

The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription

Joan S. Steffan*, Aleksey Kazantsev[†], Olivera Spasic-Boskovic[‡], Marilee Greenwald*, Ya-Zhen Zhu*, Heike Gohler[§], Erich E. Wanker[§], Gillian P. Bates[‡], David E. Housman[†], and Leslie M. Thompson*[¶]

*Department of Biological Chemistry, D240 Medical Sciences I, University of California, Irvine, CA 92697-1700; [†]Department of Biology Center for Cancer Research, Massachusetts Institute of Technology, Building E17-543, Cambridge, MA 02139; [‡]Medical and Molecular Genetics, GKT School of Medicine, King's College, 8th Floor Guy's Tower, Guy's Hospital, London SE1 9RT, United Kingdom; and [§]Max-Planck-Institut für Molekulare Genetik, Ihnestr. 73, Berlin D-14195, Germany

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Huntington's Disease (HD) is caused by an expansion of a polyglutamine tract within the huntingtin (htt) protein. Pathogenesis in HD appears to include the cytoplasmic cleavage of htt and release of an amino-terminal fragment capable of nuclear localization. We have investigated potential consequences to nuclear function of a pathogenic amino-terminal region of htt (httex1p) including aggregation, protein-protein interactions, and transcription. httex1p was found to coaggregate with p53 in inclusions generated in cell culture and to interact with p53 *in vitro* and in cell culture. Expanded httex1p represses transcription of the p53-regulated promoters, p21^{WAF1/CIP1} and MDR-1. httex1p was also found to interact *in vitro* with CREB-binding protein (CBP) and mSin3a, and CBP to localize to neuronal intranuclear inclusions in a transgenic mouse model of HD. These results raise the possibility that expanded repeat htt causes aberrant transcriptional regulation through its interaction with cellular transcription factors which may result in neuronal dysfunction and cell death in HD.

Huntington's disease (HD) is an autosomal dominant, late-onset neurodegenerative disorder characterized by motor abnormalities, cognitive dysfunction, and psychiatric symptoms (1). HD is caused by an expansion of a polyglutamine tract in the amino-terminal portion of a predominantly cytosolic protein, huntingtin (htt) (2, 3). Repeat expansions greater than approximately 38 repeats cause disease, while unaffected individuals can have repeat lengths of up to 35 repeats (4).

A transgenic mouse model for HD has shown that human huntingtin exon 1 carrying an expanded CAG repeat is sufficient to cause a HD-like phenotype (5). Preceding onset of neurological symptoms, the appearance of intranuclear inclusions, nuclear localized aggregates containing truncated htt and ubiquitin proteins, have been found in neurons of HD transgenic mice (6). These inclusions have also been identified in neurons and dystrophic neurites in HD brain tissue (7) and as cytosolic aggregates in the neuronal processes (neuropil) of both HD patient and transgenic mouse brain tissues (8, 9). Colocalization of a variety of cellular proteins, including transcription factors such as CREB-binding protein (CBP) (10), TATA-binding protein (TBP) (11), and mSin3a (12), have been shown in cell culture (CBP) and human brain (TBP and mSin3a) inclusions. The role that aggregation plays in disease progression is unclear (13–15); however, nuclear localization of the protein following cytosolic cleavage (16) has been implicated as having an important role in disease pathogenesis in both cell culture (17) and in transgenic mouse models (18).

HD results in selective neuronal degeneration, especially of the striatum and cerebral cortex (19). There is evidence that programmed cell death (apoptosis) may be involved in chronic neurodegenerative disorders including Alzheimer's, Parkinson's, and Huntington's diseases (20). Apoptotic cell death in HD has been observed using postmortem HD brain tissue (21) and in some mouse models (14), although the mechanism for cell death remains unclear.

The amino-terminal portion of htt that remains after cytoplasmic cleavage and can localize to the nucleus appears to include the polyglutamine repeat and a proline-rich region which have features in common with a variety of proteins involved in transcriptional regulation (22). Therefore, htt is potentially capable of direct interactions with transcription factors or the transcriptional apparatus and of mediating alterations in transcription. We demonstrate that mutant htt exon 1 protein (httex1p) containing an expanded repeat coaggregates with p53 in cell culture-generated inclusions using a novel approach to evaluate the presence of cellular proteins associated with aggregates, and interacts with p53 in biochemical assays. In addition, we find that expanded httex1p interacts *in vitro* with two other critical transcription factors, the coactivator CBP and the corepressor mSin3a, and may act in the nucleus to regulate transcription. The tumor suppressor protein p53 plays a central role in determining whether a cell will undergo differentiation, senescence, or apoptosis (23) and has been implicated in the regulation of neuronal apoptosis (24). Both CBP and mSin3a interact with p53 and are involved in p53-mediated transcriptional regulation (25). CBP was found to localize to neuronal intranuclear inclusions in a transgenic mouse model of HD, and independent studies have shown that mSin3a colocalizes to neuronal intranuclear inclusions in human HD brain tissue (12). Taken together, these data suggest that expanded polyglutamine repeats in the context of a disease protein may cause aberrant transcriptional regulation, leading to neuronal dysfunction and degeneration in HD and other triplet repeat diseases.

Materials and Methods

Plasmid constructs. Alternating CAG/CAA repeats, coding for either a normal range or expanded polyglutamine tract, were put into the context of either a truncated [first 17 amino acids plus poly(Q) repeat] or complete htt exon 1 (10) and subcloned into pcDNA 3.1. Repeats encoding 25 [25QP-green fluorescent protein (GFP)] or 103 (103QP-GFP) glutamines within the complete exon 1 sequence were fused in-frame to the coding sequence for an enhanced green fluorescence protein tag at the 3' end of each construct. Truncated exon 1 constructs were also created encoding a myc epitope tag at the carboxyl terminus instead of enhanced green fluorescence protein (10).

Abbreviations: HD, Huntington's disease; htt, huntingtin; httex1p, huntingtin exon 1 protein; CBP, CREB-binding protein; GST, glutathione S-transferase; GFP, green fluorescent protein; AR, androgen receptor; RXR α , retinoid X receptor alpha; TBP, TATA-binding protein; SH3, Src homology 3.

[¶]To whom reprint requests should be addressed at: Department of Biological Chemistry, D240 Medical Sciences I, University of California, Irvine, CA 92697-1700. E-mail: lmthomps@uci.edu.

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Glutathione *S*-transferase (GST)-htt exon 1 fusion proteins containing 20, 51, and 83 polyglutamine repeats were described previously (26). Truncated GST-103Q fusion protein was derived from the 103Q-myc construct by subcloning into pGEX-3X (Pharmacia Biotech), eliminating the myc coding region. pGEX-2T was used for the GST control. DNAs encoding GST fusion proteins were ligated into pcDNA3.1 for expression in mammalian cells.

Full-length p53 (1–393) (kindly provided by Eric Stanbridge), encoded within a 1.8-kb *Bam*HI fragment, was subcloned into pcDNA 6.0 *myc*/His C. To create a truncated p53 (amino acids 1–347), the previous construct was digested with *Stu*I and religated. The cDNAs for CBP in pcDNA3.1 (10) and mSin3a in pcDNA3 (kindly provided by Maureen Murphy) were used for protein synthesis. For expression in mammalian cells, htt exon 1 constructs encoding 20 (20QP) or 93 (93QP) polyglutamines in pTTL1 (27) were used.

The WAF1-luciferase fusion was created by annealing the two oligonucleotides, 5'-CGCGTGAATTCGAACATGTCCCAACATGTTGCCC-3' and 5'-GGGCAACATGTTGGGACATGTTTCAATTCA-3', containing two copies of the WAF1 p53 binding site, generating a duplex with 5' *Mlu*I and *Eco*RI sites and a 3' *Sma*I site and subcloning into the *Mlu*I-*Sma*I site of the pGL3-promoter vector (Promega).

Isolation and Biochemical Analyses of Poly(Q) Aggregates. HEK293 cells were transiently transfected with 103Q-GFP or 103QP-GFP encoding constructs using Lipofectin or GenePORTER™ II (Gene Therapy Systems). Aggregates in the media from plates of efficiently transfected cells (85–90%) showing extensive aggregate formation were pelleted by centrifugation and washed with PBS and 0.1% Triton X-100/PBS. Aggregates were incubated with rocking for 30 min in 0.1% Triton X-100/PBS, overnight in 1% Triton X-100/PBS, 2 h in 0.1% SDS/PBS, and 30 min in 1% SDS/PBS; following each incubation step, aggregates were pelleted by centrifugation. For intact cells, 0.3% NP-40 was first used to lyse the cells. Experiments were performed with and without 100 μ M PMSF in the buffers and at room temperature or at 4°C with similar results due to the stability of the aggregates. Enrichment of aggregates was followed microscopically by monitoring GFP fluorescence. Semipurified aggregates were boiled in 2 \times loading buffer containing 4% SDS, 10% β -mercaptoethanol, 10 mM DTT, 20% glycerol, 0.1 M Tris-HCl (pH 6.8), and 4 mM EDTA for 10 min. Levels of immunoreactive protein were determined for each antibody in whole-cell extracts from the transfected cells and proportionate amounts of immunoreactive protein were loaded in equivalent ratios of whole-cell extract to aggregate preparation on 8% SDS gels. The gels were transferred overnight to Immobilon-P membrane (Millipore) by standard Western blot wet transfer methods. The immobilon membrane was blocked for 1 h in 5% Fraction V BSA (United States Biochemical) in 1 \times Tris-buffered saline/Tween 20, cut into strips and incubated for 1 h in antibody, washed three times, and incubated with goat anti-mouse or goat anti-rabbit horseradish peroxidase-conjugated antibodies (The Jackson Laboratory). Antibodies used include anti-p53 (DO-1), anti-mSin3a (AK-11), anti-CBP (A-22), anti-mdm2 (SMD14), anti-retinoid X receptor alpha (RXR α) (D-20), anti-NF- κ B p65 (F-6), anti-androgen receptor (AR) (N-20) (all from Santa Cruz Biotechnology), and anti-htt polyclonal (Q51) (27). Immunoreactive bands were detected using enhanced chemiluminescence (Amersham Pharmacia Biotech). Following enhanced chemiluminescence, blots were incubated with ¹²⁵I-protein A, washed three times in Tris-buffered saline/Tween 20 and quantitated by phosphorimager analysis.

GST Pull-Down Assays. GST pull-down experiments were performed as previously described by Steffan *et al.* (28, 29). [³⁵S]Me-

thionine-labeled full-length p53 (1–393), truncated p53 (1–347), CBP, and mSin3a were synthesized *in vitro* by using rabbit reticulocyte lysate systems (Promega). The radiolabeled proteins were added in approximately equal molar amounts to GST fusion proteins preequilibrated in buffer A (20 mM Hepes-KOH, pH 7.5, 100 mM NaCl, 10% glycerol, 0.1% Triton-X-100, and 100 μ M PMSF) containing 0.2% BSA in a final volume of 200 μ l. After a 30-min rocking at room temperature, the beads were recovered and washed three times at room temperature in 1 ml of buffer A. The bound proteins were analyzed by SDS-PAGE followed by autoradiography and phosphorimager analysis.

For cell culture experiments, 2- \times 100-mm dishes of HEK293 cells were transiently transfected with a total of 40 μ g of pcDNA3.1 plasmids encoding the following proteins using Lipofectin (GIBCO/BRL); 13 μ g of either GST, GST-20QP, GST-83QP, or GST103Q, 13 μ g of p53, and 14 μ g of CBP encoding DNAs. Twenty-four hours later, cells were harvested and resuspended in 400 μ l of buffer A including 10 μ g/ml of aprotinin and leupeptin, with 100 μ l of sterile acid-washed glass beads in a 1.6-ml microfuge tube. Cells were vortexed for 30 s and then placed on ice. Lysates were spun in a microfuge at 2000 rpm for 2 min, and supernatants were collected and assayed for protein concentration by Bradford analysis. Three hundred micrograms of lysates were incubated with 30 μ l of glutathione-agarose beads (Sigma) for 30 min at 4°C rocking in buffer A. Beads were then washed five times in 1 ml of Buffer A, resuspended in 2 \times Laemmli buffer, boiled 3 min, and loaded directly onto an 8% SDS-polyacrylamide gel. Western blot analysis was performed as above.

Luciferase Assays. For WAF1-luciferase assays, SAOS-2 cells in 6-well plates were transiently transfected by calcium phosphate precipitation using 100 ng of p53 expression vector, 2 μ g of WAF1-luciferase expression vector, and 2 μ g of pcDNA3.1 vector alone or encoding 25QP-GFP, 103QP-GFP, 25Q-myc, or 103Q-myc. For MDR-1 luciferase assays in SAOS-2 cells, Lipofectin transfection in 6-well plates of 2.5 μ g of MDR1-luciferase plasmid (30) and 500 ng of pcDNA3.1 vector alone or plasmids encoding p53, 20QP, 93QP, 25Q-myc, or 103Q-myc was done using pBlueScript (Stratagene) to equalize all concentrations of DNA to 5 μ g. For all luciferase assays, lysis buffer and luciferase substrates A and B buffers were used as described by the manufacturer (PharMingen). Luciferase activity was measured for 10 s with an Analytical Luminescence Laboratory Monolight 2010 Luminometer. The protein concentrations of lysates were determined by Bradford analysis and luciferase activity was calculated per milligram of protein.

Immunohistochemistry in Transgenic Mouse Brain. The transgenic mouse line R6/2 (5) was used [Jackson code B6CBA-TgN(HDexon 1)62] and maintained by backcrossing to (CBA \times C57BL/6)F₁ mice. Genotyping and CAG repeat sizing was as described previously (31). The source and working dilutions of antibodies were as follows: S830 (1:1000) was raised against an exon 1 htt fusion protein in sheep (previously unpublished), anti-ubiquitin (1:2000; Dako), and anti-CBP (A22) (1:1000; Santa Cruz Biotechnology). Immunohistochemistry was performed as described previously (32) on 15- μ m sections cut from isopentane frozen brains using Vectastain Elite ABC kit (Vector Laboratories). The biotinylated secondary anti-sheep antibody was from the Scottish Antibody Production Unit (Carluke, Scotland).

Results

p53 Coaggregates with Expanded httex1p in Inclusions in Mammalian Cell Culture. To explore potential interactions of htt with nuclear proteins involved in transcriptional regulation, the composition of cellular proteins associated with semipurified aggregates was

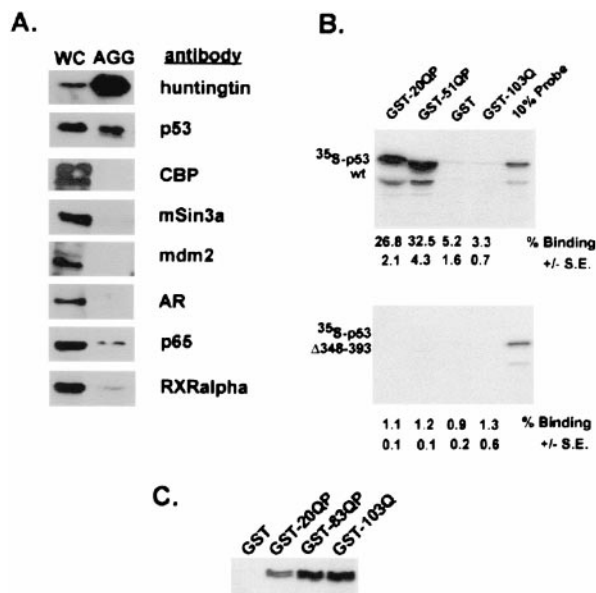


Fig. 1. p53 colocalizes with expanded httex1p in inclusions in mammalian cell culture and interacts with httex1p *in vitro* and in cell culture. (A) Western blot of solubilized proteins purified from aggregates generated in HEK293 cells expressing 103Q-GFP. Results were identical to aggregates purified from 103QP-GFP (data not shown). WC denotes the whole-cell fraction from transfected cells and AGG the aggregate preparation. Equivalent ratios of WC:AGG were run on 8% SDS gels and Western blotted. Antisera were used to examine relative levels of 103Q-GFP htt, p53, CBP, mSin3a, mdm2, AR, NF- κ B p65, and RXR α in the aggregate preparation. No immunoreactive htt protein was present in samples corresponding to the aggregate fraction isolated from 293 cells expressing 25Q-GFP (data not shown). (B) Binding of [³⁵S]methionine-labeled p53(1–393) and p53(1–347) to GST-htt fusion proteins. Equivalent amounts of [³⁵S]methionine-labeled p53(1–393) and p53(1–347) were mixed with GST-20QP, GST-51QP, GST, and GST-103Q attached to glutathione-agarose beads, incubated, and washed. The labeled proteins bound to the beads were analyzed by SDS-PAGE. Each experiment was done in triplicate. Percent binding \pm SE was calculated by phosphorimager analysis and is listed below each lane. Ten percent of the input of the labeled proteins was also analyzed as shown. Slight alteration in the pattern of p53 migration in the GST-51QP lane as compared with GST-20QP lane is because of large amounts GST-51QP comigrating with labeled p53. (C) Coprecipitation of p53 with httex1p in HEK293 cells. HEK293 cells were transiently transfected with plasmids encoding p53 and either GST, GST-20QP, GST-83QP, or GST-103Q. Cells were broken and lysates were incubated with glutathione-agarose beads. After extensive washing, beads were loaded directly on an 8% SDS gel. Western blot analysis using monoclonal anti-p53 antibody is shown.

evaluated using a novel purification technique. Expanded polyglutamine repeat containing httex1ps, epitope tagged at the carboxyl terminus with GFP, were expressed transiently in HEK293 cells and fluorescence analysis of fixed cells, both 103QP-GFP (complete exon 1 protein) and 103Q-GFP (a truncated form of exon 1 protein lacking the proline-rich region) formed inclusions predominantly in the cytosol, perinucleus, or media (data not shown). The extracellular aggregates were isolated from the media and soluble proteins were resolved by SDS-PAGE. Western blot analysis (Fig. 1A) demonstrates that htt and p53 are present in 103QP-GFP- or 103Q-GFP-generated inclusions. p53 is highly enriched compared with other cellular proteins, such as CBP and mSin3a, which have previously been shown to colocalize to inclusions by other methods (10, 12). Purification of aggregates from intact cells showed a similar enrichment for p53 (data not shown). Several other nuclear proteins were analyzed for an association with the aggregates following normalization of immunoreactivity of each protein in whole-cell lysates using ¹²⁵I quantitation. The AR and mdm2

appear to be absent in the aggregate preparation, while the p65 subunit of NF- κ B and RXR α appear to be present.

httex1p Interacts with the p53 Protein *In Vitro* and in Cell Culture. GST pull-down experiments were used to determine whether there is a direct interaction between the amino-terminal region of htt and p53. GST fusion proteins were purified from *Escherichia coli* expressing GST-20QP (complete exon 1 containing 20 glutamines), GST-51QP (exon 1 containing 51 glutamines), GST alone, and GST-103Q (truncated exon 1 containing 103 glutamines). ³⁵S-labeled full-length p53 (1–393) and truncated p53 (1–347) lacking the C-terminal region were incubated with each GST fusion protein coupled to glutathione-agarose beads, the beads were washed, and similar amounts of protein were resolved by SDS-PAGE. Fig. 1B shows autoradiography of a representative experiment. Full-length p53 binds strongly to either normal (GST-20QP) or expanded repeat (GST-51QP) protein *in vitro*. However, a long repeat containing expanded Qs (GST-103Q), but lacking the proline-rich region, shows greatly diminished binding, suggesting that binding of p53 and htt is dependent on the presence of the proline-rich region *in vitro*. p53 lacking the C-terminal amino acids 348–393 no longer interacts with htt (Fig. 1B). These data demonstrate that the amino-terminal region of htt interacts with p53 and this interaction requires the presence of the C-terminal region of p53 as well as the proline-rich region of htt.

To test for an interaction between p53 and httex1ps in cell culture, additional GST pull-down experiments were performed. GST fusion proteins, transiently coexpressed in HEK293 cells with p53, were copurified and analyzed by Western immunoblotting (Fig. 1C). p53 was found to copurify with normal range and expanded httex1p (GST-20QP and GST-83QP) as well as with a truncated form of an expanded httex1p lacking the proline-rich domain (GST-103Q), demonstrating a lack of dependence on the proline-rich region of httex1p for complex formation. This interaction of htt and p53 was also confirmed in coimmunoprecipitation experiments from cell lysates by using anti-GFP antibody (data not shown). Coimmunoprecipitations were complicated by the fact that anti-p53 polyclonal antisera were found to directly immunoprecipitate 25QP-GFP in the absence of p53, and similarly, polyclonal anti-Q51 antisera (27) could directly immunoprecipitate p53 protein in the absence of httex1p.

Expanded httex1p Represses Transcription. Because of the direct interaction observed between p53 and htt, we tested whether transient expression of httex1p has an effect upon transcription of genes regulated by p53. The expression of a *p21^{WAF1/CIP1}* reporter, WAF1-luciferase, which is transcriptionally activated by p53, was examined in SAOS-2 (p53^{-/-}) cells expressing exogenous p53, as minimal activation of this reporter occurs in the absence of p53. Repression of WAF1-luciferase by expanded httex1p (103QP-GFP) is observed, whereas normal range repeats (25QP-GFP) or htt constructs lacking the proline-rich region (25Q-myc, 104Q-myc) produced no significant repression (Fig. 2, upper), indicating a role for both the expanded polyglutamine repeat and the proline-rich region in transcriptional repression. Luciferase assays were also conducted in PC12 cells (p53^{+/+}) stably transfected with inducible htt constructs, all containing GFP epitope tags (A. Kazantsev and B. Apostol *et al.*, manuscript in preparation). Upon differentiation with nerve growth factor into a neuronal phenotype, cell lines expressing 103QP-GFP repressed transcription from both the complete *p21^{WAF1/CIP1}* promoter [WWP-luciferase (33)] and the WAF1-luciferase reporter, again in a proline- and expanded repeat-dependent manner (data not shown).

A promoter subject to p53-mediated transcriptional repression was also tested using luciferase assays conducted in transiently transfected SAOS-2 (p53^{-/-}) cells (Fig. 2, lower). The

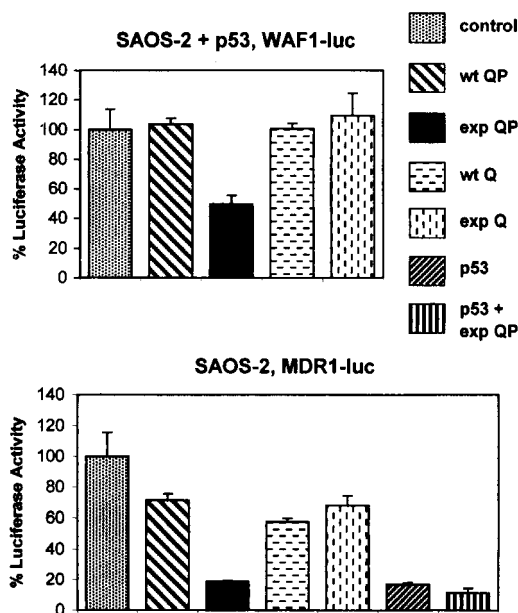


Fig. 2. Expanded httex1p represses transcription. (Upper) SAOS-2 cells were transfected with vector alone (control) or plasmids encoding 25QP-GFP, 103QP-GFP, 25Q-myc, or 103Q-myc proteins and plasmids encoding WAF1-luciferase and p53. (Lower) SAOS-2 cells were transfected with vector alone (control) or plasmids encoding 20QP, 93QP, 25Q-myc, or 103-myc proteins and the MDR1-luciferase reporter plasmid. A p53-encoding plasmid was also transfected as indicated. Each experiment was done in triplicate. Luciferase units per milligram of protein were calculated and are demonstrated graphically as percent of vector control values.

multiple drug resistance gene, *MDR-1*, has previously been shown to be transcriptionally repressed by p53 (30, 34), and this repression was shown to be dependent on the presence of the proline-rich region of p53 (35). The transcription of the *MDR-1* promoter fused to luciferase (30) was therefore examined. SAOS-2 cells, without expression of p53, showed high levels of *MDR-1* transcription. When p53 was expressed in these cells, *MDR-1* transcription was repressed (83%). When htt constructs were expressed in SAOS-2 cells, 93QP (httex1p with 93 repeats) produced extensive repression (81%) of *MDR-1* transcription, even in the absence of p53. This profile of transcriptional repression of *MDR-1* mediated by the htt constructs in the absence of p53 was similar to that observed for the WAF1-luciferase reporter in SAOS-2 cells expressing p53 and for the *p21^{WAF1/CIP1}* promoter in PC12 cells, indicating that transcriptional repression by expanded httex1p is dependent on the presence of the proline-rich region and on the length of the glutamine stretch.

Expanded httex1p Interacts *In Vitro* with CBP and mSin3a. CBP is a polyglutamine-containing transcription factor which coactivates p53 transcription through interaction with p53 residues 1–73, including the activation domain and part of the proline-rich region (36). Since expanded httex1p structurally resembles this region of p53 and since CBP aggregates in cell culture inclusions along with htt (10), GST pull-down experiments were used to examine the interaction between ³⁵S-labeled CBP and the following GST fusion proteins: GST, GST-20QP, GST-51QP, GST-103Q, and GST-p53. As shown in Fig. 3I, CBP interacts weakly with httex1p in a proline- and polyglutamine repeat length-dependent manner *in vitro*.

Analogous results were obtained for mSin3a, a corepressor that can mediate transcriptional repression through a direct

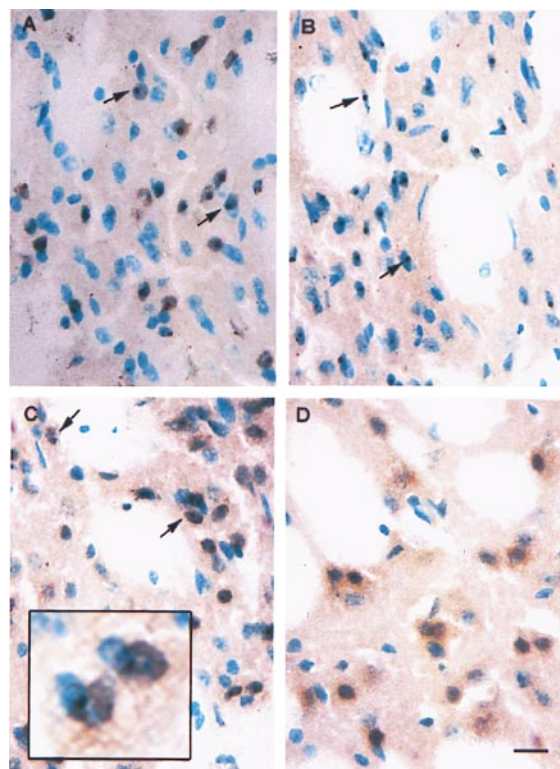
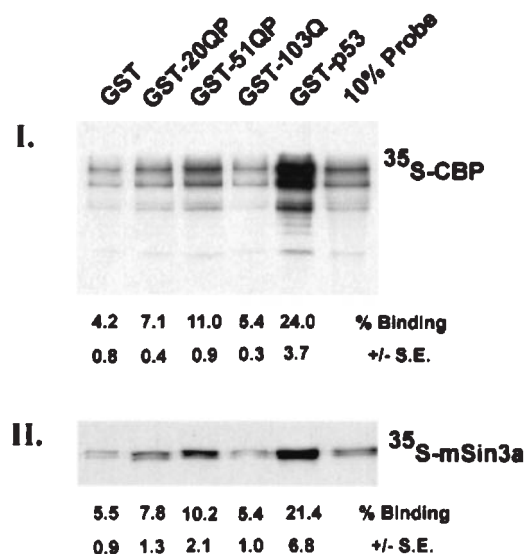


Fig. 3. CBP and mSin3a bind to httex1p *in vitro* and CBP localizes to nuclear inclusions in R6/2 mouse brains. (Top) Binding of [³⁵S]methionine-labeled CBP or mSin3a to GST-htt fusion proteins. Equivalent amounts of [³⁵S]methionine-labeled CBP (I) or mSin3a (II) were mixed with GST, GST-20QP, GST-51QP, GST-103Q, or GST-p53 attached to glutathione-agarose beads, washed, and the labeled proteins bound to the beads were analyzed by SDS-PAGE. Each experiment was done in triplicate. Percent binding ± SE was calculated by phosphorimager analysis and are listed below each lane. Ten percent of the input of the labeled proteins was also analyzed as shown. (A–D) Localization of CBP to neuronal intranuclear inclusions in R6/2 mice. Striatal sections from R6/2 (A–C) and littermate controls (D) were immunolabeled with anti-htt (A), -ubiquitin (B), and -CBP (C and D) antibodies. Nuclei were counterstained with methyl green. Nuclear inclusions are indicated by arrows. Scale bar, 15 μm.

interaction with p53 (25). mSin3a binds the amino-terminal region of p53 through an interaction with amino acids 40–160, which includes part of the activation domain and the proline-rich region of p53 (25). GST pull-down experiments, using the same GST fusion proteins described above, show that mSin3a also weakly interacts with httex1p in a proline- and polyglutamine repeat length-dependent manner *in vitro* (Fig. 3II).

CBP Localizes to Neuronal Intranuclear Inclusions in Transgenic Mice.

To demonstrate that the interactions of expanded httex1p with CBP occur *in vivo* in the neurons of a HD transgenic mouse model, immunohistochemistry of neuronal intranuclear inclusions was performed. It has previously been shown that mSin3a is present *in vivo* in neuronal intranuclear inclusions in human HD patient brain sections (12). As shown in Fig. 3, CBP (Fig. 3C) localizes to nuclear inclusions against background nuclear staining in striatal brain sections isolated from 12-week-old R6/2 transgenic mice. Inclusions from these sections also stain positive for htt (Fig. 3A) and ubiquitin (Fig. 3B) protein. CBP is also present in inclusions at 8 weeks (data not shown), suggesting that CBP localization to the aggregates is a relatively early process. The normal diffuse nuclear staining pattern of CBP is shown in wild-type littermate controls (Fig. 3D).

Discussion

We have examined the possibility that the pathogenic domain of mutant htt interacts with critical cellular transcription factors and potentially modulates p53-induced transcriptional events in cells. Our results indicate that p53 and httex1p coaggregate, interact *in vitro* and in cell culture, and that httex1p is capable of repressing transcription. In addition, the coactivator CBP and the corepressor mSin3a, known to interact with p53 and mediate p53 transcriptional regulation, were found to weakly interact with httex1p *in vitro*. Therefore, amino-terminal httex1p has functional properties which may have significant consequences for the p53 pathway in HD neurons.

It has not been determined how mutant htt acts in the neuron to cause neuronal dysfunction and subsequent degeneration, although studies suggest that selective neuronal programmed cell death (apoptosis) may be involved in a manner similar to that observed following induction by p53 (20, 21, 37). Recently, a study of Danish HD patients and controls demonstrated a significantly lower overall cancer incidence in patients with HD (38). A potential mechanism which could explain these findings is that mutant htt increases the rate of p53-dependent apoptosis in preneoplastic cells.

The mechanism by which p53 induces apoptosis is complex and not completely understood. The proline-rich region of p53 contains five PXXP motifs characteristic of Src homology 3 (SH3) domain interaction sites (39). This domain of p53 may mediate apoptosis via the transcriptional activation, repression (35, 40), or transcription-independent mechanisms (41). Deletion of all five PXXP domains in the proline-rich region of p53 inhibits its ability to induce apoptosis, whereas deletion of four of the five allows partial apoptotic activity (42). The amino-terminal region of p53 and httex1p share a proline-rich SH3 recognition site adjacent to mammalian transcriptional activation domains, a polyglutamine repeat in htt and an acidic activation domain in p53, which may have functional consequences in the pathogenic mechanism mediated by nuclear localized htt.

Recent studies suggest that the proline-rich region of p53 may interact with the C-terminal region of p53 (41, 43). Through GST pull-down experiments, we determined that both wild-type and expanded httex1p were able to interact *in vitro* with full-length but not C-terminally truncated p53, an interaction also dependent on the proline-rich region of htt.

Induction of apoptosis may rely, at least in part, on the ability of p53 to repress transcription (44). Because of the similarity between the proline-rich region and the interaction between p53 and httex1p, the possibility that mutant httex1p might mediate transcriptional repression was examined. Expanded httex1p was found to significantly repress transcription of a p53-activated promoter ($p21^{WAF1/CIP1}$) in luciferase assays and did so in a proline-dependent manner. This transcriptional repression may be due to a direct interaction with p53 interfering with transcriptional activation or to mutant htt directly or indirectly repressing transcription. To test this possibility, the transcription of an *MDR-1* reporter, which is repressed by p53, was examined (30). We found that expanded httex1p could repress transcription of *MDR-1* even in the absence of p53, potentially through mechanisms similar to p53-mediated repression.

There is precedence for transcriptional repression of specific genes in HD; expression of brain neurotransmitter receptors, in particular glutamate and D1 dopamine receptors, is decreased in brains from HD transgenic mice (45). Striatal gene expression profiles of HD transgenic mice compared with normal littermate controls, generated using DNA microarrays, show significant repression of genes involved in discrete signaling pathways (46). In addition, an interaction of the amino-terminal region of mutant htt with transcriptional repressor complex proteins may alter transcription (12).

A potential mechanism for repression of p53-regulated promoters might be through sequestration of a common coactivator, such as CBP/p300. p53 and other transcriptional activators such as NF- κ B^{p65/RelA} and the AR use the coactivators CBP/p300 (36, 47–49) to activate transcription. p53, NF- κ B^{p65/RelA}, and the viral protein E1A may all repress transcription in a sequence-independent fashion through sequestration of CBP/p300 (50, 51). Likewise, mutant htt may repress $p21^{WAF1/CIP1}$ and *MDR-1* transcription through direct interaction with CBP/p300. The direct interaction *in vitro* between CBP and expanded httex1p and the localization of CBP to neuronal intranuclear inclusions in HD transgenic mice and to inclusions in cell culture (10) lend credence to the possibility that expanded httex1p may repress transcription through direct interaction with and sequestration of CBP.

A second potential mechanism for repression of specific p53-regulated promoters might also be through interactions with components of corepressor complexes. It has been shown that p53-mediated transcriptional repression of specific promoters utilizes histone deacetylases mediated by a direct interaction of p53 with the corepressor mSin3a (25). The fact that expanded repeat htt is capable of repressing transcription, even in the absence of p53, and that htt can bind both CBP and mSin3a *in vitro*, raises the possibility that htt can mimic the function of p53 to alter cellular transcriptional events.

The transcriptional repression of the reporter constructs described here requires the presence of the proline-rich region of htt; however, coaggregation and formation of complexes in cell culture do not. This suggests that additional cellular factors may be involved in the complex formation between p53 and httex1p *in vivo*. Transcriptional repression may occur independent of inclusion formation and may precede gene expression changes caused by sequestration of basal transcription factors by aggregates. It is also possible that expanded httex1p may alter transcription-independent signaling pathways implicated in p53-mediated apoptosis which may require the SH3 domain interaction sites within the proline-rich region (41).

Selective neurodegeneration is characteristic of a number of human diseases caused by an expansion of an endogenous glutamine repeat in otherwise unrelated proteins. Besides HD, other such diseases include the spinocerebellar ataxias (spinocerebellar ataxias 1, 2, 3 (MJD), and 7), caused by expan-

sions in the respective ataxin gene, spinocerebellar ataxia 6 (CACNL1A4), spinal bulbar muscular atrophy/Kennedy's disease (AR), and DRPLA (atrophin-1) (14). With the exception of ataxin-3, for which polymorphic C termini adjacent to the polyglutamine repeat have been reported (52), each protein sequence contains proline-rich regions adjacent to the polyglutamine repeats that are expanded in the disease states. Within the proline-rich domains, these proteins contain a minimum of one consensus PXXP motif as well, although the majority contain several. The AR, in which an expanded polyglutamine repeat causes Kennedy's disease (spinal bulbar muscular atrophy), was also found to coaggregate in inclusions with CBP in transfected cell lines, spinal bulbar muscular atrophy transgenic mice, and patient brain tissue (A. McCampbell and K. Fischbeck, personal communication). It is possible that these mutant proteins may also interact with p53 and CBP and/or mimic the

function of p53 to cause neuronal cell death. Further study may elucidate a common pathogenic pathway among these diseases.

Note Added in Proof. p53 coaggregation with huntingtin in inclusions purified by CsCl gradient has been independently confirmed (Steve Suhr, Marie-Claude Senut, and Fred Gage, personal communication).

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