plex may participate directly in this repression. Intriguingly, EED, the mammalian homolog of ESC and MES-6, is involved in maintaining X-chromosome inactivation in extraembryonic tissues of female mouse embryos (*23*). How might MES-4 participate in X-chromosome repression? MES-4 on the autosomes may protect them from the binding, spreading, or action of repressors, such as the MES-2/MES-3/MES-6 complex or histone-modifying enzymes. This would serve to focus repression on the X chromosomes, which lack MES-4 protection. This model for MES-4 action is consistent with several observations, including the following: (i) *mes-4* mutants display the same sensitivity to X-chromosome dosage as *mes-2*, *mes-3*, and *mes-6* mutants; and (ii) MES-4, like MES-2, MES-3, and MES-6, is required for repression of germline expression of transgenes present in repetitive arrays (*24*). The activation of transgenes in *mes-4* mutants may be due to titration of limited levels of repressor by autosomal chromatin that in wild type does not bind the repressor. This scenario predicts that the X chromosomes are desilenced in *mes-4* mutants, as we predicted occurs in *mes-2*, *mes-3*, and *mes-6* mutants.

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Sp1 and TAFII130 Transcriptional Activity Disrupted in Early Huntington's Disease

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Huntington's disease (HD) is an inherited neurodegenerative disease caused by expansion of a polyglutamine tract in the huntingtin protein. Transcriptional dysregulation has been implicated in HD pathogenesis. Here, we report that huntingtin interacts with the transcriptional activator Sp1 and coactivator TAFII130. Coexpression of Sp1 and TAFII130 in cultured striatal cells from wild-type and HD transgenic mice reverses the transcriptional inhibition of the dopamine D2 receptor gene caused by mutant huntingtin, as well as protects neurons from huntingtin-induced cellular toxicity. Furthermore, soluble mutant huntingtin inhibits Sp1 binding to DNA in postmortem brain tissues of both presymptomatic and affected HD patients. Understanding these early molecular events in HD may provide an opportunity to interfere with the effects of mutant huntingtin before the development of disease symptoms.

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder manifested by psychiatric, cognitive, and motor symptoms typically starting in midlife and progressing toward death. HD is caused by expansion of a polyglutamine tract in the huntingtin protein. The number of diseases caused by polyglutamine expansions continues to grow, and a common mechanism could underlie these disorders. One hypothesis suggests that expanded polyglutamines result in aberrant interactions with nuclear proteins and thereby lead to transcriptional dysregulation (*1–7*). If huntingtin is involved in regulating gene transcription, it is important to determine which genes may be affected by normal and/or mutant huntingtin. Some obvious candidates are genes whose expression is altered in HD patients or in animal models of HD. Neurotransmitter receptor alterations have been described in early-stage human HD autopsy material, and many of these changes have been confirmed in transgenic mouse models of HD (*8*, *9*). Gene expression assays on DNA microarrays have shown that the scope of mRNA changes in transgenic HD mice involves several groups of genes, including neurotransmitter receptors and intracellular signaling systems (*10*). The known regulatory sequences of these genes contain binding sites for the transcription factor Sp1, suggesting that huntingtin may interfere with Sp1-mediated transcription. Sp1 is a ubiquitous transcriptional activator whose major function is recruitment of the general transcription factor TFIID to DNA (*11*). TFIID is a multisubunit complex made up of the TATA box–binding protein (TBP) and multiple TBP-associated factors (TAFs) (*12*). Involvement of one of the human TAFs, TAFII130, in activator-TAF interactions has been examined in detail (*13*, *14*). TAFII130 interacts with various cellular activators, including Sp1 and CREB, suggesting that TAFII130 may be critical for the transcriptional activation function of these factors by bridging them to the basal machinery.

Using the yeast two-hybrid system (*15*), we found that both Sp1 and TAFII130 interact with full-length huntingtin (Fig. 1).[5] The interactions between Sp1 and huntingtin are stronger in the presence of an expanded polyglutamine repeat (HttQ75) as compared to the nonexpanded repeat length (HttQ17) (Fig. 1A), whereas the interactions between TAFII130 and huntingtin are not significantly influenced by the polyglutamine tract length (Fig. 1B). Although the glutamine-rich regions of Sp1 (Sp1AB) and TAFII130 (TAFII130-M) are sufficient for their interaction with huntingtin, the presence of the COOH-terminal DNA binding domain of Sp1

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or the conserved COOH-terminal domain of TAFII130 results in stronger interaction. Because NH_2-terminal fragments of mutant huntingtin can effectively induce cell death in both in vivo and in vitro models (*16*–*19*), we examined the interactions of Sp1 and TAFII130 with the 480–amino acid NH_2-terminal fragment of huntingtin. Compared with the full-length protein, NH_2-terminal fragments showed similar, polyglutamine length–dependent interactions with Sp1, whereas their interactions with TAFII130 were independent of polyglutamine length (Fig. 1, A and B).

To further examine the strength of huntingtin/Sp1 and huntingtin/TAFII130 interactions in relation to polyglutamine length, we cotransfected HEK 293T cells with expression plasmids for normal (HttQ17) or mutant (HttQ75) full-length huntingtin and flag-tagged Sp1 or hemagglutinin (HA)–tagged TAFII130 (*15*). Coimmunoprecipitations of the transfected proteins with antibodies to huntingtin showed that Sp1 preferentially interacted with mutant huntingtin (Fig. 1C), whereas TAFII130 bound similarly to both normal and mutant huntingtin (*20*). These results, together with the yeast two-hybrid data, indicate that polyglutamine expansion enhances the interaction of Sp1, but not TAFII130, with huntingtin.

To establish whether huntingtin interacts with Sp1 and TAFII130 in the human brain, coimmunoprecipitation studies were performed using extracts from the caudate nucleus of grade 1 HD brain with antibodies to Sp1 (anti-Sp1) (Fig. 1D), to TAFII130 (anti-TAFII130) (Fig. 1E), or to huntingtin (anti-Htt) (*15*). Both anti-Sp1 and anti-TAFII130 precipitated huntingtin protein. In addition, anti-Htt coimmunoprecipitated substantial amounts of Sp1 and TAFII130 proteins. We found that the immunoprecipitated complex, in addition to TAFII130, contained other TAFs (*21*), suggesting that TAFII130 interacts with huntingtin in the context of TFIID. However, because we found TAFII130 to be expressed at higher levels in HD brain tissue, it is possible that huntingtin interacts with free TAFII130 as well (see below).

Next, we tested whether mutant huntingtin affects the interactions between Sp1 and TAFII130 in HD brain tissue. In coimmunoprecipitation experiments using anti-Sp1 and anti-TAFII130, we found a decrease in the interactions between Sp1 and TAFII130 in the postmortem human HD brain as compared to the control brain (Fig. 1F). These changes in Sp1-TAFII130 interactions were detected in postmortem HD brain tissue from presymptomatic, as well as affected, patients, suggesting early and persistent disruption of Sp1 and TAFII130 functions in the HD brain.

The interaction between huntingtin and Sp1 could also interfere with the DNA binding function of Sp1. To determine the effects of huntingtin on the binding of Sp1 to DNA, we performed an electrophoretic mobility shift assay (EMSA) with purified Sp1 and huntingtin proteins (*15*). Using a consensus Sp1 binding site as a probe, we found a 70% decrease in Sp1 binding to DNA in the presence of mutant huntingtin and a 20% decrease in the presence of wild-type huntingtin (Fig. 2A).

We had previously shown that Sp1 plays a critical role in the regulation of D2 dopamine receptor gene transcription (*22*). Thus, we tested the hypothesis that alterations in Sp1 function may be responsible for the down-regulation of D2 gene expression in HD. To examine whether mutant huntingtin affects Sp1 binding to the D2 promoter in striatal cells, we performed EMSA using nuclear extracts from primary striatal neurons transfected with wild-type (HttQ17) or mutant (HttQ75) huntingtin (*15*). Using a region of the D2 dopamine receptor promoter as a probe, we found decreased Sp1 binding in extracts expressing mutant huntingtin as compared with wild-type huntingtin (Fig. 2B). Down-regulation of D2 receptor expression has also been reported in the striata of presymptomatic, as well as affected, HD patients (*8*, *23*). Therefore, we performed EMSA using nuclear extracts isolated from the caudate and hippocampus of grade 1 and grade 4 HD brains. In grades 1 and 2 of HD, there is mild to moderate neuronal loss in the caudate nucleus, whereas the hippocampus remains relatively unaffected until later in the disease. In grade 4, the striatum, as well as other brain regions, is severely atrophic and depleted of 95% or more of its neurons (*24*). Using Sp1 binding sites in the D2 promoter as a labeled probe, we found a

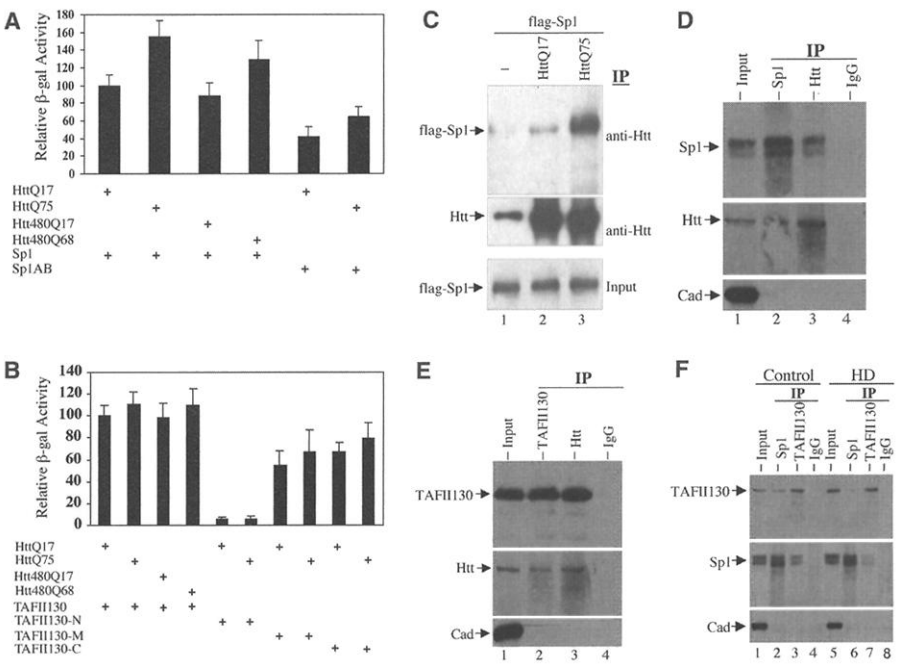

Fig. 1. Huntingtin interacts with Sp1 and TAFII130 in vitro and in human HD brain tissue. (**A**) Yeast two-hybrid experiments (*15*) were performed with full-length (HttQ17 and HttQ75) or truncated huntingtin constructs (Htt480Q17 and Htt480Q68) as prey against bait plasmids containing full-length Sp1 or Sp1 lacking the DNA binding domain (Sp1AB, amino acids 83 through 621). The β-galactosidase (β-gal) activity is displayed for each interaction as a percentage of the interaction between normal huntingtin and full-length Sp1 (set to 100%). Values are expressed as means ± SEM. (**B**) Yeast two-hybrid experiments were performed and analyzed as in (A). TAFII130 and its derivatives containing the NH_2-terminal domain (TAFII130-N, amino acids 1 through 297), the glutamine-rich domain (TAFII130-M, amino acids 270 through 700), or the COOH-terminal domain (TAFII130-C, amino acids 646 through 947) were used as baits. (**C**) Interaction between huntingtin and Sp1 in HEK 293T cells. Wild-type huntingtin (HttQ17), mutant huntingtin (HttQ75), and FLAG-tagged Sp1 were transfected, immunoprecipitated (IP) with anti-Htt, and blotted with anti-FLAG or anti-Htt (*15*). Sp1 expression is shown on a sample of lysate (10%) used for immunoprecipitation (Input). Expressed FLAG-Sp1 and huntingtin are indicated by arrows on the left. (**D**) Huntingtin and Sp1 interact in human HD brain tissue. Total homogenate was prepared from the caudate of grade 1 human HD brain tissue and immunoprecipitated with anti-Sp1 (lane 2), anti-Htt (lane 3), or nonimmune rabbit immunoglobulins (IgG, lane 4) (*15*). Lane 1 shows 10% of the input. Antibody to cadherin (Cad) was used as a control for nonspecific interaction. (**E**) Huntingtin and TAFII130 interact in human HD brain tissue. Immunoprecipitations were performed as in (D), except that anti-TAFII130 was used (lane 2) (*15*). (**F**) Reduced Sp1-TAFII130 interaction detected in human HD brain tissue. Immunoprecipitations were performed as in (D), except that anti-Sp1 (lanes 2 and 6) and anti-TAFII130 (lanes 3 and 7) were used for immunoprecipitations. A decrease in coprecipitated TAFII130 (upper panel, compare lanes 2 and 6) and Sp1 (middle panel, compare lanes 3 and 7) is seen in HD brain as compared with control brain tissue.

significant decrease in the DNA binding activity of Sp1 in the caudate nucleus of presymptomatic grade 1 HD brain tissue as compared with control brain tissue (Fig. 2C, lane 3). Similar decreases in Sp1 binding were found in extracts from grade 4 HD brain caudate (Fig. 2C, lane 4), suggesting early and persistent inhibition of Sp1 function. Binding of another transcription factor, MEF2C, to DNA was not changed in these extracts, arguing against nonspecific inhibitory effects of the extracts from HD brain tissue (fig. S1) (*15*). When EMSA was performed with nuclear extracts from the hippocampus of grade 1 HD brain tissue, no decrease in Sp1 binding was observed (Fig. 2D). The caudate-specific inhibition of Sp1 function may be, in part, due to the preferential accumulation of mutant huntingtin in the striatum in early stages of HD (*25*, *26*).

To further establish the role of huntingtin in Sp1-mediated transcription, primary striatal neurons were transfected with D2 promoter-reporter gene constructs along with mutant or normal huntingtin (*15*). Although huntingtin with normal glutamine repeats (HttQ17) had no significant effect on promoter activity, mutant huntingtin (HttQ75) produced significant inhibition (Fig. 3A). To determine whether the inhibition of Sp1-mediated transcription was dependent on increased levels of huntingtin relative to those of endogenous Sp1 and TAFII130, we overexpressed Sp1 and/or TAFII130 together with huntingtin. We found that overexpression of either Sp1 or TAFII130 alone did not significantly alter the inhibitory effects of mutant huntingtin, whereas coexpression of TAFII130 and Sp1 resulted in complete reversal of huntingtin-induced inhibition of D2 promoter activity (Fig. 3A). These effects of Sp1 and TAFII130 were dependent on Sp1 binding to the D2 promoter, because no significant effect was seen when the Sp1 functional site was deleted (pCAT D2-29) or when an Sp1 expression vector lacking the DNA binding domain (Sp1AB) was used. Similarly, no effect on promoter activity was seen with the NH_2-terminus of TAFII130, which does not affect Sp1 activity (*14*) and does not interact with huntingtin (Fig. 1B). These results suggest that mutant huntingtin inhibits Sp1-mediated transcription by interfering with Sp1/TAFII130 function. To confirm that these effects on the D2 promoter are specific, we examined whether TAFII105, a human TAF closely related to TAFII130 (*27*), and TAFII55, which binds Sp1 (*28*), affect huntingtin-mediated inhibition of the D2 promoter. When TAFII105 or TAFII55 was overexpressed together with Sp1 and mutant huntingtin in striatal cells, no significant effect was observed on D2 promoter activity (Fig. 3A) (*20*). To determine whether these effects of mutant huntingtin on the D2 promoter may simply be due to nonphysiological concentrations of overexpressed huntingtin, we performed similar experiments in striatal neurons isolated from transgenic HD mice (*15*). These mice express relatively low steady-state levels of mutant huntingtin (NH_2-terminal fragment) with 82 glutamine repeats (*17*). When these cultured striatal cells were transfected with D2 promoter constructs, similar inhibition of promoter activity was observed as in experiments where mutant huntingtin was overexpressed by transient transfection. Furthermore, overexpression of Sp1 and TAFII130 resulted in complete reversal of D2 promoter inhibition in cells derived from transgenic HD mice (Fig. 3B). As before, these effects of Sp1 and TAFII130 were dependent on Sp1 binding to its functional site on the D2 promoter (*20*). Because no toxicity was observed in cells isolated from the transgenic animals (*20*), it is unlikely that the observed decrease in transcription is a consequence of cellular toxicity caused by mutant huntingtin. To determine whether the transfection of mutant huntingtin affects endogenous D2 receptors in primary striatal neurons, we analyzed D2 mRNA levels in transfected cells (*15*). The presence of mutant huntingtin resulted in decreased expression of D2 mRNA as compared to the wild-type huntingtin. This inhibitory effect of mutant huntingtin on the endogenous D2 receptor was partially reversed by overexpression of TAFII130 and Sp1 in striatal cells (Fig. 3C). To determine whether these changes in D2 receptor mRNA lead to altered protein levels, we analyzed the expression of D2 receptors in transfected striatal neurons by immunocytochemistry, using antibody to D2 (*15*). We found that transfection of mutant huntingtin resulted in a robust decrease of D2 expression in about 70% of transfected striatal neurons, whereas neurons cotransfected with Sp1, TAFII130, and mutant huntingtin showed normal D2 expression (Fig. 3D). Taken together, these results suggest that mutant huntingtin specifically represses D2 receptor gene expression in a Sp1/TAFII130-dependent manner.

Next, we investigated the potential role of mutant huntingtin's interactions with Sp1 and TAFII130 in relation to cellular toxicity. Previous experiments demonstrated that mutant huntingtin is toxic when overexpressed in cultured striatal cells (*29*). Here, we show that when full-length mutant huntingtin (HttQ75) is expressed in striatal neurons together with Sp1 and TAFII130, huntingtin-mediated toxicity is almost completely abrogated, whereas transfection of either Sp1 or TAFII130 alone results in

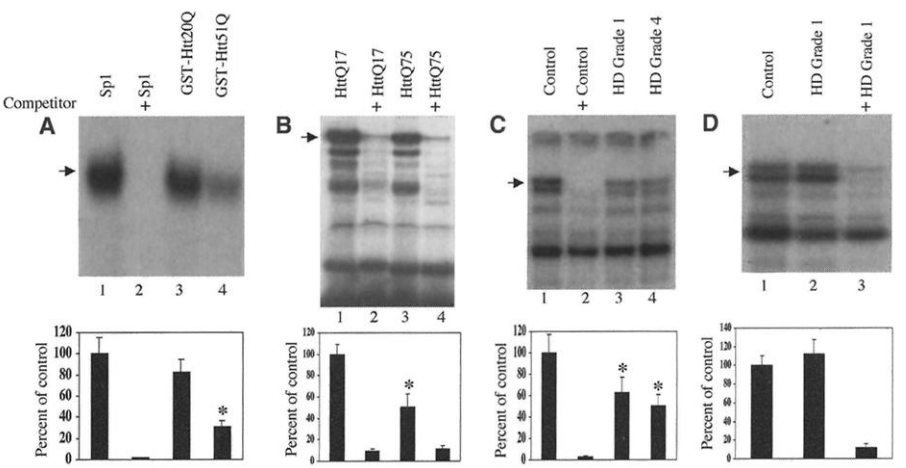

Fig. 2. Mutant huntingtin inhibits the binding of Sp1 to DNA in vitro and in vivo. (**A**) EMSAs with purified Sp1 and glutathione *S*-transferase (GST)–huntingtin fusion proteins (*15*). Binding of Sp1 to a labeled consensus Sp1 site is shown in lane 1 (arrow), and competition with a 50-fold molar excess of unlabelled probe is shown in lane 2 (plus sign). Incubation of Sp1 in the presence of GST-huntingtin with 20 glutamines (Qs) leads to a 20% reduction in Sp1 binding (lane 3), whereas huntingtin with 51 Qs inhibits Sp1 binding by 70% (lane 4). The Sp1/DNA complex was identified with anti-Sp1 and is indicated by an arrow in all panels. (**B**) Mutant huntingtin inhibits binding of Sp1 to the D2 receptor promoter in cultured striatal cells. Primary striatal cells were transfected with wild-type (HttQ17) or mutant (HttQ75) huntingtin. EMSA was performed with a labeled D2 promoter fragment containing an Sp1 binding site (*15*). Mutant huntingtin inhibits binding of Sp1 by 55% (lane 3) as compared to wild-type huntingtin (lane 1). In competition experiments, a 100-fold molar excess of double-stranded nonradioactive oligonucleotide was used (lanes 2 and 4). (**C**) Binding of Sp1 to the D2 promoter is inhibited in the caudate of the human HD postmortem brain. EMSA was performed with nuclear extracts isolated from presymptomatic grade 1 and grade 4 HD brain tissue (*15*). Binding of Sp1 to its D2 binding site is decreased by 39% in grade 1 (lane 3) and by 48% in grade 4 HD brain tissue (lane 4) as compared to control brain tissue (lane 1). Competition experiments were performed as in (B) (lane 2). (**D**) Binding of Sp1 to the D2 promoter fragment in the hippocampus of HD brain (lane 2) is not significantly altered as compared to control (lane 1). EMSA was performed as in (C). Competition with unlabeled probe is shown in lane 3. Densitometry was used to quantify the band intensities in all panels (shown as a graph below the respective autoradiogram). Values are expressed as percent of Sp1 binding shown in lane 1 and represent means ± SEM of three independent experiments, $*P < 0.001$ for comparison with lane 1 in (A), $*P < 0.01$ in (B) and (C).

minimal, statistically insignificant protection (Fig. 3E). To further establish the role of TAFII130 in huntingtin-induced cell death, we tested whether neurons could be protected against mutant huntingtin by transfecting TAFII130 lacking the COOH-terminal domain (TAFII130-ΔC). The COOH terminus of TAFII130 has been shown to mediate interactions with transcriptional activators, with other TAFs in TFIID (*30*, *31*), and with huntingtin (Fig. 1B). We found that coexpression of TAFII130-ΔC with Sp1 failed to block huntingtin-induced cell death (Fig. 3E). To determine whether the effects of TAFII130 on neuronal survival are specific, we tested TAFII105, which is structurally similar to TAFII130 (*27*). When transfected along with Sp1 and mutant huntingtin, TAFII105 did not significantly protect neurons from huntingtin-mediated toxicity.

It has previously been suggested that huntingtin interferes with gene transcription by depleting transcription factors from their normal location and sequestering them into nuclear aggregates (*32*–*35*). To examine whether the function of Sp1 is compromised through sequestration into nuclear inclusions, we performed immunocytochemistry on transgenic HD mice and human postmortem HD brains. Nuclear and cytoplasmic inclusions were strongly labeled with an antibody to the NH_2-terminus of huntingtin that specifically labels huntingtin aggregates, whereas Sp1 staining was not detected in these inclusions, suggesting that the soluble rather than the aggregated form of huntingtin interacts with Sp1 (Fig. 4A) (*15*). This finding was confirmed by Western blot analysis (*15*), showing a robust increase of Sp1 protein in the soluble fraction of caudate tissue from postmortem HD brains (Fig. 4B). Sp1 protein levels were also

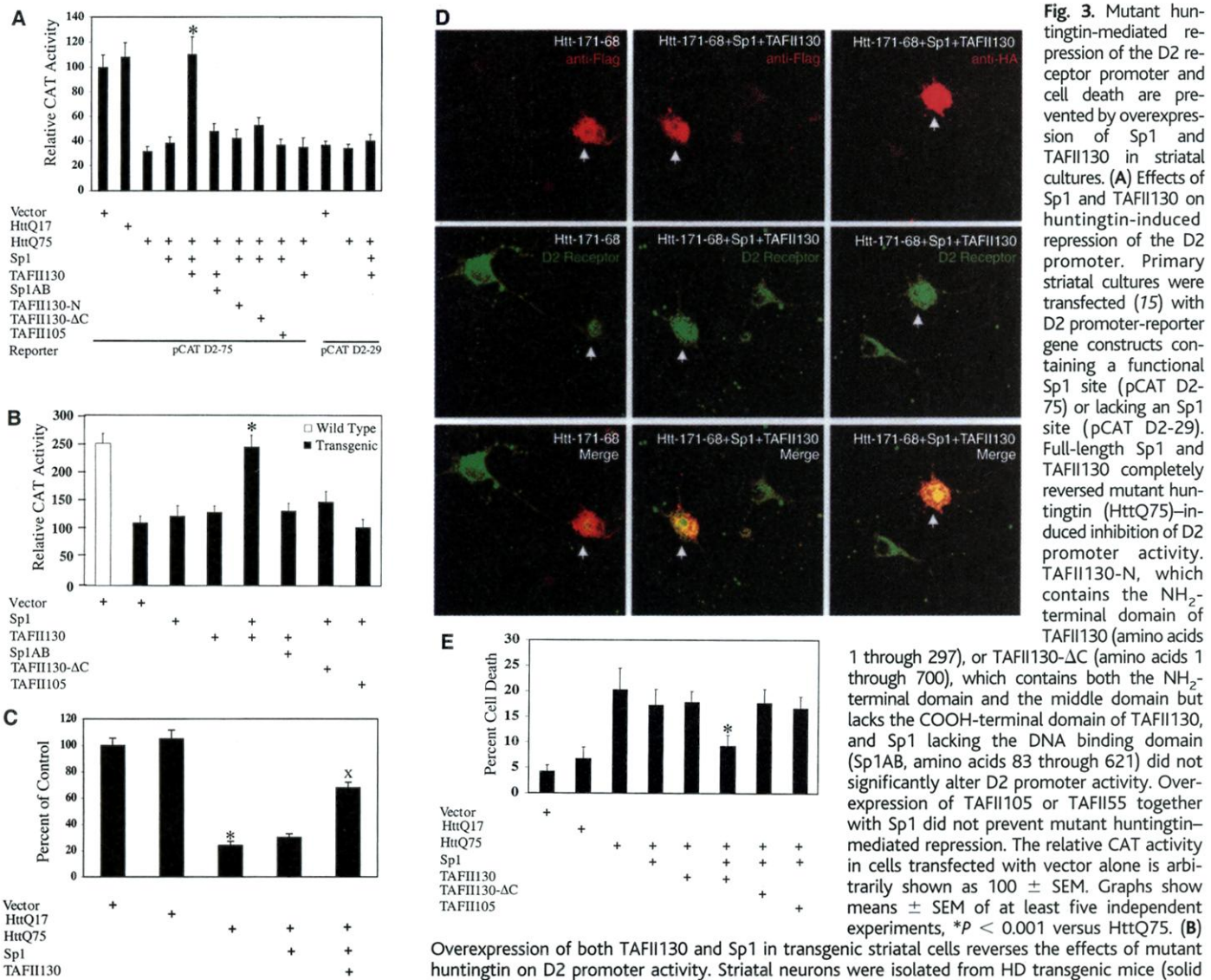

Fig. 3. Mutant huntingtin-mediated repression of the D2 receptor promoter and cell death are prevented by overexpression of Sp1 and TAFII130 in striatal cultures. (**A**) Effects of Sp1 and TAFII130 on huntingtin-induced repression of the D2 promoter. Primary striatal cultures were transfected (*15*) with D2 promoter-reporter gene constructs containing a functional Sp1 site (pCAT D2-75) or lacking an Sp1 site (pCAT D2-29). Full-length Sp1 and TAFII130 completely reversed mutant huntingtin (HttQ75)–induced inhibition of D2 promoter activity. TAFII130-N, which contains the NH_2-terminal domain of TAFII130 (amino acids 1 through 297), or TAFII130-ΔC (amino acids 1 through 700), which contains both the NH_2-terminal domain and the middle domain but lacks the COOH-terminal domain of TAFII130, and Sp1 lacking the DNA binding domain (Sp1AB, amino acids 83 through 621) did not significantly alter D2 promoter activity. Overexpression of TAFII105 or TAFII55 together with Sp1 did not prevent mutant huntingtin–mediated repression. The relative CAT activity in cells transfected with vector alone is arbitrarily shown as 100 ± SEM. Graphs show means ± SEM of at least five independent experiments, $^*P < 0.001$ versus HttQ75. (**B**) Overexpression of both TAFII130 and Sp1 in transgenic striatal cells reverses the effects of mutant huntingtin on D2 promoter activity. Striatal neurons were isolated from HD transgenic mice (solid bars) or from wild-type littermates (open bar) (*15*). Experiments were performed and analyzed as in (A). $^*P < 0.01$ versus transgenic control. (**C**) Analysis of endogenous D2 receptor mRNA in transfected striatal neurons. Primary striatal cultures were transfected as in (A), and the levels of D2 mRNA were determined by real-time reverse transcription polymerase chain reaction (*15*). The values are presented as percent change as compared to untransfected or vector-transfected striatal cells (set to 100%) ($^*P < 0.001$ versus control, $^xP < 0.01$ versus HttQ75, analysis of variance; data are from three independent experiments). All values were normalized to β-actin, whose mRNA expression was not changed in transfected neurons. (**D**) Mutant huntingtin-induced decrease of D2 dopamine receptor expression is prevented by overexpression of Sp1 and TAFII130 in striatal neurons. A series of fluorescence micrographs is shown of neurons stained for the mutant huntingtin (FLAG epitope, shown in red, left and middle panels), for the D2receptor (shown in green, all panels), and for TAFII130 (HA epitope, shown in red, right panel) (*15*). Arrows indicate transfected neurons. Decreased D2 staining is shown in the left panel, middle row. No change in D2 staining was observed in neurons transfected with huntingtin, TAFII130, and Sp1 (middle and right panels). (**E**) Sp1 and TAFII130 together protect against mutant huntingtin–induced striatal toxicity. Primary striatal cultures were isolated and transfected with the expression plasmids as in (A). Mutant huntingtin–induced cell death was significantly prevented by overexpression of both Sp1 and TAFII130 (*15*). All values are expressed as means ± SEM, $^*P < 0.01$ compared to HttQ75.

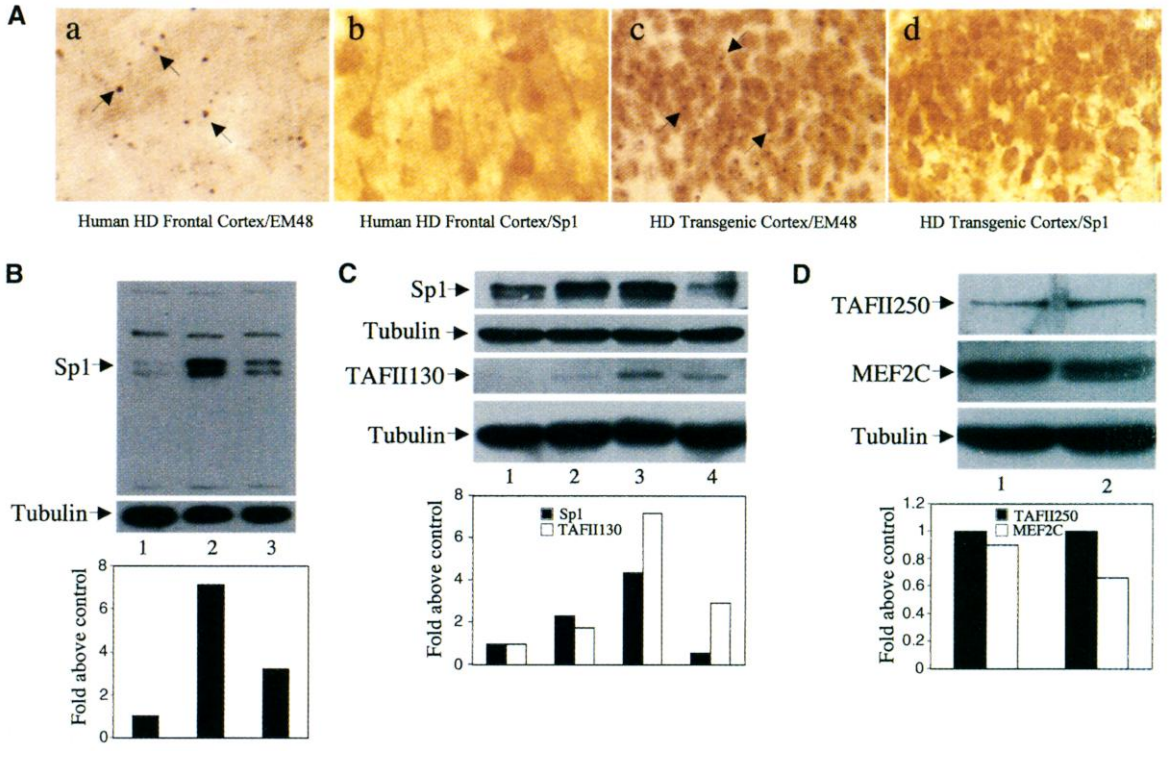

Fig. 4. Increased levels of Sp1 and TAFII130 in the caudate of the human HD brain. (**A**) Sp1 is not present in huntingtin-positive nuclear or cytoplasmic inclusions in human or transgenic HD brains. Immunohistochemistry using EM48, an antibody specific for huntingtin aggregates, shows labeling of inclusions (arrows) in postmortem grade 1 human HD brain tissue (frontal cortex, panel a) and transgenic mouse HD brain tissue (pyriform cortex, panel c) (*7*, *15*). Adjacent sections show diffuse nuclear and cytoplasmic localization of Sp1 but no evident localization of Sp1 in huntingtin aggregates. (**B**) Levels of Sp1 protein are increased in the caudate nucleus of grade 1 and grade 4 HD brains. Soluble protein fractions were collected from the caudate nucleus of a human control brain (lane 1), from a grade 1 HD brain (lane 2), and from a grade 4 HD brain (lane 3), and Western blot analysis was performed (*15*). Densitometric analysis shows that in grade 1 HD brain tissue as compared to control brain tissue, Sp1 levels are increased by 6.1 fold (compare lanes 1 and 2) and in grade 4 HD brain tissue by 2.8 fold (compare lanes 1 and 3). The brain samples used in each group were derived from postmortem tissue described in Fig. 2C. Densitometric analysis was corrected for differences in the expression of tubulin. Values are shown as fold above control. (**C**) Expression levels of Sp1 and TAFII130 in different regions of an HD brain. Postmortem brain tissues were processed and analyzed as in (B). As compared with the control brain (caudate) (lane 1), the HD brain has increased Sp1 protein in the caudate nucleus (lane 3) and cerebral cortex (lane 2) but decreased Sp1 protein in the hippocampus (lane 4). TAFII130 expression follows a similar pattern as Sp1, except that only the caudate nucleus shows a marked increase in TAFII130 expression levels (compare lanes 1 and 3). (**D**) Expression of MEF2C and TAFII250 in human HD brain tissue. Tissues were processed and analyzed as in (B). Expression of TAFII250 was not different in control (lane 1) and HD brains (lane 2), whereas MEF2C levels showed about a 30% decrease in HD brain tissue.

increased in the cerebral cortex but decreased in the hippocampus (Fig. 4C). To determine whether these changes were specific for Sp1, we examined TAFII130 protein levels as well as TAFII250 and the unrelated transcription factor MEF2C. Although TAFII130 showed an expression pattern similar to that of Sp1 (Fig. 4C), no increases in TAFII250 or MEF2C were detected in the caudate of HD brains (Fig. 4D). To exclude the possibility that these increases in Sp1 and TAFII130 represent a stress response in the diseased striatum, we examined Sp1 and TAFII130 levels in another neurodegenerative disease, progressive supranuclear palsy (PSP), which also affects the caudate nucleus. No changes in the expression of Sp1 or TAFII130 were detected in the caudate nucleus of PSP brain tissue (fig. S2) (*15*). Our finding that Sp1 and TAFII130 were specifically increased in the caudate nucleus but not the hippocampus in HD brains may represent a compensatory response to the inhibition of Sp1-regulated transcriptional activity in the presence of mutant huntingtin.

Together, the DNA binding and protein expression data suggest that the decreased function of Sp1 in HD is not due to sequestration of Sp1 into aggregates but rather to the inhibition of Sp1 by soluble mutant huntingtin. Our data are consistent with the finding of Li *et al.*, who recently showed that the soluble form of mutant huntingtin binds more tightly to Sp1 than does aggregated huntingtin (*36*).

We found that huntingtin interacts with both the glutamine-rich and the COOH-terminal domain of TAFII130 and that the COOH-terminal domain is required for protection against huntingtin-induced transcriptional dysregulation and neuronal cell death. The conserved COOH-terminal domain of TAFII130 participates in a number of protein-protein interactions, including several TAFs in the TFIID complex (*30*, *31*). These findings and the recent report that atrophin, which causes another polyglutamine disease (DRPLA), also binds to the COOH-terminal domain of TAFII130 (*37*), suggest that by competing with the critical protein interaction surface of TAFII130, polyglutamine stretches may interfere with the coupling of activator-mediated signals to the basal transcriptional machinery (fig. S3) (*15*). These contributions of TAFII130 as well as of other TAFs to gene transcription are likely to be promoter- and cell type–specific. Consistent with this idea is the finding that expression of TAFII130 varies in different regions of the central nervous system and during development (*38*), and the fact that certain TAFs appear to be required for the transcription of a subset of genes (*12*).

Gene expression analyses on DNA microarrays have shown that differential alterations in gene expression occur in transgenic HD mice as compared with wild-type mice at an early symptomatic stage (*10*). A prominent feature of these changes in gene expression is the down-regulation of genes containing putative Sp1 binding sites in their promoters (*20*), suggesting that loss of Sp1 binding and disruption of Sp1/TAFII130 activation function in early HD may lead to changes in the expression of a number of downstream genes.

Identification of such molecular mechanisms in the earliest stages of polyglutamine disorders may help identify possible targets for future therapeutics.

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Place Cells and Place Recognition Maintained by Direct Entorhinal-Hippocampal Circuitry

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Place cells in hippocampal area CA1 may receive positional information from the intrahippocampal associative network in area CA3 or directly from the entorhinal cortex. To determine whether direct entorhinal connections support spatial firing and spatial memory, we removed all input from areas CA3 to CA1, thus isolating the CA1 area. Pyramidal cells in the isolated CA1 area developed sharp and stable place fields. Rats with an isolated CA1 area showed normal acquisition of an associative hippocampal-dependent spatial recognition task. Spatial recall was impaired. These results suggest that the hippocampus contains two functionally separable memory circuits: The direct entorhinal-CA1 system is sufficient for recollection-based recognition memory, but recall depends on intact CA3-CA1 connectivity.

The hippocampus and related medial temporal lobe regions play a pivotal role in encoding, consolidation, and retrieval of associations responsible for episodic memory (*1–5*). The hippocampus consists of structurally dissimilar processing modules (subfields) that are interconnected serially as well as directly with the adjacent entorhinal cortex (*6, 7*). This arrangement suggests that individual subfields may subserve discrete computational functions, but evidence linking particular modules to specific memory operations is sparse.

The most conspicuous functional characteristic of pyramidal cells in the hippocampus is their location-specific activity (*2, 8*). Hippocampal "place cells" are influenced by experience and may form a distributed map-like mnemonic representation of the spatial environment that the animal can use for efficient navigation (*2, 9*). But how do distinct hippocampal circuits contribute to such signals, and what is the functional capacity of each part of the place-cell network? One possibility is that place-related firing is a result of intrahippocampal computations. The CA3 area may have the capacity to associate and store patterned information received from the dentate gyrus or directly from the entorhinal cortex (*1, 10–12*). However, place fields have been recorded in area CA1 after selective dentate gyrus lesions (*13*), and place fields were not affected by a moderate reduction of neuronal activity in area CA3 (*14*); these results suggest that positional information from the entorhinal cortex might bypass the dentate gyrus and perhaps also CA3. To determine whether place-related firing in area CA1 is imposed by the associative network in area CA3 or by the direct connections from the entorhinal cortex (*6, 7*), we removed the input from area CA3 so that the remaining CA1 area was innervated only by fibers from the entorhinal cortex.

We first implanted electrodes in four rats in which area CA3 had been removed bilaterally with ibotenic acid (*15*). The CA3 area was removed throughout most of the dorsal hippocampus in all animals (Fig. 1, A to C), whereas area CA1 was largely spared. Neurons appeared normal at the recording position (Fig. 1B). After surgery, the rats were trained to collect scattered food in a square open field, and unit signals were recorded from area CA1. Most of the 42 pyramidal neurons recorded in these animals had distinct and well-defined place fields that were stable for at least 1 hour and thus were similar to those of normal rats (Fig. 1D). These observations suggest that area CA3 may not be necessary for establishing and maintaining place fields in area CA1, and that spatial information from the neocortex may reach the hippocampus primarily through the alternative route: the direct pathway from layer III of the entorhinal cortex.

However, because many functions of hippocampal neurons may be performed with relatively small portions of intact hippocampal tissue (*16*), the place-specific firing in area CA1, as observed in CA3-lesioned rats, could reflect input from remaining CA3 cells at the septal pole or in more temporal parts of the hippocampus. To isolate the direct entorhinal pathway to area CA1 completely, we removed the contralateral hippocampus and made a sequence of continuous miniature razor-blade cuts between areas CA3 and CA1 of the spared hippocampus (Fig. 2). This procedure separated the recording site from area CA3 as well as from subcortical afferents entering through the fimbria-fornix (Fig. 2, M to Q). We recorded place fields from 124 CA1 cells in 11 rats with such lesions. Most units exhibited clear place specificity in the open field after postsurgical training and appeared similar to cells from normal animals (Fig. 2Y). Place fields were stable across recording sessions, even over several days (Fig. 2Y). The discharge was theta-modulated (*17*), indicating that both spatial and temporal firing patterns

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