

Depletion of CBP is directly linked with cellular toxicity caused by mutant huntingtin

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Received 27 January 2006; revised 26 March 2006; accepted 24 April 2006

Available online 12 June 2006

Huntington's disease is a neurodegenerative disease caused by an expanded polyglutamine stretch within the huntingtin protein. Transfection of mutant huntingtin causes cell toxicity and depletion of CREB binding protein (CBP) or its recruitment into huntingtin aggregates. However, the role of CBP has been controversial and the relationship between polyglutamine-induced toxicity and CBP depletion has not been examined on an individual cell basis. Using a single-cell based assay, we found that, in HT22 cells or primary neurons transfected with mutant huntingtin, cell toxicity was accompanied by CBP depletion, rather than merely recruitment. Transfection with a htt exon1 construct containing uninterrupted polyglutamine or a polyglutamine region engineered to form a compact beta structure resulted in cell toxicity. CBP depletion was accompanied by histone hypoacetylation. CBP overexpression rescued both acetylated histone levels and cell toxicity. These data suggest that CBP dysfunction and altered gene transcription contribute to mutant htt-induced neurotoxicity.

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Keywords: Huntingtin; Polyglutamine; CBP; HAT; Beta structure; Cell death; Neurodegeneration; Gene transcription

Introduction

Huntington's disease (HD) is caused by an expansion of a glutamine repeat within the huntingtin (htt) protein (Di Prospero and Fischbeck, 2005; Gatchel and Zoghbi, 2005; Paulson and Fischbeck, 1996; Ross, 2002). HD pathology is characterized by neuronal

degeneration in selected regions of the brain, including the striatum, cerebral cortex, and other regions. In addition, intracellular inclusions (or aggregates) formed by mutant htt protein have been observed in the cortex and striatum of postmortem HD brain (DiFiglia et al., 1997; Ross, 1997). Various model systems of htt aggregation and toxicity have been developed using cell culture and transgenic animals, and demonstrate that mutant htt expression often leads to cell death (Hackam et al., 1998; Saudou et al., 1998; Steffan et al., 2001).

It has been suggested that mutant huntingtin can cause cell toxicity by interfering with the function of CREB binding protein (CBP), yielding alterations in gene transcription. CBP can interact with mutant atrophin-1, the protein implicated in Dentatorubropallidoluysian Atrophy (DRPLA), in both cell culture and transgenic mouse models (Nucifora et al., 2001). Colocalization of CBP with polyQ aggregates has also been observed in cells in culture, transgenic mice, and postmortem HD brain tissue (McC Campbell et al., 2000; Nucifora et al., 2001). Moreover, CBP function may also be compromised by the loss of soluble levels of nuclear CBP (Jiang et al., 2003; McC Campbell et al., 2000; Nucifora et al., 2001). CBP can act as a histone acetyltransferase (HAT) to acetylate nucleosome-bound histones, a process that is required for remodeling of chromatin structure, and regulation of gene transcription (Bannister and Kouzarides, 1996). It has been suggested that mutant huntingtin expression reduces the acetylated level of histones, which correlates with toxicity, possibly through its effects on HATs such as CBP (McC Campbell et al., 2001; Steffan et al., 2001).

However, the role of CBP in htt toxicity has been controversial (Obrietan and Hoyt, 2004; Tallaksen-Greene et al., 2005; Yu et al., 2002). Recently, other transcription factors have been implicated (Bae et al., 2005; Dunah et al., 2002; Li et al., 2002; Schaffar et al., 2004). Furthermore, the relative contribution of CBP recruitment (i.e. incorporation into aggregates whether or not altered from its normal location) versus nuclear depletion to polyQ-induced cell toxicity is uncertain. For example, Li et al. reported that CBP

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recruitment was not observed in two different HD mouse models (Yu et al., 2002).

We reported previously that endogenous CBP can be depleted from nuclei of HT22 cells or Neuro2a cells following transfection with mutant htt (Jiang et al., 2003). This observation may result from either CBP recruitment into insoluble htt aggregates or from enhanced degradation of CBP via the ubiquitin–proteasome pathway. In addition, htt-induced toxicity was observed in HT22 cells containing nuclear or perinuclear aggregates (Jiang et al., 2003). However, these studies did not definitively establish the role of CBP depletion in polyQ-induced cell death at a single cell level. In the present study, a single-cell based assay was used to examine the relationship between CBP depletion and mutant htt-induced cell death in HT22 cells and in mouse primary cortical neurons, and the impact of CBP depletion on histone acetylation levels in HT22 cells. The data indicate that depletion of CBP from the nucleus of individual cells results in histone hypo-acetylation and parallels an increase in cytotoxicity.

Materials and methods

Plasmids and antibodies

The following constructs have been described previously: Htt-exon1-76Q, Htt-exon1-PGQ₉, Htt-exon1-PGQP (Poirier et al., 2005), and Htt-N63-148Q-myc (Cooper et al., 1998). A mouse full-length-CBP-Flag (FI-CBP-Flag) construct was a gift from Dr. Richard Goodman of Oregon Health Sciences University. To generate an in-frame deletion (amino acids 1446 to 1886) of the HAT domain of CBP (i.e. CBP Δ HAT-Flag), FI-CBP-Flag was digested with *BspE1/SgrAI* and re-ligated. Primary antibodies used include mouse anti-flag (M2) (Sigma, MO) at 1:1000, mouse anti-myc (9E10) (Roche, IN) at 1:500, mouse anti-CBP (C-1) (Santa Cruz, CA) at 1:50, rabbit anti-CBP (A-22) (Santa Cruz) at 1:200, rabbit anti-Sp1 (PEP 2) (Santa Cruz) at 1:200, rabbit anti-TBP (SI-1) (Santa Cruz) at 1:200, and rabbit anti-acetylated H4 (Lys5) (Santa Cruz) at 1:200. Secondary antibodies include FITC anti-mouse (Chemicon, CA) at 1:300, Rhodamine-anti-rabbit at 1:300, Alexa Fluor 350 anti-mouse (Molecular probe Invitrogen, CA) at 1:500. Nuclei were stained with Hoescht (Sigma).

Cell culture and transfection

HT22 cells were maintained and transfected as described previously (Jiang et al., 2003). FI-CBP-Flag and Htt-N63-148Q-myc plasmids were cotransfected at a ratio of 3:1 (Figs. 4C–E). Mouse primary cortical neurons were isolated from CD-1 outbred mice (Charles River, MA) at embryonic day 16. Primary neurons were transfected with plasmid DNA by electroporation using Nucleofector (Amaxa Inc., MD) as previously described (Poirier et al., 2005).

Indirect immunofluorescence

HT22 cells and neurons were fixed 48 h after transfection/electroporation for indirect immunofluorescence staining. Images were taken using a Zeiss conventional fluorescence microscope. CBP depletion was measured by direct comparison of soluble nuclear CBP levels with background fluorescence staining. Cell death in HT22 cells was determined via Hoescht staining of a

condensed or fragmented nucleus, often accompanied by a shrunken cell body as indicated by phase contrast. Viability of primary cortical neurons was monitored by a neurite morphology assay including the following criteria: a viable neuron must have at least one healthy neurite that is at least two times the length of the same neuronal body, with smooth (nonfragmented) neurites (Poirier et al., 2005).

Statistical analysis

Approximately 100 cells containing mutant htt aggregates were counted from randomly selected fields in each experiment (Figs. 1B, 2B, 3B, D–E, and 4B, D–E). Data shown are the average of six separate experiments and are presented as mean \pm SEM with statistical analysis performed using Standard Student's *t* test.

Results and discussion

CBP depletion is associated with htt-induced toxicity in HT22 cells and primary neurons

Transient transfection experiments were carried out to express a htt-exon1 protein containing 76 glutamine residues (i.e. Htt-exon1-76Q) in HT22 cells. Endogenous CBP can be depleted from the nucleus and recruited into a perinuclear htt aggregate, as shown in Fig. 1A (top panels, arrowhead). In response to various insults, HT22 cells undergo cell death that cannot be characterized as strictly apoptotic or necrotic (Tan et al., 1998). As shown in Fig. 1A, a cell expressing polyQ-expanded htt (arrowhead) contains a shrunken nucleus and retracted cell body with no detectable cytoplasm. This cell is unlikely to be viable. Previously, we have shown that HT22 cells with such morphological features were often TUNEL positive (Jiang et al., 2003). These cells will be referred to as nonviable throughout the text.

In contrast, images shown in the bottom panels depict HT22 cells containing htt aggregates and normal levels of endogenous CBP within the nuclei. Despite recruitment of CBP into aggregates (bottom panels, pointed by arrows), these cells appear viable. Quantification of these results (Fig. 1B) demonstrates that nuclear depletion of endogenous CBP is more often associated with the loss of viability compared to HT22 cells with no detectable changes in endogenous nuclear CBP levels. Moreover, diffuse nuclear CBP expression in HT22 cells (despite partial recruitment into htt aggregates) was observed in cells that exhibit no morphological features of viability loss. Similar results were observed in cells transfected with an N-terminal 63-residue fragment of htt containing 148 glutamines and a C-terminal myc tag, Htt-N63-148Q-myc (data not shown). Taken together, these data indicate that CBP recruitment into htt aggregates is not sufficient for toxicity, but that recruitment accompanied by nuclear depletion of CBP correlates with cytotoxicity in HT22 cells.

To confirm that CBP depletion correlates specifically with mutant htt-induced cell death, CBP expression was examined in nontransfected cells that underwent spontaneous cell death within the same culture. As shown in Fig. 1C, an untransfected and nonviable cell (1st panel, center) maintains diffuse CBP nuclear staining. This observation demonstrates that CBP depletion is not a universal feature of cell death. Quantification of these data is shown in Fig. 3F (left columns) and indicates that approximately 80% of untransfected cells that died spontaneously showed no evidence of CBP depletion. Moreover, expression of htt exon1

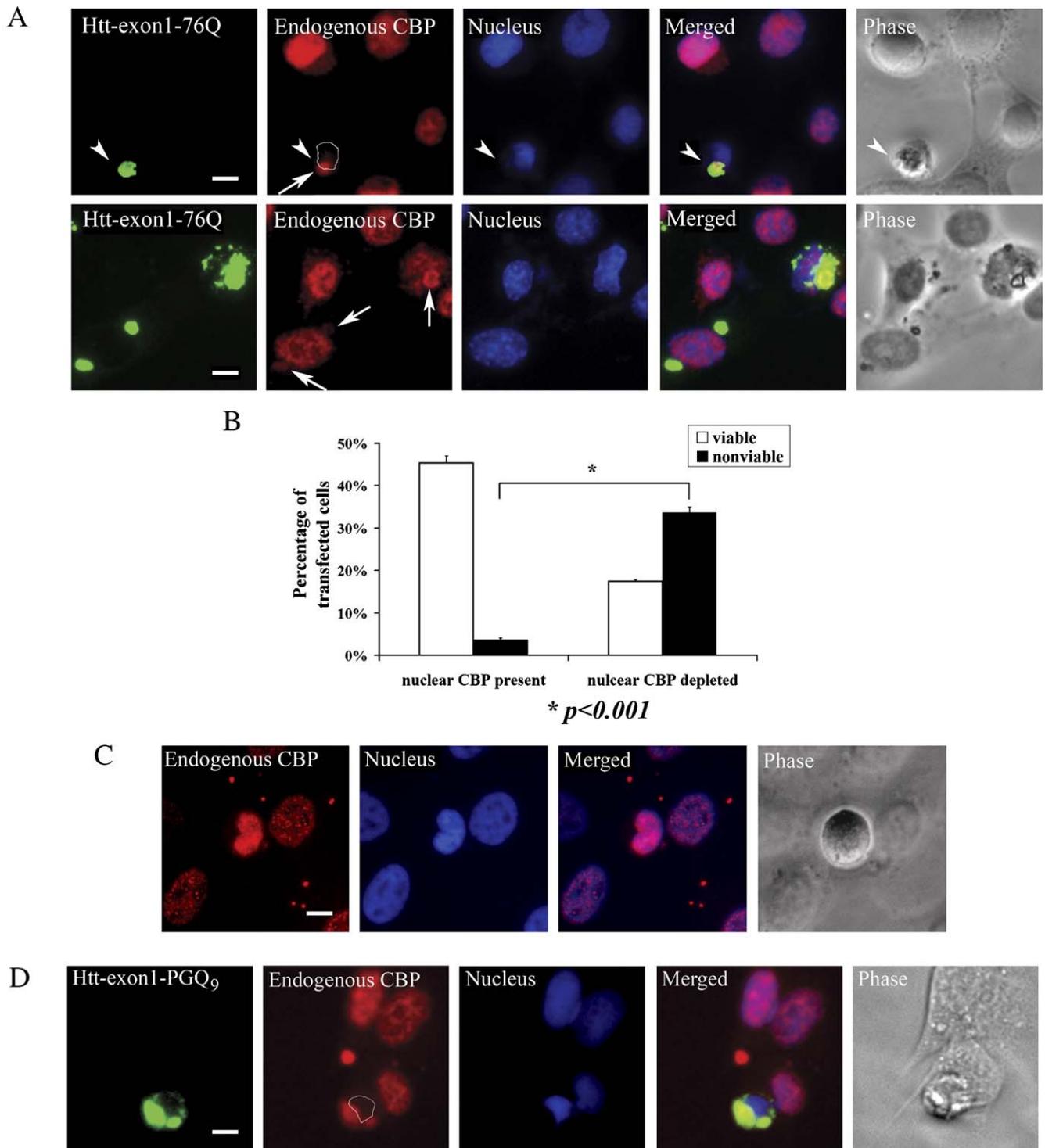


Fig. 1. Depletion of endogenous CBP parallels toxicity in HT22 cells expressing Htt-exon1-76Q. (A) Cells were fixed 48 h after transfection and mutant htt expression was detected by anti-Flag immuno-staining (green). Endogenous CBP is shown in red. A transfected cell containing a mutant htt aggregate (top row, 1st panel, arrowhead), shows nuclear depletion of endogenous CBP accompanied by recruitment into a perinuclear aggregate (top row, 2nd panel, arrow). This nonviable cell has condensed nuclear staining (top row, 3rd panel) and a rounded cell body (top row, 5th panel). Bottom panels show healthy cells with htt aggregates and normal diffuse nuclear CBP staining, despite of CBP being partially recruited in aggregates (pointed by arrows). Scale bar: 10 μ m. (B) Quantitative analysis demonstrates that mutant htt expressing cells with nuclear depletion of CBP are more likely to undergo cell death, than cells with normal levels of nuclear CBP. (C) An untransfected cell that died spontaneously (center) shows diffuse and more condensed nuclear staining of endogenous CBP. Scale bar: 10 μ m. (D) A cell containing a Htt-exon1-PGQ₉ aggregate shows evidence for nuclear depletion of endogenous CBP. This cell was nonviable at the time of analysis. Scale bar: 10 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

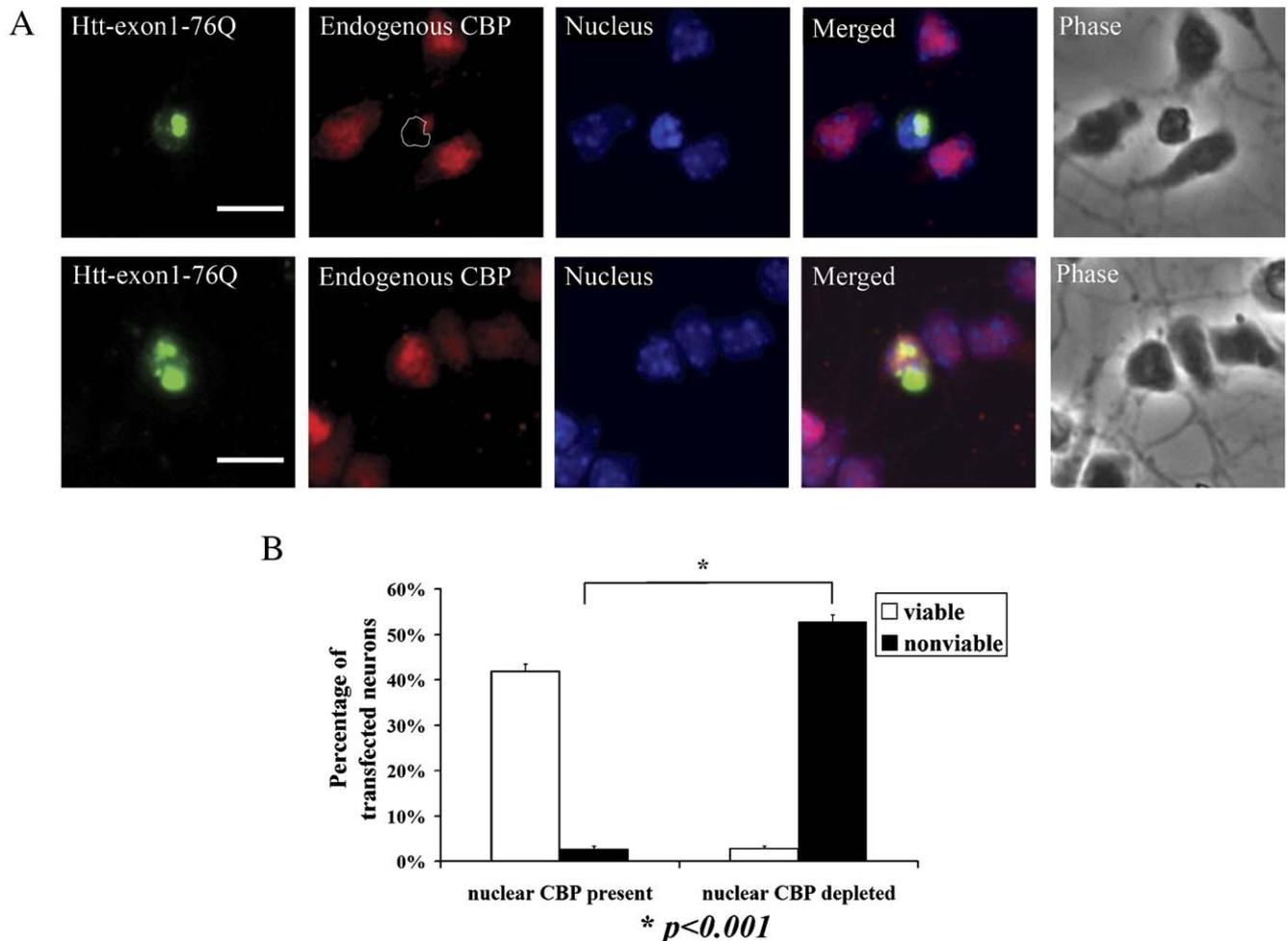


Fig. 2. Depletion of endogenous CBP parallels toxicity in primary mouse cortical neurons expressing Htt-exon1-76Q. (A) Mouse primary cortical neurons transfected with Htt-exon1-76Q were fixed 48 h after transfection and stained with an anti-Flag antibody. A neuron expressing mutant htt shows nuclear depletion of endogenous CBP with partial recruitment into a htt aggregate (top panels). This cell was nonviable at the time of analysis. A different neuron positively stained for htt aggregates has relatively diffuse nuclear CBP staining (bottom panels) and was found to be healthy. Scale bar: 10 μ m. (B) Quantitative analysis demonstrates that the majority of neurons showing nuclear CBP depletion were nonviable at the time of analysis, while neurons with normal levels of nuclear CBP were healthy.

with a normal length polyQ region (i.e. Htt-exon1-16Q) results in minimum cell death (Poirier et al., 2005), and of this small percentage, endogenous CBP is not depleted (data not shown). Therefore, CBP depletion in transfected HT22 cells was specifically correlated with mutant htt-induced cell death.

To further investigate a role for CBP dysfunction in mutant htt-mediated cell death, transient transfection experiments were performed using htt exon1 constructs with a modified expanded polyQ region. PGQ₉ has a polyQ sequence length of 77 with Pro-Gly pairs interspersed every nine residues to induce an alternating beta-strand/beta-turn structure. This design was previously shown to induce aggregation in vitro in a synthetic polyQ peptide (Thakur and Wetzel, 2002), and induced aggregation and toxicity in a htt exon1 cell culture model (Poirier et al., 2005). PGQP, designed with Pro insertions to disrupt the putative compact beta-structure, was unable to aggregate or cause cytotoxicity in the same cell model of mutant htt aggregation (Poirier et al., 2005). As shown in Fig. 1D, Htt-exon1-PGQ₉ expression resulted in both CBP depletion and in cell death. In contrast, expression of Htt-exon1-PGQP did not lead to depletion of nuclear CBP, nor did it induce toxicity

in HT22 cells (data not shown). These results suggest that formation of a compact beta-sheet structure within htt mutant polyQ may be required for CBP depletion as well as the resulting cell toxicity.

While HT22 cells are a hippocampal-derived cell line, cultured primary neurons can more closely approximate in vivo conditions. Therefore, this model system was used to investigate a possible correlation between CBP depletion and mutant htt-induced toxicity. A neuritic morphology assay previously used to monitor mutant htt-induced neuronal cell death in primary cortical neurons (Poirier et al., 2005) was used in the current study. As shown in Fig. 2A (top panels), depletion of endogenous CBP from the nucleus is accompanied by recruitment into a Htt-exon1-76Q aggregate (green). In this neuron, mutant htt expression resulted in death as assessed by an abnormal nuclear morphology and the absence of a healthy neurite (see Materials and methods). In contrast, a healthy neuron (bottom panels) maintained relatively normal CBP nuclear diffuse nuclear staining, despite an area of localized condensed CBP, which may represent an early recruitment event. Similar results were observed in Htt-N63-148Q-myc-transfected primary cortical neurons (data not shown). Quantifica-

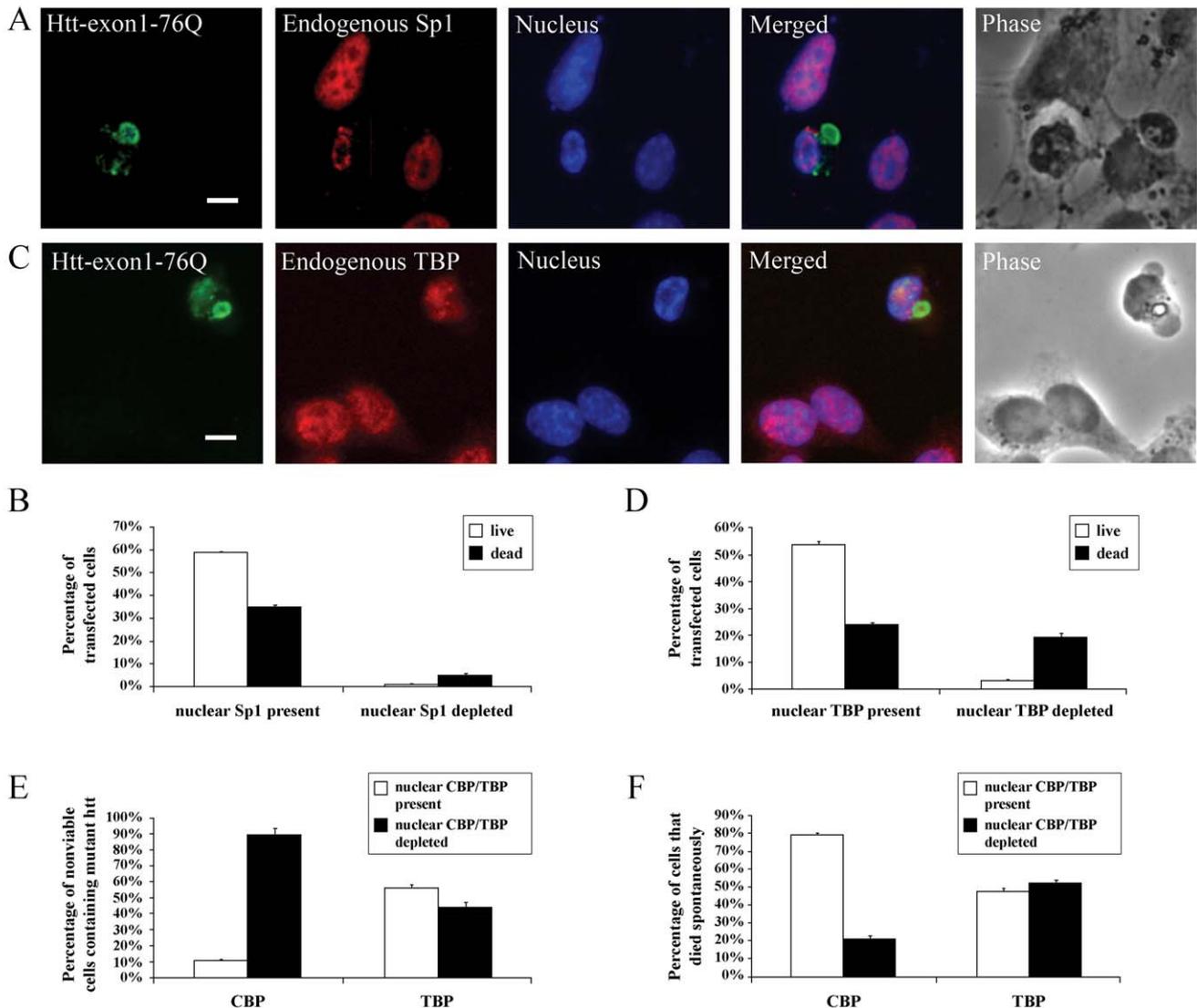


Fig. 3. Nuclear depletion of Sp1 and TBP is not specifically correlated with mutant htt toxicity in HT22 cells. (A) A cell containing mutant htt aggregates (1st panel, green) has normal levels of endogenous Sp1 (2nd panel, red). This cell was nonviable at the time of analysis. Scale bar: 10 μ m. (B) Quantitative analysis indicates that nuclear Sp1 staining pattern is not affected by mutant htt expression. (C) A cell containing a mutant htt aggregate (1st panel, green) has normal levels of endogenous TBP (2nd panel, red). This cell was nonviable at the time of analysis. (D) Quantitative analysis shows that nuclear TBP can either be depleted (20%) or present (25%) in the nuclei of nonviable cells containing mutant htt. (E) Quantitative analysis of CBP/TBP depletion. Data indicate that 45% of cells nonviable at the time of analysis showed depletion of TBP, while 90% of such cells had CBP depletion. This analysis demonstrates a lower correlation between TBP depletion and mutant htt-induced toxicity than for depletion of CBP. (F) Quantitative analysis shows that normal levels of CBP are present in the majority of untransfected cultured cells that died spontaneously. In contrast, no significant difference was observed between the percentage of cells with normal levels of TBP and those showing TBP depletion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tion of these data (Fig. 2B) demonstrates a strong correlation between CBP depletion and neuronal cell death.

The present data suggest that a minimal threshold level of soluble nuclear CBP must be maintained in order to promote neuronal survival in mutant htt-expressing cells. It is difficult to precisely define within individual cells the minimum CBP expression level required for neuronal cell survival. The severe neurological phenotypes associated with Rubinstein-Taybe syndrome (Petrij et al., 1995), a genetic disease caused by CBP haploinsufficiency, highlight the importance of maintaining appropriate levels of CBP, particularly in neurons. CBP homozygous knockout mice die at embryonic day 10.5 due to neural tube

closure defects (Kung et al., 2000; Yao et al., 1998), suggesting a specific role for CBP in neuronal development. In addition, CREB/CREM conditional double knockout mice manifest forebrain abnormalities including striatal cell apoptosis, strongly consistent with the idea that CBP dysfunction may lead to neuronal death of selected brain areas (Mantamadiotis et al., 2002).

In the current studies, both CBP depletion and cell death were not typically observed in transfected HT22 cells or in primary neurons lacking visible aggregates. However, this observation does not suggest that visible htt aggregates must be formed in cells undergoing toxicity. Previous studies have shown that mutant htt-induced cell death is correlated with the level of

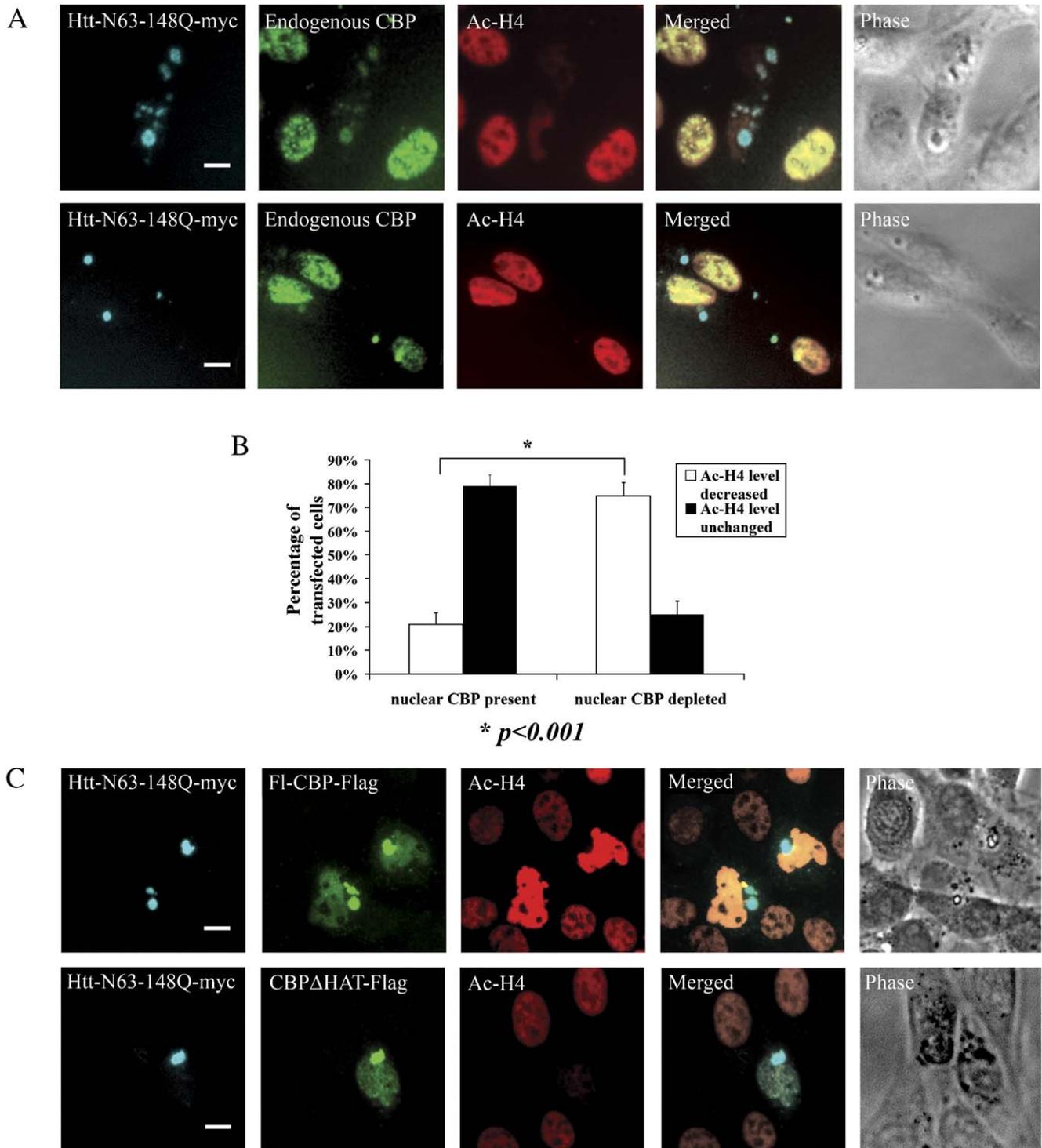


Fig. 4. CBP depletion causes histone hypo-acetylation and toxicity. (A) Transfected HT22 cells were analyzed 48 h after transfection for expression of Htt-N63-148Q-myc by immuno-staining with an anti-myc antibody (1st panels; blue). Two cells (top panels) are shown with depleted endogenous nuclear CBP (green) accompanied by decreased Ac-H4 levels (red). In contrast, two mutant htt-expressing cells (bottom panels) with diffuse nuclear CBP staining showed unaltered Ac-H4 levels. Scale bar: 10 μ m. (B) Quantitative analysis of cells positive for mutant htt expression shows that hypo-acetylation of H4 is often accompanied by depletion of nuclear CBP. Ac-H4 levels remain unaltered in cells with diffuse nuclear CBP. (C) Expression of exogenous Fl-CBP-Flag or CBP Δ HAT-Flag (2nd panels; green) was analyzed in HT22 cells transfected with Htt-N63-148Q-myc (1st panels; blue). The presence of exogenous Fl-CBP-Flag increased Ac-H4 levels in two cells expressing mutant htt (top panels), while overexpression of CBP Δ HAT-Flag did not rescue H4 hypo-acetylation (bottom panels, center cell). Scale bar: 10 μ m. (D) Quantitative analysis indicates that exogenous Fl-CBP-Flag exclusively rescued H4 hypo-acetylation induced by mutant htt, with an increase in Ac-H4 levels in 45% of the cells. In contrast, CBP Δ HAT-Flag failed to rescue H4 hypo-acetylation. (E) Quantitative analysis indicates that overexpression of Fl-CBP-Flag can rescue Htt-N63-148Q-myc-induced toxicity in HT22 cells. In contrast, CBP Δ HAT-Flag cannot rescue cell toxicity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

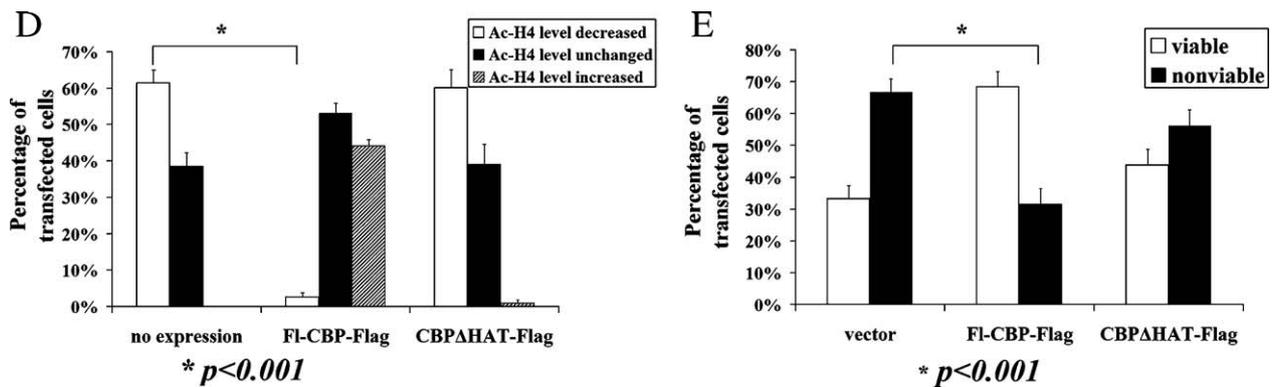


Fig. 4 (continued).

expression of mutant htt, rather than the presence of visible aggregates (Arrasate et al., 2004). The rapid formation of visible aggregates in transfected cells (i.e. within days) may result from high expression levels of mutant htt. In the present study, cells with diffuse mutant htt staining had, in general, lower levels of immunofluorescence than cells expressing nonpathological length htt polyQ. It is possible that CBP dysfunction is initiated prior to the formation of visible htt aggregates, through an aberrant interaction with soluble oligomeric forms of htt (Cong et al., 2005).

What is the structural basis for mutant htt polyQ-induced toxicity? In the present study, Htt-exon1-PGQ₉ aggregation had a similar effect on CBP depletion as the uninterrupted Htt-exon1-76Q. In contrast, Htt-exon1-PGQP, a putative beta-strand breaker, behaved as wild-type htt with a normal length polyQ region. It is tempting to speculate that htt mutant polyQ protein forms a compact beta-structure that can interact directly with CBP. Alternatively, there could be an indirect interaction with CBP or with other proteins involved in toxicity. However, we cannot exclude the possibility that these putative biochemical changes that trigger CBP loss could also be brought about by other structural features of polyQ-expanded mutant htt.

Nuclear depletion of other transcription factors is not associated with mutant htt toxicity in HT22 cells

Previous studies demonstrating on aberrant interaction between Sp1 Q rich region and TBP polyQ with mutant htt polyQ suggest a role for these transcription factors in mutant htt-induced toxicity (Dunah et al., 2002; Li et al., 2002; Schaffar et al., 2004). To test these predictions, Sp1 expression levels in individual cells were analyzed following transfection with mutant Htt-exon1-76Q. As shown in Fig. 3A, Sp1 retained a soluble nuclear localization in a nonviable cell with mutant htt aggregates. Quantification of these immunofluorescence results confirmed that Sp1 expression and localization are not affected by mutant htt expression (Fig. 3B).

Next, a potential role of TBP expression in mutant htt-induced cell death was examined. As shown in Fig. 3C, TBP was present in a nonviable cell with mutant htt aggregates. Quantification of these immunofluorescence data (Fig. 3D) demonstrates that TBP depletion was associated with ~45% of mutant htt-induced cell death compared with 90% for CBP (Fig. 3E). In addition, the percentage of transfected cells with TBP depletion is similar to that

in cells that died spontaneously (Fig. 3F). Taken together, these data imply that nuclear depletion of TBP occurs independently of mutant htt-induced cell death.

These data indicate that HT22 cells exhibit selective CBP depletion with expression of mutant htt polyQ, demonstrating that the loss of CBP function is sufficient to trigger neuronal cell death in the absence of visible effects on Sp1 and TBP. However, it is possible that Sp1 and TBP play a role in mutant huntingtin toxicity, perhaps through different mechanisms. Previous studies suggest that oligomeric forms of expanded polyQ proteins can interact with TBP and disrupt its function (Schaffar et al., 2004), and that transcription activity of Sp1 may be disrupted without apparent recruitment or loss of soluble levels in the presence of mutant htt (Dunah et al., 2002; Li et al., 2002). These observations may represent alternative mechanisms for Sp1 and TBP in mutant htt-induced toxicity.

Nuclear depletion of CBP results in hypo-acetylation of histones in HT22 cells expressing mutant htt

CBP, in addition to acting as a scaffold protein to facilitate interactions between CREB and other transcriptional regulators, has histone acetyltransferase (HAT) activity (Bannister and Kouzarides, 1996). This property allows CBP to regulate gene transcription by modulating the acetylation of histones and other transcriptional factors (Goodman and Smolik, 2000). Previous studies have reported that mutant htt expression is accompanied by histone hypo-acetylation that may contribute to toxicity (Cong et al., 2005; Igarashi et al., 2003; Steffan et al., 2001). To determine whether histone hypo-acetylation is partially due to mutant htt-induced CBP dysfunction, we examined the relationship between CBP depletion and hypo-acetylation of histone proteins (H3 and H4) in HT22 cells transfected with Htt-N63-148Q-myc. As shown in Fig. 4A (top panels), endogenous CBP (green) is depleted from two cells with Htt-N63-148Q-myc aggregates (blue). Acetylated histone H4 (Ac-H4) levels (shown in red) were also significantly decreased in both of these cells. In contrast, Ac-H4 levels remained unaltered in cells with visible htt aggregates but no CBP depletion (bottom panels). Quantification of these results, shown in Fig. 4B, demonstrates that most mutant htt-expressing cells with CBP depletion had hypo-acetylated levels of histone H4. Similar results were observed for acetylated H3 levels, and in cells expressing Htt-exon1-76Q (data not shown).

If mutant htt-mediated CBP dysfunction resulted directly in the hypo-acetylation of H4 (via loss of CBP HAT activity), one might expect CBP overexpression to restore levels of Ac-H4. To investigate this possibility, the effect of CBP overexpression on Ac-H4 levels was examined. Transient transfection experiments were carried out in HT22 cells with Fl-CBP-Flag, encoding full-length mouse CBP, and CBP Δ HAT-Flag, a HAT domain deletion mutant lacking amino acids 1446 to 1866. This region is similar to the region of CBP (amino acids 1459 to 1877) that most strongly interacts with mutant htt in vitro (Steffan et al., 2001). When Fl-CBP-Flag was transfected in HT22 cells, approximately 70% of cells had increased levels of Ac-H4. In contrast, CBP Δ HAT-Flag overexpression did not cause an increase in Ac-H4 levels, indicating that HAT activity was abolished (data not shown). When cotransfected with Htt-N63-148Q-myc, exogenous Fl-CBP-Flag expression leads to an increase in Ac-H4 levels in cells with mutant htt aggregates (Fig. 4C, top panels). In contrast, expression of CBP Δ HAT-Flag (bottom panels) did not rescue H4 hypo-acetylation, despite the presence of CBP Δ HAT-Flag in the nucleus. Quantification of these data (Fig. 4D) demonstrates that Fl-CBP-Flag overexpression efficiently rescued H4 hypo-acetylation. Moreover, Ac-H4 levels were increased in 45% of the cells with mutant htt aggregates. As shown in Fig. 4E, overexpression of Fl-CBP-Flag, but not CBP Δ HAT-Flag, rescued mutant htt toxicity. Taken together, these data support the hypothesis that CBP dysfunction leads to histone hypo-acetylation in HT22 cells expressing mutant htt and directly compromises cell viability.

Controversy regarding CBP dysfunction in HD pathogenesis

There has been controversy about the role for CBP dysfunction in HD pathogenesis. Results from cell culture studies have shown CBP loss in cells expressing polyQ-expanded htt, androgen receptor, or atrophin-1 (McC Campbell et al., 2000; Nucifora et al., 2001). However, most of these studies were carried out with transiently overexpressed CBP. In this study, levels of endogenous CBP were examined and found to be depleted from the nucleus in cells undergoing htt-induced toxicity, providing further support for a role for CBP in HD pathogenesis.

Analyses of CBP function in HD transgenic mice have generated conflicting results. For example, a number of studies provided evidence for CBP recruitment in transgenic mouse brain (McC Campbell et al., 2000; Nucifora et al., 2001), while others found a lack of CBP recruitment and depletion (Li et al., 2002; Obrietan and Hoyt, 2004; Yu et al., 2002). One possible explanation for this disparity is that disease phenotypes seen in transgenic mouse models may not fully mimic those observed in HD patients. For example, HD transgenic mouse brains do not usually present substantial neuronal loss (Schilling et al., 1999; Yu et al., 2003), although HD patient brain shows significant loss in the striatum. Therefore, mouse models of HD may not be best suited for cell death studies. In addition, transient transfection of mutant htt in cultured cells causes cell death within days, while disease progression in HD transgenic mice can typically last several months. CBP dysfunction in vivo may be a more subtle process, with recruitment into aggregates being more difficult to detect than in cell culture. Furthermore, while CBP may still be present in the nucleus, CBP function could be disrupted due to aberrant interactions with either monomeric or oligomeric forms of mutant htt. CBP dysfunction rather than CBP depletion may cause neuronal abnormalities in HD transgenic mouse models.

Our results strongly suggest that CBP dysfunction via loss of CBP HAT activity is sufficient to cause histone hypo-acetylation and may contribute to mutant htt-induced cell death. Studies on CBP dysfunction may lead to novel HD therapeutics targeted to a decrease in HAT activity. Steffan et al. (2001) reported that HDAC inhibitors can rescue neurodegeneration of retinal neurons in an HD transgenic model in *Drosophila*. In addition, HDAC inhibitors can rescue disease phenotype in HD transgenic mouse models (Ferrante et al., 2003; Gardian et al., 2005), and in a transgenic mouse model of spinal and bulbar muscular atrophy (SBMA) (Minamiyama et al., 2004). HDAC inhibitors are promising therapeutic agents for HD and other polyglutamine diseases. It is possible that targeting HDACs most directly related to CBP could provide additional specificity.

Acknowledgments

Dr. David Schubert (Salk Institute) is thanked for the kind gift of HT22 cells and Dr. Richard Goodman (Oregon Health Sciences University) for Fl-CBP-Flag construct. This work is supported by NINDS (NS 16375), HDSA Coalition for the Cure, and HDF.

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