Huntingtin Regulates RE1-silencing Transcription Factor/Neuron-restrictive Silencer Factor (REST/NRSF) Nuclear Trafficking Indirectly through a Complex with REST/NRSF-interacting LIM Domain Protein (RILP) and Dynactin p150Glued*

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Huntingtin has been reported to regulate the nuclear translocation of the transcriptional repressor RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF). The REST/NRSF-interacting LIM domain protein (RILP) has also been shown to regulate REST/NRSF nuclear translocation. Therefore, we were prompted to address the question of how two distinct proteins could have the same function. We initially used a yeast two-hybrid screen to look for an interaction between huntingtin and RILP. This screen identified dynactin p150Glued as an interacting protein. Coimmunoprecipitation of proteins in vitro expressed in a reticulocyte lysate system showed an interaction between REST/NRSF and RILP as well as between RILP and dynactin p150Glued. Coimmunoprecipitation analysis further showed a complex containing RILP, dynactin p150Glued, and huntingtin. Huntingtin did not interact directly with either REST/NRSF or RILP, but did interact with dynactin p150Glued. The N-terminal fragment of wild-type huntingtin did not affect the interaction between dynactin p150Glued and RILP; however, mutant huntingtin weakened this interaction. We further show that HAP1 (huntingtin-associated protein-1) prevents this complex from translocating REST/NRSF to the nucleus. Thus, this study suggests that REST/NRSF, dynactin p150Glued, huntingtin, HAP1, and RILP form a complex involved in the translocation of REST/NRSF into the nucleus and that HAP1 controls REST/NRSF cellular localization in neurons.

Huntington disease (1–4) is a choreic-psychiatric neurodegenerative disease caused by a mutation in the huntingtin protein (5) in which expansion of a CAG tract (>35 repeats) at the 5'-end of the gene is translated into a toxic polyglutamine (polyQ)2 stretch (6). The pathogenic mechanisms induced by mutant polyQ-huntingtin are still not clearly understood (6, 7). However, it was reported that huntingtin interacts with the transcriptional regulator RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) (8), which is a major silencing transcription factor regulating the expression of numerous neuronal genes (9, 10). The interaction between huntingtin and REST/NRSF was reported to retain REST/NRSF in the cytosol, thereby preventing REST/NRSF target gene repression.

REST/NRSF (9, 10) binds to a 21-bp DNA cis-regulatory element within the regulatory regions of its target genes (11–13) known as RE1 (repressor element-1) or the neuron-restrictive silencer element (NRSE). There are >1000 genes containing RE1/NRSE and even more when one considers functional RE1/NRSE-like elements. These include genes that encode proteins with fundamental importance for neuronal function, including signaling proteins, adhesion molecules, synaptic vesicle proteins, neuronal receptors, and neurotransmitter synthetic enzymes (14) and channel proteins (15). REST/NRSF is also involved in the regulation of neural cell fate determination and the repression of neuron-specific genes in differentiated nonneuronal cells (9, 10, 12, 13).

Recently, we described a novel protein that interacts with the zinc finger domains of REST/NRSF (16). This protein, the REST/NRSF-interacting LIM domain protein (RILP), is predominantly a nuclear protein that is required for the translocation of REST/NRSF into the nucleus. It thus seemed surprising that two distinct proteins, huntingtin and RILP, would have the same function and act independently. In the present study, we identify dynactin p150Glued as an interacting protein of both RILP and huntingtin and demonstrate formation of a complex between these proteins that, in neurons, additionally contains HAP1. This study provides new insights into the role of RILP, huntingtin, dynactin p150Glued, and HAP1 in the regulation of neuron-specific gene expression by REST/NRSF.

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2The abbreviations used are: polyQ, polyglutamine; REST/NRSF, RE1-silencing transcription factor/neuron-restrictive silencer factor; NRSE, neuron-restrictive silencer element; RILP, REST/NRSF-interacting LIM domain protein; siRNA, small interfering RNA; TBS, Tris-buffered saline; Ni-NTA, nickel-nitrilotriacetic acid.
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EXPERIMENTAL PROCEDURES

Yeast Two-hybrid System—A yeast two-hybrid screen was conducted with the MATCHMAKER Yeast Two-hybrid System 3 (Clontech) using a C-terminal fragment (amino acids 391–831) of human RILP as bait. A DNA fragment encoding amino acids 391–831 of human RILP was amplified from its cDNA using PfuTurbo Hotstart DNA polymerase (Stratagene) and primers TCACCCAACCTAGACACTCTGAAGTGAG-GTACGGATGTG and CCCATCGCTGTAGCGCTGT. This fragment was first inserted into the Smal site in pBluescript and then inserted in-frame into the bait vector pGBK7. A human brain MATCHMAKER cDNA library constructed in the pGAD10 vector (Clontech) was used for screening. The sequences were confirmed by DNA sequencing. Positive clones were identified by nutritional selection (synthetic defined medium) and confirmed by DNA sequencing. Positive clones were identified by nutritional selection (synthetic defined medium) and confirmed by DNA sequencing.

Plasmid Construction—HAP1 and dynactin p150Glued cDNAs were obtained from American Type Culture Collection (Manassas, VA). HAP1 cDNA was amplified by PCR and subcloned into the pCR2.1-TOPO vector (Invitrogen). The plasmid was digested with EcoRI/XbaI and cloned into pcDNA3.1 (Invitrogen). The cDNAs of wild-type (containing 15 polyQs) and mutant (containing 138 polyQs) huntingtin proteins in pHM830 was obtained from Dr. R. Truant (McMaster University). These plasmids were digested with Xhol/NotI, and the fragments were inserted into pcDNA3.1. The sequences were confirmed by DNA sequencing.

Pulldown Assay of RILP and Dynactin p150Glued—A full-length RILP cDNA was cloned into pcDNA3.1-His, which contains an N-terminal His tag (Invitrogen). A full-length cDNA of dynactin p150Glued (American Type Culture Collection) was cloned into the expression vector pCMVTag2 (Stratagene). The sequences of both constructs were confirmed by DNA sequencing. FLAG-tagged dynactin p150Glued and/or His-tagged RILP was expressed in the TN T coupled rabbit reticulocyte lysate protein expression system (Promega) using the manufacturer’s protocol. Lysates were applied to a Ni-NTA–agarose column using 20 mM phosphate buffer, followed by washing with this buffer. The absorbed proteins were eluted with 200 mM imidazole in the phosphate buffer.

Coimmunoprecipitation Analysis—Lysates from HeLa and NT2 cells were prepared in lysis buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 1.0% Nonidet P-40). NT2 cells were incubated in medium containing 10 μM all-trans-retinoic acid (Sigma) to induce a neuronal phenotype, NT2 cells were treated with 10 μM all-trans-retinoic acid in Dulbecco’s modified Eagle’s medium for 4 weeks. The medium was changed every 2–3 days.

Construction and Transfection of Small Interfering RNA—Double-stranded small interfering RNA (siRNA) was purchased from Ambion. The oligonucleotide sequence of HAP1 siRNA (17) is 5′-GAAGTATGTCTCAGAAA-3′. The following scrambled random oligonucleotide were used as a negative control: 5′-CGCGCTTTGTAGGGATTCG-3′. Transfection was performed using the Silencer™ siRNA transfection kit (Ambion) according to the manufacturer’s protocol.

Reporter Gene Analysis—The EcoRI/Xhol fragment of the human cholinergic gene locus was inserted into the multiple cloning site of the pXP2 vector. pXP2-EX is a construct containing a 2200-bp 5′-extension of the vesicular acetylcholine transporter gene. After transfection of these constructs as well as the pCMV-β-galactosidase plasmid into cells using Effectene reagent (Qiagen), cell lysates were prepared, and luciferase activity was measured by a luminescence assay (18). β-Galactosidase activity was measured using the Galacto-Light system (Applied Biosystems) according to the manufacturer’s procedure. Luminescence was detected using an Opticomp I luminometer (GEM Biomedical, Inc.).

Intracellular Localization of RILP—HeLa and NT2 cells were cultured in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum at 37 °C in 5% CO2. To transfect cells, cells were plated on glass coverslips (22 × 22 mm) in 6-well plates and transfected the next day. The transfected cells were grown for 24 h, fixed in dry ice/methanol for 2 min, washed three times with phosphate-buffered saline, and blocked in Tris-buffered saline (TBS)-T-M (20 mM Tris-HCl (pH 7.6), 137 mM NaCl, 0.1% Tween 20, and 5% skim milk). Fixed cells were incubated with antibodies (anti-NRSF antibody (12C11-1) at 1:50 dilution, with antibodies (anti-NRSF antibody (12C11-1) at 1:50 dilution, and anti-HAP1 antibody at 1:50 dilution) in TBS-T-M at 25 °C for 30 min. Cells were washed three times with TBS-T-M, followed by incubation with Alexa Fluor 594- or Alexa Fluor 488-linked IgG (Molecular Probes) in TBS-T-M. Coverslips were mounted in VECTASHIELD H-1200 (Vector Laboratories), which contains 4′,6-diamidino-2-phenylindole for staining nuclei. Fluorescence was observed using a Nikon E600 epifluorescent microscope. Images were analyzed by confocal microscopy. Quantification was performed using Leica LCS software from three line scan intensity measurements through each cell. The average intensity of nuclear or cytosolic REST in each scan was used to calculate the fraction of REST in the nucleus relative to REST in the cytosol. The values of the averaged cell measurements are reported as means ± S.D. (n = 12).
RESULTS

To elucidate the function of RILP and its relationship to huntingtin, we screened for interacting proteins using a yeast two-hybrid system. A C-terminal RILP fragment (amino acids 391–831) was used as bait to screen a human brain MATCHMAKER cDNA library. As noted previously, the N terminus of RILP contains LIM domains, which are REST/NRSF-interacting domains, whereas the C-terminal domain is important for nuclear localization of RILP and REST/NRSF (16, 18). This yeast two-hybrid screen yielded a single partial clone of dynactin p150Glued. To further study the interaction between dynactin p150Glued and RILP, a full-length cDNA of human dynactin p150Glued was cloned into pCMVTag2, in which a FLAG epitope tag was added to the N terminus. The cDNA for RILP was cloned into pcDNA3.1-His, in which an N-terminal His tag was added. His-RILP and FLAG-dynactin p150Glued were then coexpressed in vitro using the TNT coupled reticulocyte lysate system. An aliquot of the lysate was applied to Ni-NTA-agarose, washed, and eluted with 200 mM imidazole, with the fractions subjected to Western blot analysis using anti-FLAG or anti-His antibodies. As a result, both RILP and dynactin bound to Ni-NTA-agarose and were found in eluates (Fig. 1, E lanes), suggesting that they directly interact.

Because the above data show that RILP interacts with dynactin p150Glued, we further analyzed the physiological interaction of endogenous RILP, dynactin p150Glued, and huntingtin. Lysates from HeLa cells were prepared and incubated with anti-RILP, anti-dynactin p150Glued, or anti-huntingtin antibodies and pulled down with protein A/G. Western blot analysis of the immunoprecipitates detected RILP, dynactin p150Glued, and huntingtin in the same immunoprecipitate (Fig. 2), indicating they form a complex.

Although it was reported that REST/NRSF interacts with both mutant huntingtin and wild-type huntingtin and that this interaction controls REST/NRSF function (8), a direct interaction was not demonstrated. To determine whether a direct interaction between wild-type or mutant huntingtin and REST/NRSF occurs, they were coexpressed in vitro. As shown in Fig. 3a, in vitro expressed RILP and REST/NRSF were coimmunoprecipitated with anti-RILP or anti-REST/NRSF antibodies, confirming our previous results. RILP and dynactin p150Glued were also coimmunoprecipitated with anti-RILP or anti-dynactin p150Glued antibodies (Fig. 3a), confirming a direct interaction between RILP and dynactin p150Glued. In contrast, when we analyzed for an interaction between REST/NRSF and either wild-type or mutant huntingtin using in vitro translated pro-
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The endogenous RILP-dynactin p150Glued-huntingtin complex was also found in differentiated NT2 cells, a neuron-like cell line that expresses, in addition, the neuron-specific huntingtin-associated protein HAP1 (19, 20). As shown in Fig. 5a, HAP1 was coimmunoprecipitated with the endogenous complex from NT2 cells. To study direct interactions, wild-type or mutant huntingtin, dynactin p150Glued, and RILP were expressed in vitro in the presence or absence of HAP1. After immunoprecipitation with anti-huntingtin or anti-RILP antibodies, Western blot analysis was conducted using anti-huntingtin or anti-RILP antibodies. As shown in Fig. 5b, huntingtin was coimmunoprecipitated with RILP in the presence or absence of HAP1, but mutant huntingtin was precipitated only in the absence of HAP1 (Fig. 5b, fourth and eighth lanes). The ratio of band densities of huntingtin versus RILP were 3.15 (Fig. 5b, seventh lane) and 1.79 (eighth lane). Expression of mutant huntingtin caused a decrease of ~50% in the interaction of RILP and wild-type huntingtin. This suggests that HAP1 affects the interaction between mutant huntingtin, dynactin p150Glued, and RILP. In vitro translated HAP1 and REST/NRSF or HAP1 and RILP did not coimmunoprecipitate (Fig. 5c), showing that neither REST/NRSF nor RILP interacts directly with HAP1.

To further analyze the effect of HAP1 on complex formation, mouse striatal cells expressing wild-type or mutant huntingtin were used for coimmunoprecipitation analysis. Lysates from each cell line were prepared and incubated with anti-RILP, anti-dynactin p150Glued, anti-HAP1, or anti-huntingtin antibodies and pulled down with protein A/G. As shown in Fig. 6 (upper panel), Western blot analysis of the immunoprecipitates detected RILP, dynactin p150Glued, HAP1, and huntingtin in the same immunoprecipitate, indicating they form a complex. In striatal cell lines expressing mutant huntingtin, most of the RILP did not coimmunoprecipitate with anti-dynactin p150Glued, anti-HAP1, or anti-huntingtin antibodies, suggesting that mutant huntingtin causes a dissociation of RILP from the complex.

FIGURE 4. RILP interacts with huntingtin through dynactin p150Glued. Each protein was coexpressed and subjected to immunoprecipitation (IP) using the appropriate antibodies as shown in Fig. 3. Immunoprecipitation in the absence (a) or presence (b) of dynactin p150Glued was carried out with anti-RILP (α-RILP), anti-dynactin p150Glued (α-p150), or anti-huntingtin (α-Htt) antibodies. Aliquots of immunoprecipitates were subjected to Western blot analysis with anti-RILP, anti-p150, or anti-Htt antibodies. + and −, presence and absence, respectively, of each protein in the lysate; S, starting lysate for immunoprecipitation; wild, wild-type; mut, mutant. The input was one-fifth of what was applied to immunoprecipitation.

FIGURE 5. Effect of HAP1 on the interaction between RILP and huntingtin. α, RILP coimmunoprecipitated with dynactin p150Glued, HAP1, and huntingtin in NT2 cells. NT2 cell lysates were prepared in lysis buffer and subjected to immunoprecipitation (IP) with anti-RILP, anti-dynactin p150Glued (p150), anti-huntingtin (Htt), anti-HAP1, or nonimmune antiserum. Nonimmune antibodies were from goat and rabbit. Aliquots of immunoprecipitates were subjected to Western blot analysis with antibodies. INPUT is one-tenth what was applied to immunoprecipitation. b, proteins were coexpressed and subjected to immunoprecipitation using anti-huntingtin or anti-RILP antibodies as described in the legend to Fig. 3. Immunoprecipitation was carried out with anti-RILP (α-RILP) or anti-huntingtin (α-Htt) antibodies. Aliquots of immunoprecipitates were subjected to Western blot analysis with anti-RILP or anti-Htt antibodies. + and −, presence and absence, respectively, of each protein in the lysate; S, starting lysate for immunoprecipitation. The input was one-fifth of what was applied to immunoprecipitation. c, the interaction between REST/NRSF and HAP1 or RILP and HAP1 was analyzed. Immunoprecipitation was carried out with anti-HAP1 (α-HAP), anti-RILP, or anti-NRSF (α-NRSF) antibodies. Aliquots of immunoprecipitates were subjected to Western blot analysis with anti-RILP, anti-NRSF, or anti-HAP1 antibodies.

proteins, they were not coimmunoprecipitated (Fig. 3b), showing that REST/NRSF and wild-type or mutant huntingtin do not interact directly.

We then looked for an interaction between huntingtin and RILP to which REST/NRSF could bind. Wild-type or mutant huntingtin and RILP were coexpressed, followed by immunoprecipitation with anti-huntingtin or anti-RILP antibodies. As shown in Fig. 4a, neither wild-type nor mutant huntingtin interacted directly with RILP. However, when mutant or wild-type huntingtin and RILP were coexpressed in the presence of dynactin p150Glued and immunoprecipitated with anti-huntingtin, anti-dynactin p150Glued, or anti-RILP antibodies, all three proteins were found (Fig. 4b). These results show that mutant or wild-type huntingtin and RILP interact indirectly through dynactin p150Glued. Comparing the intensities of bands, the interaction between RILP and mutant huntingtin appeared weaker than the interaction between RILP and wild-type huntingtin.

In vitro translated HAP1 and REST/NRSF or HAP1 and RILP did not coimmunoprecipitate (Fig. 5c), showing that neither REST/NRSF nor RILP interacts directly with HAP1.
Because HAP1 is expressed predominantly in neuronal cells, it may play an important role in regulating the activity of the complex. The effect of HAP1 on the translocation of REST/NRSF to the nucleus was thus studied using an RE1/NRSE-containing reporter gene. This reporter gene (pXP2-EX) is composed of the cholinergic gene locus RE1-containing promoter element linked to a luciferase reporter gene (14). As shown in Fig. 7a, expression of this pXP2-EX reporter gene was suppressed in HeLa cells, whereas the same construct in which the RE1 element was inactivated by mutation (pXP2-EXmut) was not repressed. This confirms that in HeLa cells REST/NRSF localizes to the nucleus and binds to RE1/NRSE, leading to repression of RE1/NRSE-containing genes. Interestingly, overexpression of HAP1 in HeLa cells led to a significant increase in pXP2-EX expression almost to the level of inactive pXP2-EXmut. This shows that, in the presence of HAP1, REST/NRSF is not functional and likely was not translocated to the nucleus. As expected, an siRNA for HAP1 did not change reporter gene expression in HeLa cells because these cells do not express HAP1. Similarly, overexpression of HAP1 in NT2 cells did not significantly change the expression of both pXP2-EX and pXP2-EXmut. However, lowering HAP1 expression with an siRNA in NT2 cells led to repression of reporter gene expression (Fig. 7a), consistent with the requirement for HAP1 to prevent REST/NRSF nuclear translocation.

To directly show the effect of HAP1 expression on the intracellular localization of REST/NRSF, expression or repression of HAP1 by siRNA was performed. HeLa or NT2 cells were treated with HAP1 siRNA or a HAP1 expression construct for 24 h, and then REST/NRSF was transected into cells. The localization of REST/NRSF was determined by immunostaining with anti-REST/NRSF antibody followed by Alexa Fluor 594-conjugated goat anti-rabbit IgG, whereas HAP1 was analyzed using anti-HAP1 antibody followed by Alexa Fluor 488-linked antibody. As shown in Fig. 8, in HeLa cells, REST/NRSF was detected in the nucleus. Expression of HAP1 in HeLa cells caused a shift of REST/NRSF and HAP1 to the cytosol. Used as a control, expression of HAP1 siRNA did not change REST/NRSF localization in HeLa cells. No cross-reactivity of anti-HAP1 and anti-REST/NRSF antibodies was determined. On the other hand, in NT2 cells, REST/NRSF was detected in the cytosol (Fig. 8, upper panels). Expression of HAP1 was also detected in the cytosol. Repression of HAP1 in NT2 cells treated with siRNA caused REST/NRSF to mislocalize to the nucleus. HAP1 was barely detectable, indicating that siRNA treatment effectively lowers intracellular HAP1.

**DISCUSSION**

A new role for the huntingtin protein had been elucidated by showing its modulation of the nuclear translocation of the transcriptional repressor REST/NRSF (8). Loss of this activity in mutant huntingtin may contribute to disease pathogenesis in Huntington disease (21). However, a direct interaction between huntingtin and REST/NRSF was not shown, and the mechanism by which huntingtin controls REST/NRSF localization was unclear. We reported that RILP is also required for the translocation of REST/NRSF into the nucleus and controls neuronal gene expression (16). Thus, both RILP and huntingtin appear to have the same function. To elucidate the role of RILP, we identified dynactin p150Glued as its interacting protein. Sim-
It was reported that dynactin p150Glued and huntingtin interact (22, 23). It was also suggested that REST/NRSF interacts with huntingtin, whereas mutant huntingtin causes abnormal neuronal REST/NRSF nuclear localization (8). We have employed coimmunoprecipitation analysis using HeLa cells to show that RILP, dynactin p150Glued, and huntingtin interact and form a complex.

Using *in vitro* expression of these proteins, we have shown that huntingtin does not directly interact with REST/NRSF, but instead interacts with dynactin p150Glued, which interacts in turn with RILP, a protein shown to directly interact with REST/NRSF. Thus, our data suggest that REST/NRSF, dynactin p150Glued, huntingtin, and RILP form a quaternary complex involved in the translocation of REST/NRSF, likely through a microtubule pathway, into the nucleus.

In NT2 neuronal cells, we found that the complex additionally contains HAP1, consistent with reports that HAP1 interacts with REST/NRSF, dynactin p150Glued, huntingtin, and RILP form a quaternary complex involved in the translocation of REST/NRSF, likely through a microtubule pathway, into the nucleus.

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This study further suggests that mutant polyQ-huntingtin binding to the REST/NRSF–RILP–dynactin–HAP1 complex alters its conformation, permitting REST/NRSF to inappropriately be transported to the nucleus, resulting in the repression of a host of neuronal genes. To support this hypothesis, we used mouse striatal cell lines established from wild-type and mutant huntingtin knock-in embryos (25). Gel filtration of extracts from these cells showed that RILP in the cell line expressing wild-type huntingtin is detected in a high molecular weight fraction indicative of a complex with dynactin p150Glued, huntingtin, and RILP. However, in mutant huntingtin-expressing cells, the majority of RILP is detected in a lower molecular weight fraction than found in wild-type huntingtin-expressing cells. This is consistent with the data in this study showing that HAP1 affected the interaction between mutant huntingtin, dynactin p150Glued, and RILP in the complex and caused release of RILP from the complex.

Reporter gene analysis showed that when HAP1 is overexpressed in a non-neuronal cell line such as HeLa, REST/NRSF gene repression is reversed. Conversely, lowering HAP1 expression in a neuronal cell such as NT2 leads to REST/NRSF repression of an RE1/NRSE-containing gene. In accordance with these data, our results clearly show that in non-neuronal cells, which do not express HAP1, REST/NRSF localizes to the nucleus. However, REST/NRSF localizes to the cytosol when HAP1 is overexpressed. In NT2 neuronal cells, REST/NRSF localizes normally to the cytosol. Repression of HAP1 causes REST/NRSF to abnormally localize to the nucleus. These results show that HAP1, likely through its interaction with the RILP-dynactin-huntingtin complex, prevents the complex from translocating REST/NRSF to the nucleus, making HAP1 a key regulator of REST/NRSF activity.

Thus, the paradox of how both RILP and huntingtin affect REST/NRSF trafficking to the nucleus appears to be answered.

3 M. Shimojo, manuscript in preparation.

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**FIGURE 8. Effect of HAP1 expression on REST/NRSF localization.** HeLa and NT2 cells were grown on a coverslip and transfected with HAP1 siRNA or HAP1 expression vector. After 24 h of culture, REST/NRSF was transfected, followed by culturing for an additional 24 h. The cells were then fixed with dry ice/cold methanol and incubated with anti-REST/NRSF (12C11-1) and anti-HAP1 antibodies followed by fluorescently labeled IgG. Red fluorescence is due to RILP; and blue fluorescence is due to nuclear 4',6-diamidino-2-phenylindole (DAPI) staining. Upper panels, cells transfected with REST/NRSF as well as with a non-related sequence control siRNA; middle panels, cells treated with REST/NRSF following transfection with REST/NRSF; lower panels, cells transfected with HAP1 siRNA followed transfecting with REST/NRSF. The subcellular distribution of REST was examined by confocal microscopy as described under “Experimental Procedures.” The relative staining intensities yielded the following percentages of nuclear REST in HeLa cells: 91.24 ± 3.17% (REST), 20.19 ± 4.42% (REST and HAP1), and 91.86 ± 1.70% (REST and HAP1 siRNA). The percentages of nuclear REST in NT2 cells were as follows: 9.73 ± 2.09% (REST), 9.71 ± 2.20% (REST and HAP1), and 81.73 ± 2.92% (REST and HAP1 siRNA).
This study, coupled with others, shows that both RILP and huntingtin interact directly with dynactin p150Glued to form a complex. REST/NRSF binds to this complex through a direct interaction with RILP. In the absence of HAP1, as in non-neuronal cells, this complex traffics REST/NRSF to the nucleus. In neuronal cells, HAP1 binds to huntingtin and causes the retention of the complex in the cytosol. There are undoubtedly other proteins involved in this process, which can now be sought. Finally, this study has described a previously unidentified trafficking complex; provided new insights into the roles of huntingtin, HAP1, dynactin p150Glued, and RILP in the regulation of neuron-specific gene expression by controlling REST/NRSF trafficking; and suggested a new target for Huntington disease.

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