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Regulation of RE1 Protein Silencing Transcription Factor (REST) Expression by HIP1 Protein Interactor (HIPPI)*[S]

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Background: HIPPI, along with its molecular partner HIP1, can regulate transcription of the caspase-1 gene.
Results: HIPPI binds to the promoter of *REST* and increases its expression in neuronal and non-neuronal cells and in a Huntington disease cell model.
Conclusion: HIPPI is a novel transcription regulator of *REST*.
Significance: This study provides a novel mechanistic interpretation of HD pathogenesis through HIPPI-mediated transcriptional regulation of *REST*.

Earlier we have shown that the proapoptotic protein HIPPI (huntingtin interacting protein 1 (HIP1) protein interactor) along with its molecular partner HIP1 could regulate transcription of the caspase-1 gene. Here we report that RE1-silencing transcription factor/neuron-restrictive silencer factor (*REST/NRSF*) is a new transcriptional target of HIPPI. HIPPI could bind to the promoter of *REST* and increased its expression in neuronal as well as non-neuronal cells. Such activation of *REST* down-regulated expression of REST target genes, such as brain-derived neurotrophic factor (*BDNF*) or proenkephalin (*PENK*). The ability of HIPPI to activate *REST* gene transcription was dependent on HIP1, the nuclear transporter of HIPPI. Using a Huntington disease cell model, we have demonstrated that feeble interaction of HIP1 with mutant huntingtin protein resulted in increased nuclear accumulation of HIPPI and HIP1, leading to higher occupancy of HIPPI at the *REST* promoter, triggering its transcriptional activation and consequent repression of REST target genes. This novel transcription regulatory mechanism of REST by HIPPI may contribute to the deregulation of transcription observed in the cell model of Huntington disease.

Huntington disease is an autosomal dominant progressive neurodegenerative disorder of the central nervous system (CNS) characterized by uncontrolled movement, psychiatric abnormalities, and cognitive deficits (1). The molecular basis of the disease lies in the expansion of CAG repeats in the first exon of the huntingtin gene (*HTT*) (2). The mutated protein contains an abnormally long polyglutamine (poly(Q)) stretch at the N terminus, which is highly self-associative and forms intracellular aggregates (3, 4). Mutant HTT aggregates as well as the soluble form of the protein are believed to be toxic and deregulate various cellular processes and ultimately induce death of the striatal neurons possibly by apoptosis (5, 6).

The exact mechanism by which mutant HTT gives rise to the pathogenic condition is not clearly known. The dominant pattern of inheritance of the disease suggests a toxic gain of function of the mutant protein (7). However, loss of wild type protein function has also been implicated in the disease (8). *In vitro* and *in vivo* studies indicate that wild type HTT has a prosurvival role. Overexpression of wild type HTT ameliorates mutant HTT-induced toxicity in cells (9, 10), whereas depletion of wild type HTT renders the cells more sensitive to apoptotic insults (11). Direct evidence of wild type HTT loss of function comes from the work of Zuccato *et al.* (12), who demonstrated that wild type HTT but not the mutant protein promotes transcription of *BDNF*, a neurotrophin required for survival and function of cortico-striatal neurons. Expression of *BDNF* has been reported to be down-regulated in neurons affected with HD[2] (12). Subsequent studies have established that *BDNF* transcription is negatively regulated by REST, a transcription repressor that binds to the repressor element 1 (RE1) site present in the *BDNF* promoter (13). Wild type HTT sequesters REST in the cytoplasm by forming a multiprotein complex with RILP (REST/NRSF-interacting LIM domain protein), HAP1 (huntingtin-associated protein 1), and dynactin $p150^{glued}$ (14), leading to normal *BDNF* transcription. Mutation in HTT, however, disrupts this complex, resulting in increased nuclear accumulation of REST and down-regulation of *BDNF* expression along with other essential neuronal genes (13, 15).

HIPPI-HIP1-mediated aberrant activation of the apoptotic cascade provides another instance of loss of function of wild type HTT (16). HIP1 is a proapoptotic protein that preferentially interacts with wild type HTT (17). Under diseased conditions when HTT is mutated, HIP1 dissociates from mutant HTT and interacts with HIPPI, a novel pseudo-death effector domain (pDED)-containing protein. HIPPI-HIP1 heterodimer then recruits procaspase-8, followed by its activation and induction of the downstream apoptotic cascade (16, 18).

While investigating the HIPPI-mediated apoptotic pathway in detail, Majumder *et al.* (18) observed that HIPPI positively

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[2] The abbreviations used are: HD, Huntington disease; pDED, pseudo-death effector domain; NLS, nuclear localization signal; IOD, integrated optical density.

regulates the expression of several caspases, including caspase-1, -3, -7, and -8 in a cell model. HIPPI directly interacts *in vitro* and *in vivo* with a specific 9-bp DNA sequence, AAAGACATG, present at the putative promoter of the caspase-1 gene (19, 20). The protein lacks any conventional nuclear localization signal (NLS) and is carried to the nucleus by the NLS present at the C terminus of HIP1 (21). The HIPPI-HIP1 heterodimeric complex is then recruited to the promoter of caspase-1 to regulate its transcription (21).

The emergence of HIPPI as a new transcription regulator for caspase-1 motivated us to investigate the global change in gene expression brought about by HIPPI. Transcriptomic profiling of HeLa cells in the presence of exogenous HIPPI revealed elevated expression of *REST* together with other genes.[3] In our present study, we report that HIPPI could directly bind to the upstream sequence of *REST* to increase its expression in neuronal and non-neuronal cells. We also have shown that, due to lower affinity of HIP1 for mutant HTT, HIPPI and HIP1 predominantly localized to the nucleus in ST*Hdh*$^{Q111/Q111}$ cells, a cell model of HD (22), compared with ST*Hdh*$^{Q7/Q7}$ cells. Finally, we have demonstrated that occupancy of HIPPI in the *REST* promoter was higher in ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells, which led to increased expression of *REST* and a consequent up-regulation of REST-mediated neuronal gene repression in ST*Hdh*$^{Q111/Q111}$ cells.

EXPERIMENTAL PROCEDURES

Antibodies and Chemicals—Geniticin, Hygromycin, and anti-β-actin antibody (A2228, clone AC-74, lot number 107K4791) were obtained from Sigma. The anti-mouse and anti-rabbit secondary antibodies conjugated with horseradish peroxidase and protein A-agarose beads were purchased from Bangalore Genei (India). Anti-HIP1 antibody was purchased from Novus Biologicals (NB300-204, 1B11, lot number A), anti-HIPPI (ab5205-100, lot number 63362), and anti-lamin B antibodies (ab16048-25, lot number 393854) were purchased from Abcam. Anti-REST antibody (sc-25398) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Immobilon-P Transfer membrane was from Millipore; Taq polymerase was from Bioline; and restriction enzymes (BamHI, SalI, HindIII, BglII, and KpnI) were from Promega. Protease inhibitor mixture was purchased from Roche Applied Science. TRIzol reagent was obtained from Invitrogen. Other molecular biology grade fine chemicals were procured locally.

Construction of Plasmids—Constructions of GFP-Hippi, GFP-wpDED (coding for amino acids 335–429 of full-length HIPPI) and GFP-mpDED (R393E) clones have been described earlier (21). Construction of wild type huntingtin exon 1 cloned in DsRedC1 vector (DsRed-16Q) was described earlier (23). HIP1 clone in pcDNA3 (pcDNA3-Hip1) was kindly provided by Prof. T.S Ross (University of Michigan Medical School). The NLS mutant clone of HIP1 (R1005E) in pcDNA3 was kindly provided by Dr. Ian Mills (CRUK Uro-Oncology Research Group, Cambridge, UK).

For the luciferase assay, we cloned the 5′ upstream promoter region of the mouse *REST* gene from position −4773 to −4216 (encompassing the HIPPI binding site from position −4430 to −4422) in pGL3 basic vector (designated as Luc-RESTups) between the restriction sites of BglII and KpnI. The primer sequences used for constructing this clone are given in supplemental Table S1.

Cell Culture and Transfection—HeLa and Neuro2A cells were routinely grown in minimal essential medium (Himedia, Mumbai, India) supplemented with 10% fetal bovine serum (Biowest) at 37 °C in 5% CO_2 atmosphere under humidified conditions. Immortalized striatal HD cell lines, ST*Hdh*$^{Q111/Q111}$ and ST*Hdh*$^{Q7/Q7}$ cells (22) were grown in DMEM (Himedia) supplemented with 10% FBS and 400 μg/ml G418 (Invitrogen) at 33 °C in humidified conditions and 5% CO_2. Transfection of cells was performed using Lipofectamine 2000 (Invitrogen). Unless otherwise mentioned, for the single transfection experiment, 2.5 μg (60-mm plate) or 5 μg (100-mm plate) of DNA constructs as well as 5 or 10 μl of Lipofectamine 2000, respectively, were used. After 24 h, transiently transfected cells were checked for transfection efficiency by monitoring either GFP or DsRed expression under a fluorescence microscope and were used for experiments. Transfection efficiency varied from 70 to 90%.

Knockdown of HIP1 and HIPPI by siRNA—Knockdown of *HIP1* by gene-specific siRNA in HeLa and Neuro2A cells was described earlier (21).

For siRNA mediated knockdown of *HIPPI* in Neuro2A and ST*Hdh*$^{Q111/Q111}$ cells, the mouse *HIPPI* cDNA sequence was submitted to the online GenScript siRNA designing tool. Among the various sequences retrived by the software for siRNA, we choose the sequence 537-GTCACGATTAGGACTGACA-519. The scrambled sequence (5′-GACGCGTGACCAAGATTAT-3′) for the siRNA was also designed using the GenScript sequence scrambler tool. The complete sequence that was inserted into the expression vector pRNA-U61Hygro (Genescript) was 5′-GTCACGATTAGGACTGACATTCAAGAGATGTCAGTCCTAATCGTGAC-3′ (designated "HippiSi") with a termination signal and appropriate restriction site linkers (BamHI and HindIII; not shown) and an insert for loop formation (underlined). The entire sequence for the scramble siRNA was 5′-GACGCGTGACCAAGATTATTTGATATCCGATAATCTTGGTCACGCGTC-3′ (designated "HippiScr"). Both HippiSi and HippiScr were cloned in our laboratory using the restriction enzymes BamHI and HindIII.

HippiSi and HippiScr clones were transfected in Neuro2A and ST*Hdh*$^{Q111/Q111}$ cells using Lipofectamine 2000 (Invitrogen) using a protocol provided by the manufacturer. Transfected cells were selected for hygromycin resistance. Knockdown of *HIPPI* was confirmed by RT-PCR using sequence-specific primers for *HIPPI*. A list of various cell lines generated either by stable or transient transfection is given in supplemental Table S2.

Subcellular Fractionation and Western Blot Analysis—Methods for subcellular fractionation and Western blot were essentially the same as described previously (21). Briefly, cells grown in 100-mm Petri dishes were washed with ice-cold PBS and harvested at 300 × *g* for 3 min at 4 °C. Cytosol was extracted using cytosol extraction buffer (50 mM Tris-HCl, pH 7.5, 10 mM NaCl, 2 mM EDTA, 1 mM PMSF, and 1× protease inhibitor

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mixture, 0.25% Nonidet P-40). The nuclear pellet was suspended in nuclear extraction buffer (50 mM Tris-HCl, pH 7.5, 400 mM NaCl, 2 mM EDTA, 1 mM PMSF, and 1× protease inhibitor mixture) and kept on ice for 40 min followed by centrifugation at 13,000 × *g* for 20 min at 4 °C. The supernatant was kept as nuclear extract. For preparing whole cell extracts, cell lysis was carried out using lysis buffer (50 mM Tris-HCl, pH 7.5, 2 mM EDTA, 100 mM NaCl, 0.1% Triton X-100, and PMSF with 100 μg/ml final concentrations). Protein concentration was measured by Bradford assay. The samples were boiled with SDS gel loading buffer, run on SDS-polyacrylamide gel, transferred to membrane, and probed with antibodies. β-Actin was used as an internal control for cytoplasmic and whole cell extracts, whereas lamin B was used as loading controls for nuclear fractions. Each experiment was repeated three times. Integrated optical density (IOD) of each band was calculated using Image Master VDS software (Amersham Biosciences). Whenever necessary, IOD was normalized with that of the loading control.

Chromatin Immunoprecipitation (ChIP) Assay—Methods used for the ChIP experiments were described earlier (21). In brief, ST*Hdh*$^{Q111/Q111}$ and ST*Hdh*$^{Q7/Q7}$ cells expressing endogenous HIPPI and HeLa and Neuro2A cells expressing exogenous GFP-Hippi or GFP-wpDED or GFP-mpDED were cross-linked with 1.1% formaldehyde for 10 min at room temperature. This cross-linking reaction was stopped using 125 mM glycine. Cells were washed with ice-cold PBS and harvested at 300 × *g* for 3 min at 4 °C. Cytosol was extracted with Buffer C (20 mM HEPES, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, and 1 mM PMSF). Nuclei were harvested at 13,000 × *g* for 10 min at 4 °C, and the pellet was resuspended in breaking buffer (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 150 mM NaCl, 1% SDS, and 2% Triton X-100) and sonicated twice (two pulses of 10 s each). Contents were then centrifuged. Triton buffer (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 150 mM NaCl, and 0.1% Triton X-100) was added to the nuclear extract. The immunoprecipitation reaction was carried out with either anti-HIPPI antibody (for ST*Hdh*$^{Q111/Q111}$ and ST*Hdh*$^{Q7/Q7}$ cells and cells expressing GFP-Hippi) or anti-GFP antibody (for GFP-wpDED- or GFP-mpDED-transfected Neuro2A cells) followed by the addition of BSA-soaked Protein G-agarose beads. The immunoprecipitated complex was washed, followed by decross-linking, phenol chloroform extraction, and ethanol precipitation of the DNA. PCR amplification of the eluted DNA was carried out using sequence-specific primers for *REST*. The primer sequences are given in supplemental Table S1.

Semiquantitative RT-PCR—Total RNA was extracted from cells using TRIzol reagent (Invitrogen). Two μg RNA was reverse transcribed using random hexamer primer (Fermentas) and murine leukemia virus reverse transcriptase (Fermentas). Semiquantitative RT-PCR was carried out using Red TaqDNA polymerase (Bioline) for 35 cycles. Expression of β-actin was taken as endogenous control. The densitometry of the bands was carried out using Image Master VDS software (Amersham Biosciences).

The gene-specific primers used for RT-PCR were designed using Primer Express software (Applied Biosystems). Primer sequences used for amplification of the target genes are given in supplemental Table S1.

Luciferase Assay—The method for the luciferase assay was described previously (24). Briefly, cells grown in 35-mm plates were transfected with 500 ng of pGL3 construct (empty pGL3 vector or Luc-RESTups construct) and GFP construct (empty GFP vector, GFP-Hippi, GFP-wpDED, or GFP-mpDED). Twenty-four h after transfection, the luciferase assay was carried out using the luciferase reporter assay system (Promega) according to the manufacturer's protocols and detected by a Sirius Luminometer (Berthold Detection Systems). Five μg of protein was used for each assay. Transfection efficiency was normalized by measuring GFP fluorescence at 510 nm (Fluromax-3, Jobin Yvon Horiba).

Statistical Analysis—For statistical analysis, an unpaired *t* test was carried out to compare the means of two experimental groups using the on-line software GraphPad QuickCalc.

RESULTS

Induction of REST Gene Transcription by HIPPI in HeLa Cells—From microarray experiments, we observed that exogenous expression of HIPPI increased *REST* expression together with that of many other genes in HeLa cells.[3] To validate this observation, we measured *REST* expression in GFP-Hippi-expressing HeLa cells by semiquantitative RT-PCR (Fig. 1, *A* and *B*). *REST* expression was 2-fold increased ($p = 0.0002$, $n = 3$) in GFP-Hippi-transfected HeLa cells (supplemental Table S2, Hippi) compared with the parental HeLa cells (HeLa). Our earlier work has established HIP1 as the nuclear translocator of HIPPI (21). We, therefore, checked the expression of *REST* in the presence of HIPPI in *HIP1*-knocked down HeLa cells (Hip1SiHi). Generation of *HIP1*-knocked down HeLa cells has already been reported (21). *HIP1* knockdown prevented nuclear entry of HIPPI, and as a result, HIPPI-mediated up-regulation of *REST* was lost in Hip1SiHi cells (Fig. 1, *A* and *B*). Our result, thus, suggests that up-regulation of *REST* by HIPPI was dependent on the presence of HIP1. To address the functional consequence of such HIPPI-mediated up-regulation of the *REST* gene in HeLa cells, we measured the expression level of *PENK*, a gene negatively regulated by REST (15). *PENK* expression was down-regulated ($p = 0.002$, $n = 3$) in GFP-Hippi-transfected HeLa cells compared with parental HeLa cells (Fig. 1, *A* and *B*). On the contrary, *PENK* expression in Hip1SiHi cells did not alter significantly from the corresponding untransfected cells (Hip1Si). Thus, HIPPI-mediated *REST* up-regulation resulted in repression of REST downstream target gene in HeLa cells. Further analysis of the *REST* promoter region revealed the presence of a putative 9-bp HIPPI binding site (20) from position −1264 to −1256 in the upstream promoter of the human *REST* gene (Fig. 1*C*). To test whether this binding site was functional, we carried out chromatin immunoprecipitation from GFP-Hippi-expressing HeLa cells. The result showed that HIPPI could bind to this site (Fig. 1*D*, $n = 3$), indicating that *REST* could be a direct transcriptional target of HIPPI in HeLa cells.

HIPPI Enhances REST Gene Transcription by Binding to Its Upstream Promoter in Neuro2A Cells—To investigate the effect of HIPPI on neuronal *REST* expression, we transfected GFP-

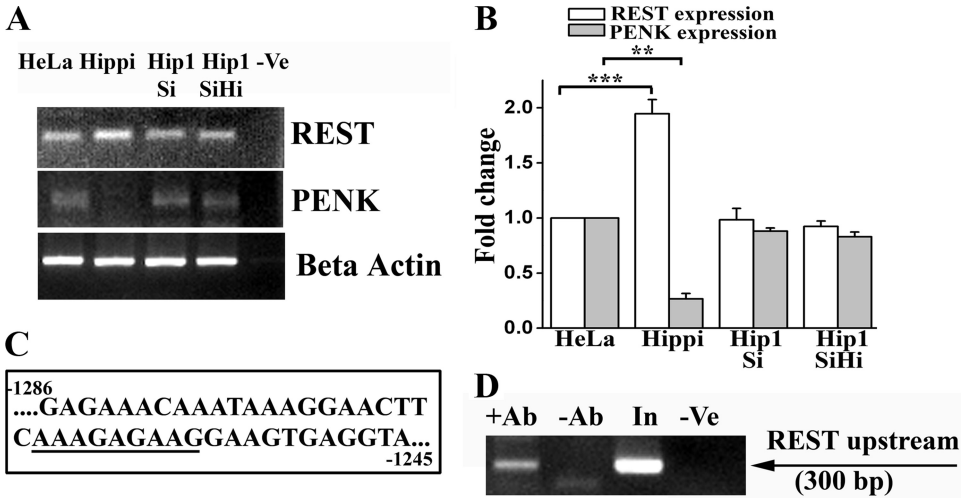

FIGURE 1. **HIPPI increases *REST* expression in HeLa cells.** *A*, gel image representative of three ($n = 3$) independent experiments for sqRT-PCR of *REST* and *PENK* expression in empty GFP vector-transfected HeLa cells (HeLa), GFP-Hippi-transfected HeLa cells (Hippi), empty GFP vector-transfected *HIP1*-knocked down HeLa cells (*Hip1Si*), and GFP-Hippi-transfected *HIP1*-knocked down HeLa cells (*Hip1SiHi*). Expression of β-actin was taken as endogenous control. *B*, *bar graph* representing the mean IOD of bands obtained in *A*. The expression levels of *REST* and *PENK* in a sample were normalized by the corresponding β-actin expression level. -Fold change was calculated by considering the relative expression level of *REST* and *PENK* in HeLa cells to be 1. *Error bars*, S.D. The statistical significance level between various experimental pairs is indicated (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). *C*, presence of HIPPI binding site in the upstream promoter of the human *REST* gene (ENSG00000084093). Sequence was retrieved from ENSEMBL Biomart. The putative 9-bp HIPPI binding sequence (−1264 to −1256) is *underlined*. *D*, chromatin immunoprecipitation showing *in vivo* interaction of HIPPI with the *REST* promoter in GFP-Hippi-transfected HeLa cells. Immunoprecipitation was carried out with anti-HIPPI antibody, and precipitated DNA was PCR-amplified using *REST* upstream sequence-specific primers. *Lane +Ab*, PCR amplification was carried out using chromatin immunoprecipitated by anti-HIPPI antibody. *Lane −Ab*, PCR amplification was carried out using chromatin immunoprecipitated by IgG alone. *Lane In*, PCR amplification was carried out using DNA isolated from GFP-Hippi-expressing HeLa cells. *Lane −ve*, PCR amplification was carried out without adding any template.

Hippi in the mouse neuroblastoma cell line, Neuro2A (N2A). *REST* expression was 2-fold higher ($p = 0.0001$, $n = 3$) in HIPPI-transfected N2A cells (N2A Hippi) compared with the parental N2A cells (Fig. 2, *A* and *B*). Because REST is a transcription repressor, such up-regulation of *REST* expression by HIPPI would alter the expression of its downstream targets. Therefore, we measured the expression of *BDNF*, a RE1-containing gene known to be targeted by REST (15). REST regulates *BDNF* expression from *BDNF* promoter II (13). We observed that *BDNF* expression was reduced ($p = 0.02$, $n = 3$) in N2A Hippi cells compared with N2A (Fig. 2, *A* and *B*). However, HIPPI was unable to increase *REST* expression in *HIP1*-knocked down N2A cells (Fig. 2, *A* and *B*, *N2AHIP1SiHi*). Knockdown of *HIP1* in Neuro2A cells has already been shown (21). To address this further, we knocked down endogenous *HIPPI* in Neuro2A cells using specific siRNA. Knock-down of *HIPPI* was confirmed by RT-PCR, and specificity of knockdown was checked by using scrambled siRNA control (supplemental Fig. S1, *A* and *B*, N2ASi and N2AScr). *HIPPI* knockdown also resulted in down-regulation ($p = 0.006$, $n = 3$) of endogenous *REST* (supplementary Fig. S1, *A* and *C*) and consequent up-regulation of *BDNF* ($p = 0.02$, $n = 3$). Next, we attempted to recover HIPPI expression in *HIPPI*-knocked down cells by transiently transfecting GFP-Hippi in N2ASi cells. HIPPI expression recovery also recovered *REST* expression ($p = 0.0003$, $n = 3$), suggesting a specific nature of regulation (supplemental Fig. S1, *A* and *C*, N2ASiHi). We then searched the upstream promoter of the mouse *REST* gene for the presence of the HIPPI binding motif. A putative 9-bp HIPPI binding sequence, 5′-AAAGACATT-3′, was present at positions −4430 to −4422 (Fig. 2*C*). Chromatin immunoprecipitation from GFP-Hippi-transfected N2A cells indicated association of HIPPI with this promoter (Fig. 2*D*, $n = 3$). To investigate it further, we determined the level of *REST* and *BDNF* expressions at different time points after HIPPI transfection in N2A cells. The rationale behind this is that if *REST* is a direct target of HIPPI, then one would expect *REST* up-regulation to occur at a much earlier time point compared with *BDNF* down-regulation, which is downstream of *REST* up-regulation. Results showed that *REST* was significantly up-regulated ($p = 0.02$, $n = 4$) within 3 h of GFP-Hippi transfection (Fig. 2*E*), reached its peak within 6 h ($p = 0.001$, $n = 4$), and remained almost at the same level until 24 h of transfection ($p = 0.0001$, $n = 4$). *BDNF* expression, on the other hand, was unaltered until 12 h post-transfection, but significant reduction was observed after 14 h ($p = 0.006$, $n = 4$) and at 24 h ($p = 0.02$, $n = 4$) of transfection. Similar early response (peaks within 3–6 h) was obtained with caspase-1 gene expression (supplemental Fig. S2), an already reported direct transcriptional target of HIPPI (21). Thus, *REST* up-regulation by HIPPI was an early event indicating that *REST* could be a direct transcriptional target of HIPPI.

Effect of HIP1 and HIPPI Mutations on REST and BDNF Expression in Neuro2A Cells—It has been reported earlier that HIPPI does not contain an NLS, and its nuclear entry is mediated by HIP1 (21). A mutation (R1005E) in the NLS domain of HIP1 prevents nuclear entry of HIPPI, and consequently HIPPI-driven transcription regulation of caspase-1 is blocked (21). To check whether this could be true for *REST* also, we first measured the level of *REST* and *BDNF* expressions in N2A cells stably overexpressing wild type HIP1 protein (N2A HIP1). Overexpression of wild type HIP1 increased nuclear accumulation of HIPPI (21), and as a result, *REST* expression was elevated ($p = 0.001$, $n = 3$), and *BDNF* expression was reduced ($p = 0.02$, $n = 3$) in N2A HIP1 cells compared with the parental N2A (Fig.

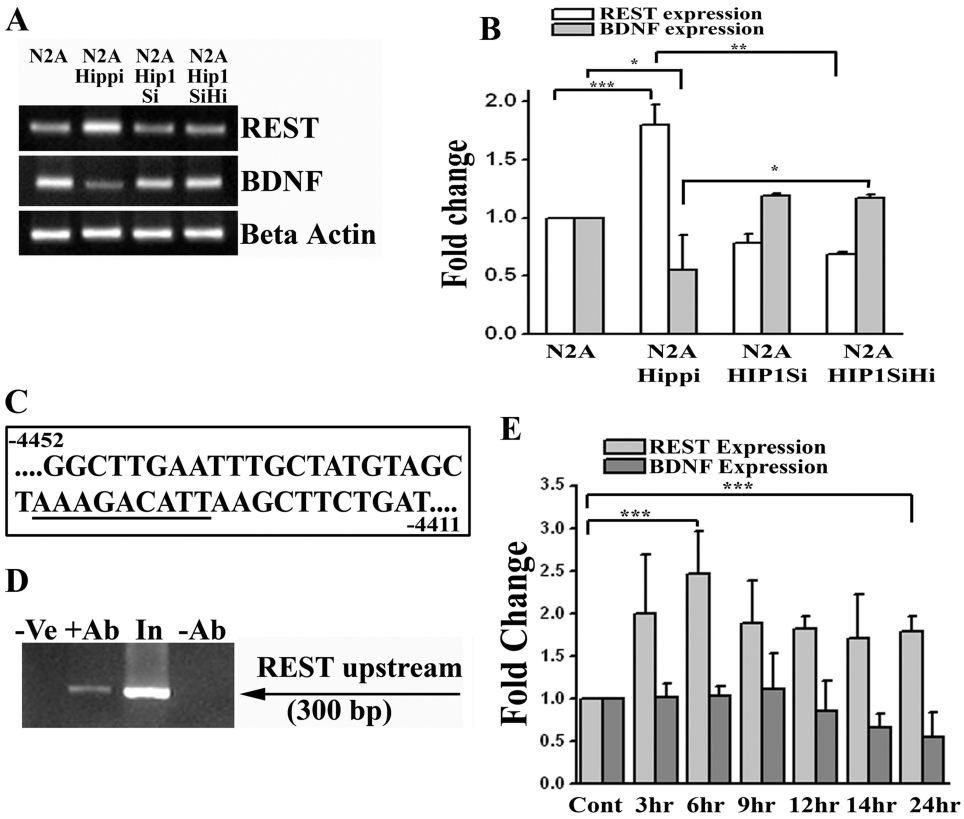

FIGURE 2. **HIPPI-mediated *REST* up-regulation and its interaction with *REST* promoter in Neuro2A cells.** *A*, gel image representative of three ($n = 3$) independent experiments for sqRT-PCR of *REST* and *BDNF* expressions in parental Neuro2A cells (*N2A*), GFP-Hippi-transfected Neuro2A cells (*N2AHippi*), *HIP1*-knocked down Neuro2A cells (*N2AHIP1Si*), and GFP-Hippi-transfected *HIP1*-knocked down Neuro2A cells (*N2AHIP1SiHi*). Expression of β-actin was taken as endogenous control. *B*, *bar graph* representing the mean IOD of bands obtained in *A*. The expression levels of *REST* and *BDNF* in a sample were normalized by the corresponding β-actin expression level. -Fold change was calculated by considering the relative expression level of target genes in N2A to be 1 (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). *C*, presence of the HIPPI binding site in the upstream sequence of the mouse *REST* gene (ENSMUSG00000029249). Sequence was retrieved from ENSEMBL Biomart. Putative 9-bp HIPPI binding sequence (−4430 to −4422) is *underlined*. *D*, *in vivo* interaction of HIPPI with the *REST* promoter in Neuro2A cells detected by ChIP assay. Immunoprecipitation was carried out with anti-HIPPI antibody, and precipitated DNA was PCR-amplified using *REST* upstream sequence-specific primers. The *lane markings* are as described in the legend to Fig. 1*D*. *E*, sqRT-PCR ($n = 3$) for *REST* and *BDNF* expression in GFP-Hippi-transfected Neuro2A cells at different time points after transfection. β-Actin expression level was considered as endogenous control. -Fold change was calculated by considering the relative expression level of *REST* and *BDNF* in control untransfected N2A cells (*cont*) to be 1. *Error bars*, S.D.

3*A*). However, cells stably overexpressing the NLS mutant of HIP1 (N2A 1005E) showed significant lowering of *REST* expression ($p = 0.0008$, $n = 3$) and consequently *BDNF* up-regulation ($p = 0.01$, $n = 3$) compared with N2A cells (Fig. 3*A*). Therefore, the presence of intact NLS in HIP1 was a prerequisite of HIPPI-mediated up-regulation of *REST*.

HIPPI interacts with DNA through its pDED (21). A mutation in the pDED replacing an arginine residue at position 393 of the full-length protein by glutamic acid (R393E) abolishes the DNA binding activity of HIPPI (21). To check the effect of such a mutation on *REST* expression, we transiently transfected GFP-tagged wild type pDED of HIPPI (N2A wpDED) and R393E mutant pDED of HIPPI (N2A mpDED) in N2A cells. Expression of wild type pDED was as effective as the full-length HIPPI protein in increasing *REST* expression ($p = 0.03$, $n = 3$) and *BDNF* down-regulation ($p = 0.0004$, $n = 3$) in N2A cells (Fig. 3*B*). On the contrary, expression of the mutant pDED was unable to alter either of the genes (Fig. 3*B*). A similar trend was observed in the protein level expression of REST, as detected by Western blot analysis (Fig. 3, *C* and *D*). Wild type HIP1, HIPPI, and wpDED of HIPPI were able to increase REST expression significantly in Neuro2A cells (designated as "Hip1," "Hippi," and "wpDED," respectively), whereas the mutant pDED (mpDED) could not. The NLS mutant of HIP1 (1005E), on the other hand, showed significant reduction in REST expression compared with either parental N2A cells or N2A cells overexpressing wild type HIP1. Next to see the DNA binding of these two proteins (wpDED and mpDED), we carried out luciferase reporter assay with the upstream sequence of the *REST* gene encompassing the HIPPI binding site (Luc-RESTups). Expression of full-length GFP-HIPPI in N2A cells (N2A Hippi) increased luciferase activity by ~2-fold ($p = 0.01$, $n = 4$) compared with empty GFP-transfected N2A cells (Fig. 3*E*, *N2A GFP*), indicating the interaction of HIPPI with *REST* upstream sequence followed by transcriptional activation. A similar increase in luciferase activity ($p = 0.002$, $n = 4$) was observed with wild type pDED of HIPPI (Fig. 3*E*), reflecting its intact DNA binding and transactivation ability. Mutant pDED HIPPI, on the other hand, was compromised in its ability to interact with *REST* upstream, and consequently luciferase activity remained at the level of N2A GFP cells. This was also evident from the chromatin immunoprecipitation result (Fig. 3*F*, $n = 3$). The ChIP experiment showed that wild type pDED HIPPI (wpDED) could immunoprecipitate *REST* upstream DNA from N2A cells where as mutant pDED (mpDED) could not.

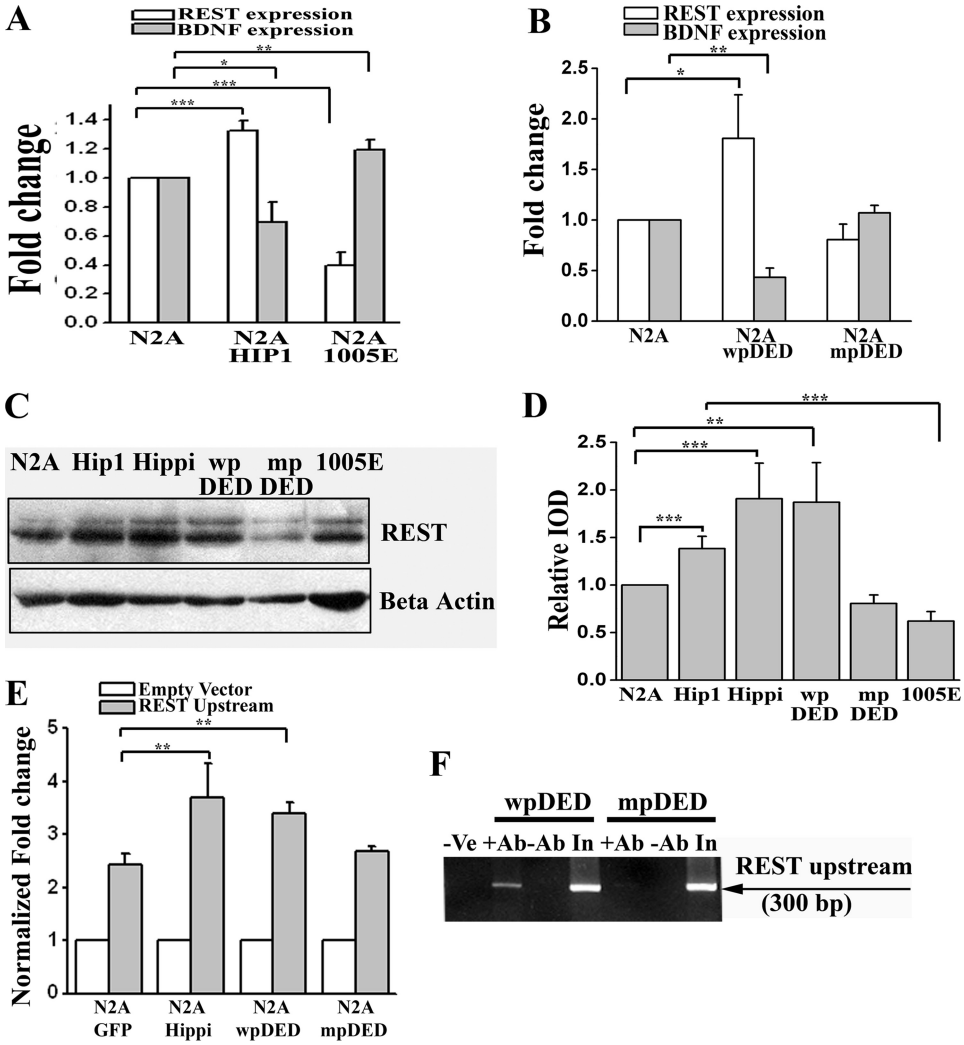

FIGURE 3. **Role of HIP1 and HIPPI mutations on *REST* and *BDNF* expression in Neuro2A cells.** *A*, sqRT-PCR ($n = 3$) for *REST* and *BDNF* expression in parental Neuro2A cells (N2A), Neuro2A cells stably overexpressing wild type HIP1 (N2A HIP1), and Neuro2A cells stably overexpressing NLS mutant of HIP1 (N2A 1005E). Expression of β-actin was taken as endogenous control. -Fold change was calculated as described earlier. *B*, sqRT-PCR ($n = 3$) for *REST* and *BDNF* expression in parental Neuro2A cells (*N2A*) and Neuro2A cells transiently transfected with GFP-wpDED (*N2AwpDED*) and GFP-mpDED (*N2AmpDED*). *C*, Western blot analysis for the expression of REST in parental Neuro2A cells (*N2A*) and Neuro2A cells overexpressing wild type HIP1 (*Hip1*), GFP-Hippi (*Hippi*), GFP-wpDED (*wpDED*), GFP-mpDED (*mpDED*), and the 1005E mutant of HIP1. β-Actin was used as loading control. *D*, *bar graph* showing the mean IOD ($n = 3$) of bands obtained for REST in *C*. The IOD of each REST band was normalized by the corresponding β-actin band. -Fold change was calculated by considering relative expression of REST in N2A to be 1. *E*, luciferase reporter assay ($n = 3$) of the REST upstream cloned in pGL3 vector in Neuro2A cells transiently transfected with empty GFP vector (N2A GFP), GFP-Hippi (N2A Hippi), GFP-wpDED (N2A wpDED), and GFP-mpDED (N2A mpDED). Luciferase activity of the above cells was normalized by the luciferase activity of the corresponding empty pGL3 vector-transfected cells. *Error bars*, S.D. The statistical significance level between various experimental pairs is shown (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). *F*, the wild type pDED of HIPPI and not the mutant pDED interact with REST promoter *in vivo*. Neuro2A cells were transiently transfected either with GFP-wpDED (*wpDED*) or GFP-mpDED (*mpDED*). ChIP was carried out with anti-GFP antibody, and precipitated DNA was PCR-amplified using *REST* upstream sequence-specific primers. The *lane markings* are as described in the legend to Fig. 1*D*.

Increased Nuclear Abundance of Endogenous HIPPI and HIP1 Increases REST Expression in STHdh$^{Q111/Q111}$ Cells—To check whether feeble interaction between HIP1 and mutant HTT might result in increased nuclear accumulation of HIPPI-HIP1, we measured the cytoplasmic and nuclear content of the two proteins in ST*Hdh*$^{Q111/Q111}$ and ST*Hdh*$^{Q7/Q7}$ cells. The relative expression levels of HIPPI and HIP1 did not vary significantly in the two cell lines (supplemental Fig. S3). However, it was observed that nuclear fraction of HIP1 was increased ($p = 0.02$, $n = 3$) from 40% in ST*Hdh*$^{Q7/Q7}$ cells to 60% in ST*Hdh*$^{Q111/Q111}$ cells (Fig. 4, *A* (*left*) and *B*). Similarly, nuclear HIPPI content increased ($p = 0.02$, $n = 3$) to 57% in ST*Hdh*$^{Q111/Q111}$ cells from 43% in ST*Hdh*$^{Q7/Q7}$ cells (Fig. 4, *A* (*left*) and *C*). To test whether expression of wild type HTT could prevent such increased nuclear localization of HIPPI and HIP1 in ST*Hdh*$^{Q111/Q111}$ cells, we exogenously transfected DsRed-16Q in both of the cell lines. In ST*Hdh*$^{Q7/Q7}$ cells, nuclear localization of HIPPI and HIP1 decreased in the presence of DsRed-16Q (Fig. 4, *A–C*) compared with the parental ST*Hdh*$^{Q7/Q7}$ cells, but the change was not statistically significant ($p = 0.07$, $n = 3$). This could be because of the fact that in presence of wild type HTT in ST*Hdh*$^{Q7/Q7}$ cells, HIP1 remained mostly cytoplasmic; the addition of more wild type HTT fragments therefore exhibited only a small change in HIP1 subcellular distribution. However, in ST*Hdh*$^{Q111/Q111}$ cells, there was a significant accumulation of both HIP1 ($p = 0.001$, $n = 3$) and HIPPI ($p = 0.008$, $n = 3$) in the cytoplasm following DsRed-16Q expression (Fig. 4, *A–C*).

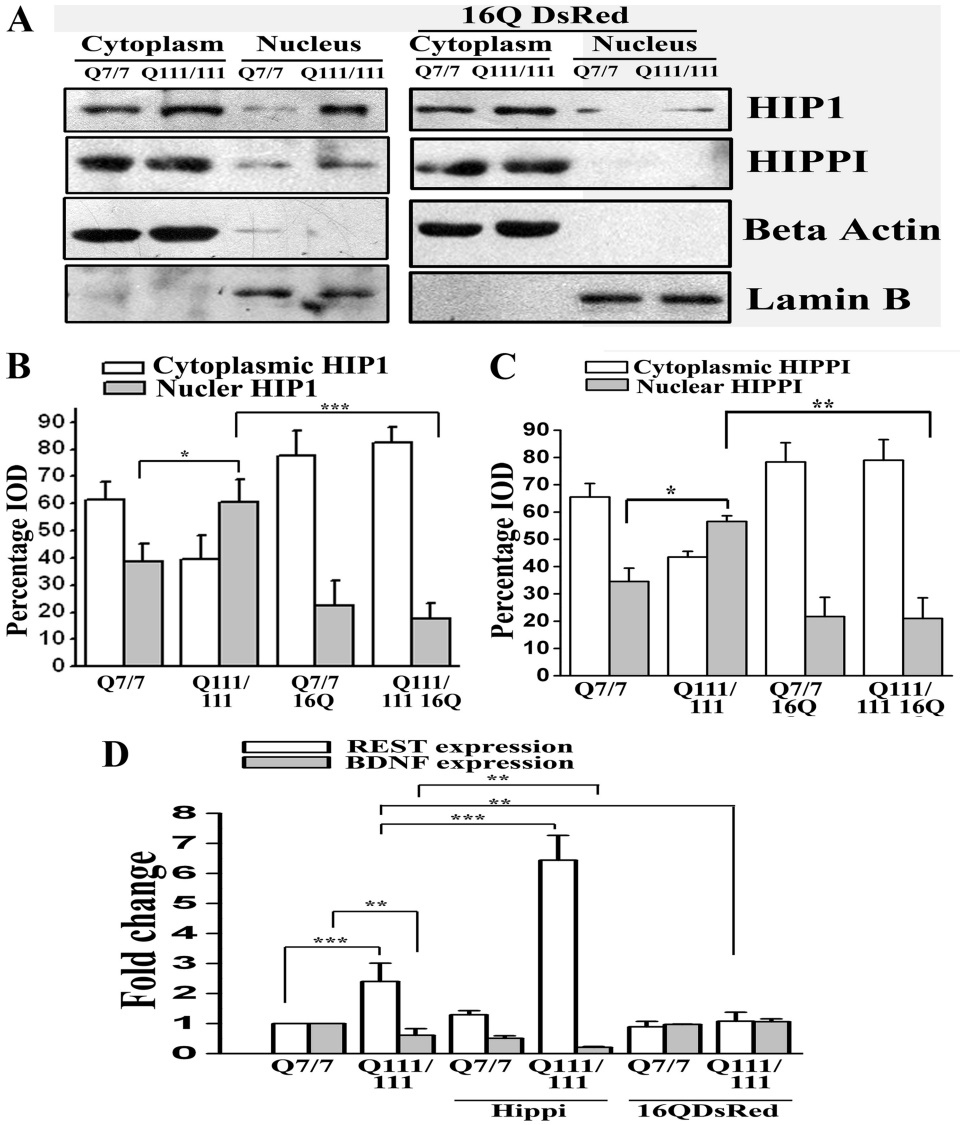

FIGURE 4. **Increased nuclear compartmentalization of HIPPI and HIP1 in ST*Hdh*$^{Q111/Q111}$ cells results in increased expression of *REST*.** *A*, Western blot analysis showing the subcellular distribution of endogenous HIP1 (*top panel*) and HIPPI (*second panel*) in ST*Hdh*$^{Q7/Q7}$ cells, ST*Hdh*$^{Q111/Q111}$ cells, ST*Hdh*$^{Q7/Q7}$ cells transfected with DsRed-16Q, and ST*Hdh*$^{Q111/Q111}$ cells transfected with DsRed-16Q. β-Actin (*third panel*) and lamin B (*bottom panel*) were used as loading controls for cytoplasmic (*Cytoplasm*) and nuclear extract (*Nucleus*), respectively. *B*, *bar graph* showing the mean IOD ($n = 3$) of bands obtained with anti-HIP1 antibody. IODs obtained in each fraction were normalized by the IOD values obtained with anti-β-actin (cytoplasmic) or lamin B (nuclear) antibodies. The proportion of HIP1 proteins in the cytoplasm and nucleus was determined by dividing the corresponding normalized IOD in cytoplasm and nucleus with the total IOD in the two compartments. *C*, *bar graph* showing the mean IOD ($n = 3$) of bands obtained with anti-HIPPI antibody. The proportion of HIPPI in the cytoplasm and nucleus was determined as described above. *D*, sqRT-PCR for *REST* and *BDNF* expression in ST*Hdh*$^{Q7/Q7}$ (Q7/7) cells, ST*Hdh*$^{Q111/Q111}$ (Q111/111) cells, Q7/7 cells exogenously transfected with GFP-Hippi (Q7/7Hi), Q111/111 cells exogenously transfected with GFP-Hippi (Q111/111Hi), Q7/7 cells exogenously transfected with DsRed-16Q (Q7/7 16Q), and Q111/111 cells exogenously transfected with DsRed-16Q (Q111/111 16Q). β-Actin was used as endogenous control. -Fold change was calculated by considering the relative expression of target genes in Q7/7 cells to be 1 (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). *Error bars*, S.D.

Thus, although the expression levels of HIPPI and HIP1 remained unchanged in ST*Hdh*$^{Q111/Q111}$ and ST*Hdh*$^{Q7/Q7}$ cells, subcellular distribution of the two proteins altered significantly in the two cell lines, nuclear content of both the proteins being greater in ST*Hdh*$^{Q111/Q111}$ cells. This indicated that HIPPI-mediated transcriptional activation would be greater in ST*Hdh*$^{Q111/Q111}$ cells. To investigate that possibility, we measured the relative transcript levels of *REST* and *BDNF* in the two cell lines. *REST* expression was ~2.5-fold higher ($p = 0.0008$, $n = 5$) in ST*Hdh*$^{Q111/Q111}$ cells (Q111/111) compared with ST*Hdh*$^{Q7/Q7}$ cells (Fig. 4*D*, *Q7/7*), whereas *BDNF* expression was reduced by ~40% in ST*Hdh*$^{Q111/Q111}$ cells ($p = 0.01$, $n = 4$). At the protein level, REST expression was increased 2-fold in Q111/Q111 cells compared with Q7/Q7 cells, as evident from Western blot analysis (supplemental Fig. S4). Down-regulation of *BDNF* in ST*Hdh*$^{Q111/Q111}$ cells has already been reported by Zuccato *et al.* (15), due to increased nuclear localization of REST in ST*Hdh*$^{Q111/Q111}$ cells. Our data suggest that increased expression of *REST* along with its increased nuclear localization might contribute to REST-mediated down-regulation of *BDNF* in ST*Hdh*$^{Q111/Q111}$ cells. Ectopic expression of HIPPI in ST*Hdh*$^{Q7/Q7}$ cells (Q7/7Hi) increased *REST* expression, but the change was not significant (Fig. 4*D*). This could be due to the unavailability of HIP1 to transport excess overexpressed HIPPI

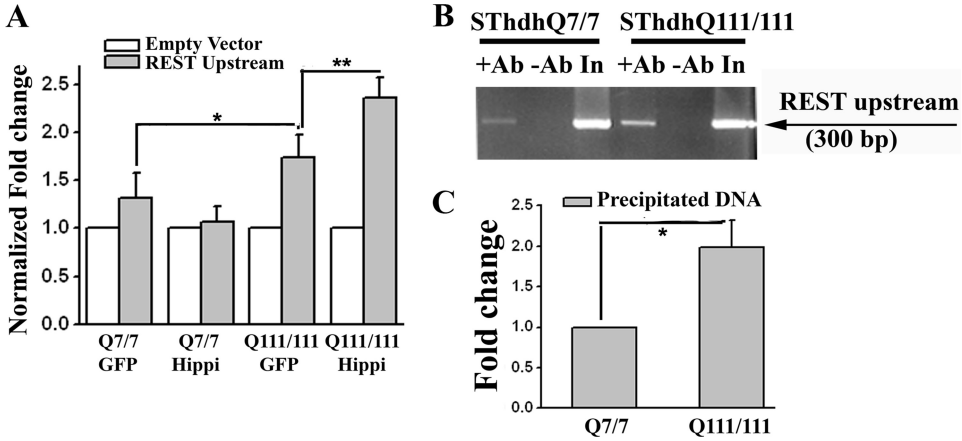

FIGURE 5. **Relative occupancy of HIPPI in the *REST* promoter is more in ST*Hdh*$^{Q111/Q111}$ cells.** *A*, luciferase reporter assay ($n = 4$) of the *REST* upstream cloned in pGL3 vector in empty GFP vector-transfected ST*Hdh*$^{Q7/Q7}$ (*Q7/7GFP*) cells, GFP-Hippi-transfected ST*Hdh*$^{Q7/Q7}$ (*Q7/7Hippi*) cells, empty GFP vector-transfected ST*Hdh*$^{Q111/Q111}$ (*Q111/111GFP*) cells, and GFP-Hippi transfected ST*Hdh*$^{Q111/Q111}$ (*Q111/111Hippi*) cells. Luciferase activity of the above cells was normalized by the luciferase activity of the corresponding empty pGL3 vector-transfected cells. *Error bars*, S.D. The statistical significance level between various experimental pairs is shown (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). *B*, comparative ChIP analysis showing relative occupancy of endogenous HIPPI in the *REST* promoter was greater in ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ cells. Immunoprecipitation was carried out with anti-HIPPI antibody, and the immunoprecipitated DNA was PCR-amplified using primers specific for *REST* upstream sequence. The *lane markings* are as described earlier. *C*, *bar graph* representing the mean IOD ($n = 2$) of PCR bands obtained in *B*. The IOD for the *+Ab lane* was normalized by the IOD of the corresponding input (*In*) *lane*. -Fold change was calculated by considering the normalized IOD of ST*Hdh*$^{Q7/Q7}$ cells as 1.

into the nucleus. However, in ST*Hdh*$^{Q111/Q111}$ cells, *REST* expression was ~6.5-fold up-regulated ($p = 0.0007$, $n = 3$) in the presence of HIPPI (Fig. 4*D*, *Q111/111Hi*). Exogenous expression of DsRed-16Q in ST*Hdh*$^{Q111/Q111}$ cells (Q111/111 16Q) arrested HIP1 in the cytoplasm (Fig. 4, *A* and *B*); consequently, HIPPI could not enter into the nucleus, resulting in lowering of *REST* expression ($p = 0.002$, $n = 4$) and a consequent elevation of *BDNF* expression ($p = 0.02$, $n = 3$) compared with Q111/111 cells (Fig. 4*D*). siRNA-mediated knockdown of endogenous *HIPPI* in Q111/111 cells also reduced ($p = 0.004$, $n = 3$) *REST* expression (supplemental Fig. S5, *A* and *C*, *Si*), and a consequent up-regulation of *BDNF* was observed ($p = 0.001$, $n = 3$). The effect could be reversed by transient transfection of GFP-Hippi in Si cells (supplemental Fig. S5, *A–C*, *SiHi*), which recovered *HIPPI* expression ($p = 0.0001$, $n = 3$) as well as that of *REST* ($p = 0.001$, $n = 3$).

Occupancy of HIPPI in the REST Promoter Is Greater in STHdh$^{Q111/Q111}$ *Cells*—Previously, it was reported that many transcription factors have increased occupancy to their cognate binding sites in ST*Hdh*$^{Q111/Q111}$ cells. We therefore investigated whether the *REST* promoter was transcriptionally more active in ST*Hdh*$^{Q111/Q111}$ cells than ST*Hdh*$^{Q7/Q7}$. Luciferase reporter assay using the Luc-RESTups construct revealed that luciferase activity was ~2-fold higher ($p = 0.04$, $n = 4$) in ST*Hdh*$^{Q111/Q111}$ cells compared with ST*Hdh*$^{Q7/Q7}$ (Fig. 5*A*). When we transfected GFP-Hippi exogenously in both of the cells, luciferase activity increased prominently ($p = 0.004$, $n = 4$) in ST*Hdh*$^{Q111/Q111}$ cells (Fig. 5*A*, *Q111/111 Hippi*). Finally, we checked the relative occupancy of endogenous HIPPI in the promoter of *REST* in the two cell lines by comparative ChIP analysis. Results showed that occupancy of HIPPI in the *REST* promoter in ST*Hdh*$^{Q111/Q111}$ cells was 2-fold higher ($p = 0.04$) compared with ST*Hdh*$^{Q7/Q7}$ cells (Fig. 5, *B* and *C*). This indicated that HIPPI-mediated transactivation of *REST* was indeed higher in ST*Hdh*$^{Q111/Q111}$ cells.

DISCUSSION

In the present study, we have identified HIPPI as a novel transcription regulator for *REST*. Using both neuronal and non-neuronal cell lines, we have demonstrated that exogenous expression of HIPPI increased expression of *REST* by binding to the upstream promoter of the gene. The ability of HIPPI to regulate *REST* expression was dependent on HIP1 because HIPPI-mediated *REST* up-regulation was lost in *HIP1*-knocked down cells or cells expressing the NLS mutant of HIP1. We also report that altered nuclear and cytoplasmic distribution of endogenous HIPPI and HIP1 in ST*Hdh*$^{Q111/Q111}$ cells led to higher occupancy of HIPPI in the *REST* promoter and consequently increased its expression in these cells. Expressions of two known targets of REST (viz. *PENK* and *BDNF*) were also decreased in the presence of HIPPI in our study, as an effect of REST up-regulation.

The transcription repressor REST regulates myriads of protein coding and noncoding genes (26–29). Involvement of REST in neuronal development is well studied (30, 31). REST expression remains high in neuronal progenitor cells, leading to repression of neuronal genes. However, during terminal differentiation of progenitor cells to mature neurons, REST expression is turned off, allowing expression of the essential neuronal genes (32). Recent work has demonstrated the role of the functional REST regulatory network in maintaining pluripotency of embryonic stem cells (28). REST is, therefore, a master regulator that maintains cells in an undifferentiated state. Although a large number of studies have been carried out to elucidate REST function, studies depicting control of *REST* gene expression are limited. In neuronal cells, *REST* expression is activated by YY1, DYRK1A, and Wnt/TCF signaling (33–35). Recently, transcription factor Sp1 has been shown to regulate *REST* expression in undifferentiated NG108 cells as well as in a mouse model of HD (36). Additionally, a double negative feedback loop involving REST-dependent expression of microRNA 9 and

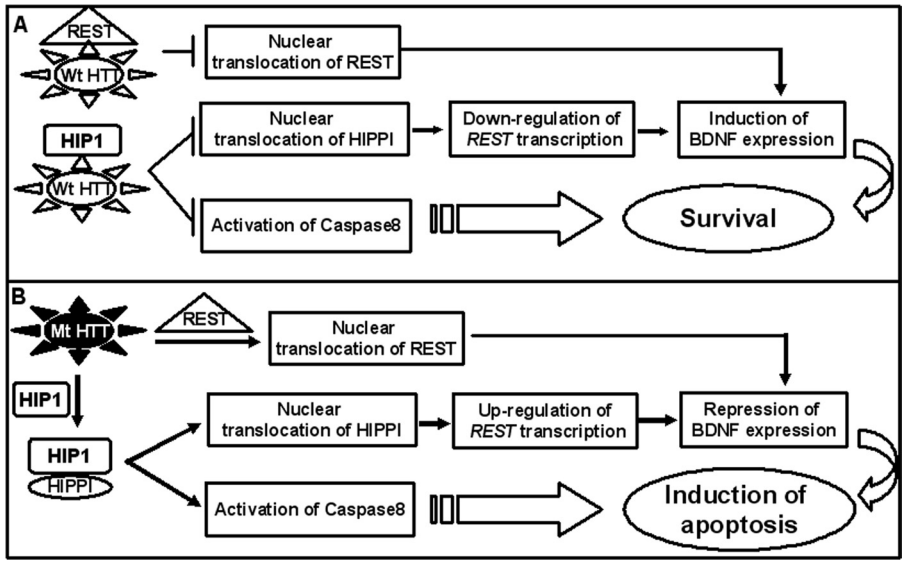

FIGURE 6. **Schematic representation of the involvement of HIPPI-HIP1 in the pathogenesis of HD.** *A*, the stronger interaction between wild type HTT and HIP1 prevents formation of HIPPI-HIP1 heterodimer, thereby blocking nuclear localization of HIPPI. *REST* expression remains in the basal level. Also wild type HTT can sequester REST protein in the cytoplasm. This leads to activation of REST target genes like *BDNF*, which is essential for the survival of striatal cells. Additionally, the absence of HIPPI-HIP1 heterodimer blocks caspase-8-mediated apoptosis induction, leading to cell survival. *B*, mutation in HTT favors release of HIP1 from the HTT-HIP1 complex and facilitates formation of HIPPI-HIP1 heterodimer. The heterodimer enters into the nucleus, where HIPPI interacts with the *REST* promoter to increase its expression. Unlike wild type HTT, mutant HTT cannot sequester REST protein in the cytoplasm. Thus, increased expression along with increased nuclear localization of REST causes repression of the *BDNF* gene. Also, formation of HIPPI-HIP1 heterodimer triggers activation of caspase-8-mediated apoptosis, leading to cell death.

9* controls *REST* expression in HD (37). Our initial observation that exogenous expression of HIPPI increased *REST* expression in HeLa cells in a microarray study prompted us to investigate whether HIPPI could act as a transcription regulator of REST. Overexpression of HIPPI in both HeLa and Neuro2A cells up-regulated *REST* expression in these cells. Knockdown of *HIP1*, the nuclear transporter of HIPPI, or overexpression of NLS-mutated HIP1 prevented this activation, indicating that nuclear translocation of HIPPI was essential for HIPPI-mediated *REST* up-regulation. Analysis of *REST* promoter sequence revealed the presence of a 9-bp HIPPI binding site in the upstream promoter of the *REST* gene. Using a luciferase reporter assay and chromatin immunoprecipitation, we confirmed that HIPPI could bind to this site and induce *REST* gene transcription. To address the functional consequence of this regulation, we measured expression of two REST target genes (viz. *PENK* and *BDNF*) under different experimental conditions. A consistent negative correlation between *REST* and *PENK*/*BDNF* expression was observed in the study, suggesting that HIPPI-mediated transcription activation of *REST* could affect the downstream cellular processes.

Involvement of transcription deregulation is well documented in HD. Several mechanisms for transcription deregulation have been elucidated, which include sequestration of cellular transcription factors and co-factors in mutant HTT aggregates, nuclear translocation, and aberrant nonspecific DNA binding of pathogenic N-terminal fragment of mutant HTT and also loss of wild type HTT-mediated transcriptional control (38). Involvement of REST in HD falls in the last category (12). The interaction of wild type HTT with REST through RILP, HAP1 (huntingtin-associated protein 1), and dynactin $p150^{glued}$ sequesters the complex in the cytoplasm (14), allowing expression of essential neuronal genes that are otherwise targets of REST-mediated repression (12, 13). Mutation in HTT, however, disrupts this interaction, which facilitates nuclear entry of REST and formation of the repressor complex on the RE1 site followed by target gene silencing. Many of the REST target genes have been shown to be down-regulated in HD (15). Moreover, there are reports indicating an overall increase in REST expression in various models of HD (25, 36, 39). The importance of REST in regulating HD pathogenesis inspired us to investigate whether HIPPI-mediated up-regulation of *REST* plays any role in HD. Feeble interaction of HIP1 with mutant HTT results in increased heterodimerization of HIP1 with HIPPI (16). Keeping this fact in mind, we hypothesized that increased heterodimerization of HIPPI-HIP1 might increase the nuclear content of the two proteins, which in turn could activate *REST* transcription in HD. Using ST$Hdh^{Q111/Q111}$ cells, we observed that it was indeed the case. Nuclear localization of both HIPPI and HIP1 was significantly enhanced in ST$Hdh^{Q111/Q111}$ cells compared with the wild type ST$Hdh^{Q7/Q7}$ cells. As a result, occupancy of HIPPI in the *REST* promoter was increased 2-fold, resulting in induction of *REST* gene expression. The observation could be reversed by overexpressing wild type HTT exon 1 (DsRed16Q) in ST$Hdh^{Q111/Q111}$ cells, where nuclear fractions of HIPPI and HIP1 were significantly reduced compared with untransfected ST$Hdh^{Q111/Q111}$ cells. Wild type HTT sequestered HIP1 in the cytoplasm, preventing its nuclear entry, along with HIPPI, which in turn repressed *REST* expression. On the other hand, expression of HYPK (huntingtin-interacting protein K), a chaperone-like protein capable of reducing mutant HTT aggregates (23), was unable to reduce *REST* expression in ST$Hdh^{Q111/Q111}$ cells (data not shown), indicating the process to be independent of mutant HTT aggregation. Therefore, our results demonstrate that HIPPI-mediated aberrant transcriptional activation of *REST* in ST$Hdh^{Q111/Q111}$ cells could be

attributable to the loss of wild type HTT function in these cells. This was further corroborated by the observation that overexpression of HIPPI in the wild type ST*Hdh*$^{Q7/Q7}$ cells could not activate *REST* transcription significantly. Retention of HIP1 in the cytoplasm by wild type HTT created a deficiency of nuclear transporter for HIPPI in ST*Hdh*$^{Q7/Q7}$ cells. Overexpression of HIPPI therefore exhibited no effect on *REST* expression.

Down-regulation of BDNF in cell and animal models of HD resulting from increased nuclear localization of REST has been reported previously by Zuccato *et al.* (12, 13). Our results predict an additional mechanism for *REST* activation and *BDNF* down-regulation in the HD cell model. The complete loss of wild type HTT-mediated sequestration of HIP1 in ST*Hdh*$^{Q111/Q111}$ cells caused redistribution of HIPPI and HIP1 in the nucleus, leading to induction of *REST* gene expression. The elevated level of REST protein in the cytoplasm could not be sequestered by mutant HTT, as depicted by Zuccato *et al.* (13), resulting in increased nuclear localization of REST, which in turn could reduce *BDNF* expression in ST*Hdh*$^{Q111/Q111}$ cells. HIPPI may therefore participate in HD pathogenesis by two mechanisms (Fig. 6). First, increased HIPPI-HIP1 heterodimerization in the presence of mutant HTT may induce caspase-8-mediated apoptosis as reported by Gervais *et al.* (16). Second, the heterodimer may translocate to the nucleus, leading to activation of *REST* transcription, which in turn may repress prosurvival genes like *BDNF* and induce neuronal death (Fig. 6).

Based on our results, we show, for the first time, the HIPPI-mediated transcriptional regulation of the *REST* gene and establish its connection with HD pathogenesis. In addition, the work of Houde *et al.* (40) has shown that *HIPPI* knock-out mice are embryonic lethal and exhibit defects in the Sonic hedgehog (Shh) pathway. Shh is a trophic factor that promotes neurogenesis from adult mice stem cells (41). It remains to be determined whether aberrant interaction of HIPPI and HIP1 in HD sequesters HIPPI from its normal function, resulting in alteration of the Shh pathway (40). Additionally, increased HIPPI-HIP1 interaction may induce transcriptional activation of the *REST* gene, leading to down-regulation of neuronal gene expression. Taken together, this study sheds light on a novel mechanistic interpretation of HD pathogenesis through HIPPI-mediated transcriptional regulation of *REST*.

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