAC activity in an mSin3-dependent manner and appears to function as an essential cofactor in the maintenance of mSin3-associated HDAC activity, a function conserved in yeast and mammalian cells (26, 28). Recent studies have shown that genetic inactivation of mSds3 in the mouse generates a cell-lethal condition characterized by severe proliferation defects, increased apoptosis, and impaired chromosome segrega-
tion, leading to rampant aneuploidy (29). In this study, we provide evidence that mSds3 interacts with p35 enabling Cdk5/p35-directed phosphorylation of mSds3 serine 228. This mSds3 phosphorylation residue appears critical in mSds3-mediated transcriptional repression and in the rescue of the mSds3 null phenotype. The identification of mSds3 as a Cdk5 substrate reveals a novel role for Cdk5 in the regulation of mSds3-directed activities including transcriptional regulation.

EXPERIMENTAL PROCEDURES

Plasmids and Antibodies—Complementary DNA (cDNA) encoding full-length p35 was subcloned into the yeast GAL4 DNA-binding vector, pAS2–1 (Clontech, Palo Alto, CA), to construct GAL4bd-p35, which was used as bait in the yeast two-hybrid screen. Similarly, the cDNAs encoding p25 and p10 were subcloned into pAS2–1. Partial cDNA fragments of mSds3 (encoding amino acids 1–170, 171–328, or 59–170) were amplified using mouse cDNA by PCR, subcloned into GAL4 transcriptional activation vector, pACT2 (Clontech), and used in the experiment described in Fig. 1. Full-length mSds3 was subcloned into expression vector pMT21 or pcDNA3-His6 for overexpression experiments. The GST-mSds3 and His-mSds3 fusion constructs were generated by subcloning full-length mouse mSds3 into pGEX-6P-2 and pETH-32, respectively (Amersham Biosciences). Point mutations (e.g. serine 228 to alanine (S228A)) were generated by PCR using complementary primers containing the mutations and subcloned into pGEX-6P-2. All of the constructs were confirmed by sequencing. Sds3 antibody was purchased from Bethyl (Montgomery, TX), whereas antibodies specific for Cdk5, p35, HA, and His were from Santa Cruz Biotechnology (Santa Cruz, CA).

Yeast Two-hybrid Screen—Yeast two-hybrid screen was performed following the Matchmaker two-hybrid screen protocol (Clontech). pP5 was used as bait to screen a mouse muscle cDNA library constructed in GAL4 transcriptional activation vector (pACT2). The bait and library plasmid was transformed into yeast strain Y190. The transformants were selected on SD-Trap-Leu-His plates, and the selected clones were subjected to β-galactosidase filter assay. The Sds3 clone isolated from the two-hybrid screen encodes the full-length protein including 130 nucleotides at the 5′ end of the initiation codon. A construct without this extra sequence still interacts with p35 (data not shown), confirming that these extra nucleotides are not necessary for the interaction between p35 and Sds3. Subsequent two-hybrid interaction analyses were carried out by co-transformation of plasmids containing the GAL4 DNA-binding (pAS2–1) and activation (pACT2) domains into yeast.

Cell Culture and Transfection—COST, NIH3T3, 293T, and immortalized mouse embryonic fibroblasts (MEF) cells were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen). Because Cdk5 is contained in the coiled-coil domain but not for the protein lacking this domain (−). The coiled-coil domain and mSin3 interaction domains are denoted by shaded and solid bars, respectively.

with 40 μl of protein G-Sepharose at 4 °C for 1 h. The samples were washed with buffer A and resuspended in SDS sample buffer, and co-immunoprecipitated proteins were detected using Western blot analysis. In vitro co-immunoprecipitation studies using brain lysates were performed as described previously (30). Nuclear and cytoplasmic fractions of brain and muscle were prepared following the instruction of the manufacturer (Sigma).

Fusion Protein and Pull-down Assay—GST-mSds3 was expressed in BL21 strain and purified using a glutathione-Sepharose 4B column following the instruction of manufacturer (Amersham Biosciences). His-mSds3 was purified according to the protocol of the manufacturer (Qiagen). The binding assay was carried out as described previously (30). For pull-down studies, 5 μg of GST or GST fusion proteins were first immobilized onto glutathione-Sepharose beads in phosphate-buffered saline supplemented with 5 μg/ml bovine serum albumin. After incubation at 4 °C with end-to-end rotation for 1 h, the beads were washed three times with phosphate-buffered saline. GST-p25 proteins were then incubated with His-mSds3, whereas GST-mSds3 was incubated with 100 μg of COS7 extracts overexpressing p35 in buffer A at 4 °C for 1 h or with P5 brain lysate at 4 °C overnight. The beads were washed 5 times with buffer A, and bound proteins were resuspended in SDS loading buffer, resolved by SDS-PAGE, and analyzed by Western blot analysis.

RNA Extraction and Northern Blot Analysis—Total RNA was prepared from rat tissues by lithium chloride/urea extraction (31). Northern blot analysis was performed as described previously (32). Full-length cDNA fragment of mSds3 was used as the probe.

In Vivo Phosphorylation Assay—The kinase assay was performed at 30 °C for 30 min in kinase buffer (20 mM MOPS, pH 7.4, 15 mM MgCl2, 100 μM ATP) containing 1 μCi of [γ-32P]ATP. 200 μg of His-mSds3 or GST-mSds3 fusion proteins (including wild type and the S228A mutant) were utilized as the substrates for Cdk5/p25 in the same assay. The phosphorylated proteins were separated on a SDS-PAGE and visualized by autoradiography.

Reporter Assay—Reporter assays were carried out as described previously (26). Subconfluent NIH3T3 cells were transfected with GAL4BD-mSds3 construct and different amounts of the p35 construct along with the luciferase reporter plasmid. Cells were lysed 48 h posttransfection, and assays for luciferase activity. Transfection efficiencies were normalized using an internal β-galactosidase control.

Rescue Experiment—The rescue experiment was performed in mSds3 null fibroblasts as described previously (29). Immortalized MEFs of mSds3+/− were transduced with retrovirus encoding wild type or mutant mSds3 and infected with a retrovirus encoding Cre recombinase. The growth of each transduced cell line was compared. For each genotype, two independent cell lines were tested.

RESULTS

Identification of mSds3 as a p35-interacting Protein—A positive clone encoding full-length mSds3 was identified from the yeast two-hybrid screen of mouse muscle cDNA library using p35 as a bait. mSds3 encodes a polypeptide of 328 amino acids containing two domains, a coiled-coil domain (amino acids 59–170), and the mSin3 interaction domain (SID, amino acids 188–226) (Fig. 1) (26). To map the regions of mSds3 that interact with p35, GAL4-AD constructs containing various regions of mSds3 were generated and tested for their ability to interact with p35 in yeast. As summarized in Fig. 1, the N-
terminal region of mSds3 (amino acids 1–170), but not the C-terminal region (amino acids 171–328), interacted with p35. The interaction domain of mSds3 with p35 was delimited further to amino acids 59–170, the mSds3 coiled-coil domain that enables homodimerization (26). This N-terminal region of mSds3 is dispensable for the interaction with mSin3B, which has been mapped previously to the mSds3 C-terminal region (amino acids 188–226), designated SID (26). To characterize further the interaction between p35 and mSds3, the N-terminal (p10) and C-terminal (p25) regions of p35 were tested for their ability to bind with mSds3. The p25 protein is a cleavage product of p35 that is a potent activator of the Cdk5 kinase yet lacks the N-terminal 100 residues present in p35, which is necessary for membrane targeting (33). mSds3 was found to interact with the p25 protein and not the p35-specific N-terminal region (Fig. 1, p.10).

mSds3 Interacts with p35—GST pull-down assays were performed to validate further the mSds3–p35 interaction in vitro. GST-p25 was found to bind to His-mSds3 (Fig. 2A). mSds3–p35 association was similarly documented in vitro. Recombinant mSds3, but not GST, was able to pull down p35 protein from the p35-overexpressing COS7 cells or from the normal P5 brain (Fig. 2B). To further define the mSds3–p35 interaction in mammalian cells, COS7 cells were transiently transfected with mSds3 and p35. p35 was immunoprecipitated from the cell lysate using p35 antibody, and the presence of mSds3 in the complex was detected using mSds3 antibody. We found that p35 was able to interact with mSds3 when co-transfected in the COS7 cells (Fig. 2C). Finally, anti-p35 immunoprecipitates from embryonic day 19 and adult brains contained mSds3 protein, suggesting that endogenous mSds3 interacts with p35 in vivo (Fig. 2D).

mSds3 Is Prominently Expressed in Brain and Muscle—The spatial and temporal expression profiles of mSds3 in rat tissues were examined. The level of mSds3 transcript and protein remained relatively unchanged in brain during development (Fig. 3, A and B). On the other hand, mSds3 mRNA and protein levels were most abundant in developing muscle during embryonic development and had declined thereafter (Fig. 3, A and B). Consistent with our previous observation (16), the level of p35 protein in muscle was considerably lower compared with the protein level in brain and was below the detectable limit in total muscle lysate (Fig. 3B). To examine the subcellular localization of mSds3 protein, fractionation experiments were carried out. Consistent with the identification of mSds3 as an integral component of the Sin3-HDAC co-repressor complex, the protein was mainly detected in the nucleus of rat brain and muscle during different developmental stages (Fig. 3C, P2, P10, and adult (Ad)). p35 protein was detected in both nuclear and cytosolic fractions of brain (Fig. 3C) (20). Interestingly, a detectable level of mSds3 was expressed in the cytoplasmic fraction of adult rat brain (Fig. 3C). Nuclear localization of mSds3 was also observed in cultured cortical neurons or C2C12 myotubes (Fig. 3D).

mSds3 Is Phosphorylated by Active Cdk5—Cdk5 is a proline-directed serine/threonine kinase, prompting in vitro kinase assays to determine whether mSds3 is a substrate for active Cdk5. As shown in Fig. 4, recombinant mSds3 was efficiently phosphorylated by purified Cdk5/p25 or Cdk5/p35 complex, an observation in line with the presence of serine 228 in the context of preferential phosphorylation consensus sequence ((S/T)PX(K/H/R)). Consistent with the capacity of Cdk5 to phosphorylate mSds3 at this site, we demonstrate that an mSds3 S228A mutant is refractory to Cdk5-directed phosphorylation (Fig. 4B), a finding suggesting that mSds3 serine 228 serves as the major phosphorylation site for Cdk5.

p35 Expression Increases the Homodimerization of mSds3—We next sought to examine the impact of Ser-228 phosphorylation on mSds3 function. The presence of the phosphorylation site (serine 228) next to the SID (amino acids 188–226) raises the possibility that the phosphorylation of mSds3 could modulate its ability to bind mSin3 proteins. However, as shown in Fig. 5A, the phosphorylation-deficient mutant of mSds3 (S228A) or the phosphomimetic mutant of mSds3 (S228D) did not affect interaction with mSin3A. As a negative control, mSds3ΔSID mutant (with the deletion of SID) could not pull down mSin3A in the same assay. Furthermore, the mSds3 S228A mutant exhibited a similar ability to associate with mSin3B when compared with that of wild type mSds3 (Fig. 5B). The interaction of mSds3 with mSin3A or mSin3B was further examined in muscle and brain prepared from E18 Cdk5-deficient mice. The association between mSds3 with mSin3A or mSin3B in Cdk5-deficient muscle and brain was similar to that observed in wild type mSds3 (Fig. 5C). These findings suggest that the phosphorylation of mSds3 at serine 228 does not modulate the incorporation of mSds3 into the mSin3 co-repressor complex. Since mSds3 was previously reported to homodimerize through direct protein-interaction via the coiled-coil domains, the same
region responsible for its association with p35 (Fig. 1) (26), we further examined whether p35 could modulate the homodimerization of mSds3. By overexpressing different epitope-tagged mSds3 with p35, we showed that the presence of p35 protein could augment the interaction between His-mSds3 and HA-mSds3 (Fig. 5D). On the other hand, Cdk5 and a closely related Cdk, Pctaire1, did not affect the ability of mSds3 to form homodimers. Notably, the dimerization of the phosphorylation-deficient mutant of mSds3 (S228A) was not greatly enhanced by p35 when compared with wild type (Fig. 5D). This finding suggests that the phosphorylation of mSds3 by active Cdk5 increases the homodimerization potential of mSds3.

**p35 Increases mSds3 Repressor Activity**—Given the ability of mSd3 to modulate mSin3-associated histone deacetylase activity (26) as well as our observation that p35-Cdk5 can phosphorylate mSds3, we investigated the possibility that p35 could regulate histone acetylation in vivo. We showed that p35 overexpression led to a significant and reproducible decrease in histone H3 acetylation (Fig. 6A), whereas Cdk5 overexpression had no effect. Although we cannot exclude the possibility that p35 overexpression might also affect other HDAC-containing complexes, this observation, together with the ability of Cdk5/p35 to phosphorylate mSds3, strongly suggests that p35 is able to modulate mSin3-associated histone deacetylase activity through mSds3 phosphorylation. To assess the consequences of p35/Cdk5 on mSds3 biological activities, we first sought to determine whether the p35 overexpression modulates mSds3-associated transcriptional repression activity. As mSds3 can repress transcription in reporter gene assays (26), we assessed the impact of p35 overexpression on the transcriptional repression activity of a GAL-DBD-mSds3 fusion protein. p35 overexpression did not significantly affect the level of transcription driven by GAL-DBD alone. In contrast, p35 overexpression increased GAL-DBD-mSds3-mediated repression activity in a dose-dependent manner (Fig. 6B). These results suggest that p35 increases the repressor activity of mSds3 in the reporter assay.

**Phosphorylation-deficient Mutant of mSds3 Cannot Rescue the Lethality Caused by mSds3 Null Mutant**—mSds3 protein expression was shown to be necessary for cell growth and survival in mouse fibroblasts (29). To examine the biological importance of the mSds3 Ser-228 residue, we assessed the capacity of various mSds3 point mutants to rescue cell growth and viability in a mSds3/L−/−-immortalized cell line rendered null by Cre-mediated deletion of the remaining wild type mSds3 allele (29). Although wild type mSds3 rescued cell growth and survival, the growth defects persisted in cells transduced with the mSds3 S228A or S228D mutants (Fig. 6C). The mSds3/L−/−-immortalized cells failed to grow after being

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**Fig. 3. Temporal and spatial expression profiles of mSds3.** A, Northern blot analysis of mSds3 in developing rat muscle and brain. E, embryonic; P, postnatal; Ad, adult. B, Western blot analysis of mSds3 in developing rat muscle and brain. C, Western blot analysis of mSds3 in cytosolic and nuclear fractions of P2, P10, and adult brain and muscle. D, Western blot analysis of mSds3 and p35 in fractions prepared from cortical neurons cultured for 21 days and C2C12 myotubes. Cyt, cytoplasmic; Mem, membrane; Nuc, nucleus.

**Fig. 4. In vitro phosphorylation of mSds3 by Cdk5/p25 or Cdk5/p35 complex.** A, purified His₆-mSds3 was utilized as the substrate for recombinant Cdk5/p25 or Cdk5/p35 in the kinase assay. The kinase assay was performed in the absence (−) or presence (+) of an increasing amount of Cdk5/p25 (0.2–1 μg) or Cdk5/p35 (1 μg). B, Cdk5/p25 complex phosphorylated the GST protein encoding mSds3 (WT) but not the serine mutant (M) S228A (upper panel). Lower panel, Coomassie Blue staining.

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**Cdk5/p35 Regulates mSds3-mediated Repression**

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FIG. 5. Cdk5/p35 did not affect the interaction between mSds3 and mSin3 but enhanced mSds3 homodimerization. 

A, interaction between mSds3 or its mutants and mSin3A. Upper panel, Western blot of the co-immunoprecipitated mSin3A proteins with an anti-FLAG antibody following transfection of various FLAG-tagged mSds3 constructs (mSds3flag, mSds3ΔSIDflag, mSds3S228Aflag (serine 228 changed to alanine), or mSds3S228Dflag (serine 228 changed to aspartate)) into 293T cells. Lower panel, mSds3 proteins used in the co-immunoprecipitation analysis.

B, Western blot analysis of mSin3B co-immunoprecipitated using an anti-HA antibody following transfection of HA-tagged mSds3 constructs into 293T cells. Ad Br, adult brain.

C, association of mSds3 with mSin3A or mSin3B in Cdk5 knock-out muscle and brain. Immunoprecipitation of the brain and muscle extract of embryonic day 18 Cdk5 knock-out mice was performed using Sds3 antibody followed by immunoblotting with mSin3A antibody (upper panel) or mSin3B antibody (middle panel). The blot was reprobed using mSds3 antibody as control.

D, overexpression of p35 increases the association of HA-mSds3 with His-mSds3. Different expression constructs were co-transfected into COS7 cells as indicated. The association between epitope-tagged Sds3 molecules (wild type (mSds3) or S228A mutant (mSds3-M)) was demonstrated by immunoprecipitation with HA antibody followed by immunoblotting using His antibody. IB, immunoblotting; IP, immunoprecipitated.
**FIG. 6.** mSds3 harboring phosphorylation-deficient mutations cannot rescue the lethality caused by mSds3 null mutation. A, p35 reduces the acetylation of histone H3. Expression constructs encoding p35 or Cdk5 were transfected into 293T cells. The acetylation status of histone H3 was examined by Western blot analysis using acetyl-histone H3 antibody (top panel). Western blots for p35, Cdk5, and α-tubulin are shown in lower panels. B, p35 enhances mSds3-mediated repression. NIH3T3 cells were transfected with GAL-DBD-mSds3 expression construct and different amounts of p35 expression construct along with the luciferase reporter plasmid (Cdk expression construct was used as control). Shown are the means of three experiments performed in duplicate. Error bars represent the mean ± S.D. for each point. The amount of p35 and Cdk5 used and the fold of repression relative to GAL4-DBD are shown. *, p < 0.05, significantly different from the vector control using Student’s t test. C, growth curve of immortalized MEFs mSds3ΔSID or with point mutation at serine 228. Altogether, these results suggest that the dynamic phosphorylation of serine 228 of mSds3 is necessary for sustained cell growth and viability. Whereas the actions of Cdk5 was originally thought to be largely restricted to neuronal cells, recent emerging studies reveal its unanticipated functions in non-neuronal cells, such as regulation of apoptosis in leukemia cells and prostate cancer cells (34, 35). Thus, whereas our study clearly assigns relevance to serine 228 phosphorylation in mSds3 function, it remains to be determined whether endogenous Cdk5 activity or an as yet unidentified kinase is responsible for this modification in MEFs and other non-neuronal cell types in vivo.

**DISCUSSION**

This study reports the physical interaction of mSds3 and p35 as evidenced by yeast two-hybrid assays, in vitro pull-down assays, and cellular and tissue co-immunoprecipitations. The data are consistent with a model of which the Cdk5/p35 complex phosphorylates serine 228, a residue positioned in the coiled-coil domain of mSds3, thereby modulating mSds3 homodimerization and in turn affecting mSds3 function, possibly via its HDAC maintenance activity of the mSin3 co-repressor complex. Our findings provide a new avenue in exploring the molecular mechanism by which mSds3 regulates gene repression as well as cell viability.

Acetylation of core histones contributes to the regulation of gene expression by conferring accessibility of the DNA template to the transcriptional machinery for gene expression (36). It is tightly controlled by histone acetyltransferase and HDAC. Acetylation by histone acetyltransferase is associated with gene activation, whereas HDAC modifies the chromatin folding and is associated with gene silencing. mSds3 is a recently identified integral component of the Sin3-HDAC complex and was reported to promote functional HDAC1 activity in the mSin3 complex (26). However, the precise mechanism(s) through which mSds3 operates is not clear. When tethered to DNA, mSds3 exhibits potent transcriptional repression activity in reporter assays and this activity is only partly neutralized by the HDAC inhibitor, trichostatin A, or by deletion of the putative SID of mSds3 (26). Thus, it is possible that mSds3 might recruit repressive activities that are independent of class 1 HDACs.

In this study, we have mapped the interacting region of mSds3 and p35 to the coiled-coil domain, which is dispensable for mSds3-Sin3 interaction. Mutation of putative Cdk5 phosphorylation site on mSds3 does not affect its association with mSin3 proteins, suggesting that Cdk5 phosphorylation of mSds3 is not necessary for the interaction of these proteins. Interestingly, mSds3 harboring the phosphorylation mutations cannot rescue the lethality caused by mSds3 null mutation. This finding suggests that this residue and, by inference, its phosphorylation play an important role in mSds3-dependent cell growth and survival. Moreover, we found that p35 can enhance the ability of mSds3 to form homodimers. This finding is consistent with the previous observation that the coiled-coil domain, which is indispensable for the interaction between p35 and mSds3, is a homodimerization interface (26). However, the functional consequence of mSds3 homodimerization on its repressor activity is not clear. It is possible that mSds3 is a scaffold protein between HDAC1/2 and an as yet unidentified HDAC-activating factor or that mSds3-mediated homodimerization is essential for the functional presentation of the mSin3-associated HDAC activity. The serine 228 phosphorylation of mSds3 might be essential for this recruitment to affect the enzymatic activity of the complex and subsequent cell growth and survival. Further studies on the regulation of
mSds3 phosphorylation in vivo will shed the light on the molecular mechanisms controlling the mSds3-Sin3-mediated gene repression. Although a number of proteins have been identified to associate with Cdk5/p35 (7), the majority of these proteins mediate their interaction with Cdk5 through p35, suggesting that p35 may act not only as an activator of Cdk5 but also as an adaptor to associate Cdk5 with its regulators and physiological targets. By interacting with mSds3, p35 might bring Cdk5 to close proximity to the mSds3-Sin3-HDAC1 complex, whereby Cdk5 can then phosphorylate mSds3 or other proteins of the complex and regulate its transcriptional activity.

Whereas Cdk5/p35 is mainly associated with membrane or cytoskeleton, detectable Cdk5/p35 was reported in the nuclear fractions of neurons and muscle (19, 20). In addition, Cdk5 is suggested to be involved in nuclear functions, especially during the process of neurodegeneration. It was reported that neurotoxicity results in the enrichment of Cdk5 kinase activity in nucleus (23, 33). A number of transcription factors, including retinoblastoma, p53, myocyte enhancer factor-2, and STAT3, are closely related to the regulation of neuronal apoptosis (39, 40). Delineating the molecular mechanism by which Cdk5 regulates mSds3-mediated repression have far-reaching implications (37). For example, the status of histone acetylation in hippocampus was recently shown to be regulated during the process of memory formation (38), whereas other studies have shown that E2F-dependent gene repression and derepression are closely related to the regulation of neuronal apoptosis (39, 40). Given the important role of histone acetylation in neuronal development and neurodegenerative diseases, our novel findings on the involvement of Cdk5 in regulating mSds3-mediated repression will shed the light on the molecular mechanisms controlling the mSds3-Sin3-mediated gene repression.

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