Huntingtin Interacting Protein 1 Induces Apoptosis via a Novel Caspase-dependent Death Effector Domain*

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Huntington disease (HD)1 is a progressive neurodegenerative disease caused by the expansion of a polymorphic glutamine tract in huntingtin. The huntingtin interacting protein (HIP-1) was identified by its altered interaction with mutant huntingtin. However, the function of HIP-1 was not known. In this study, we identify HIP-1 as a proapoptotic protein. Overexpression of HIP-1 resulted in rapid caspase 3-dependent cell death. Bioinformatics analyses identified a novel domain in HIP-1 with homology to death effector domains (DEDs) present in proteins involved in apoptosis. Expression of the HIP-1 DED alone resulted in cell death indistinguishable from HIP-1, indicating that the DED is responsible for HIP-1 toxicity. Furthermore, substitution of a conserved hydrophobic phenylalanine residue within the HIP-1 DED at position 398 eliminated HIP-1 toxicity entirely. HIP-1 activity was found to be independent of the DED-containing caspase 8 but was significantly inhibited by the antiapoptotic protein Bcl-xL, implicating the intrinsic pathway of apoptosis in HIP-1-induced cell death. Coexpression of a normal huntingtin fragment capable of binding HIP-1 significantly reduced cell death. Our data identify HIP-1 as a novel proapoptotic mediator and suggest that HIP-1 may be a molecular accomplice in the pathogenesis of Huntington disease.

1 The abbreviations used are: HD, Huntington disease; DED, death effector domain; HIP-1, huntingtin interacting protein 1; DISC, death-inducing signaling complex; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide; DEVD-CHO, N-acetyl-Asp-Glu-Val-Asp-aldehyde; DAB, 3,3′-diaminobenzidine.

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The Journal of Biological Chemistry Vol. 275, No. 52, Issue of December 29, pp. 41299–41308, 2000

This paper is available online at http://www.jbc.org

Received for publication, September 13, 2000
Published, JBC Papers in Press, September 27, 2000, DOI 10.1074/jbc.M008408200

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† Supported by a Medical Research Council of Canada postdoctoral fellowship.

‡ Supported by the Huntington Disease Society of America.

§ Supported by the Huntington Disease Society of America and National Science Foundation Grant NS35255.

∥ Supported by a Hereditary Disease Foundation postdoctoral fellowship.

* Supported by a fellowship from the Huntington Disease Society of America.

‡ Supported by the Medical Research Council of Canada.

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EXPERIMENTAL PROCEDURES

Subcloning and Mutagenesis—The identification of human HIP-1 cDNA by yeast two-hybrid analysis and cloning into the mammalian expression vector pCRI (Promega) was described in Kalchman et al. (3). The 5’-end of the HIP-1 sequence was extended by reverse transcriptase-polymerase chain reaction from human total brain RNA (CLONTECH) using Superscript II reverse transcriptase, according to the manufacturer’s protocol (Life Technologies, Inc.). The sense primer 5’-GCCCTCGGTACATGATGGAGAATCCCTGCATTCTTCCG-3’, encompassing the Kozak consensus sequence and ATG start site of splice variant HIP1-2 (28), was used in combination with the antisense primer 5’-GACATGTCCAGGGATGCAAGATG-3’ to amplify this 533-base pair fragment that was then cloned into the SnaI site of pBluescript SKII (Stratagene). The Ndel–HpaI fragment of HIP-1 (3), containing the 5’-end of HIP-1, was isolated and ligated adjacent to the 5’-end using EcoRI–HpaI digestion. Subsequently, a NotI–KpnI fragment containing full-length HIP-1 was cloned into the mammalian expression vector pCRI (Promega) for in vitro studies.

The DED expression construct, corresponding to amino acids 376–457 of HIP-1, was amplified using the sense primer 5’-CTAGAGCCAGCATGGGCCGACGTCATGCTGGAGGCG-3’, which included the Kozak consensus sequence, initiating methionine, and XbaI restriction site for subcloning, and the antisense primer, 5’-GGCCGCTAGAGCTTCTTCGACTTATTGTTTTGGTGTTCCTGATCTCATTCCG-3’, which included the sequence for the c-Myc epitope tag, a termination codon, and a NotI restriction site. The polymerase chain reaction product was subcloned into the cloning vector pCR2.1 (Invitrogen), sequenced, and then rescubloned into the mammalian expression vector pCRI.

Three HIP-1 DED mutants, E397G, F398G, and F398Y, were created using a polymerase chain reaction-based mutagenesis strategy. Primers corresponding to the HIP-1 sequence were designed with the indicated base change, corresponding to amino acids 397 and 398 in the DED (E397G; 5’-GCGGCCGACGACTGTGGAT-3’; F398G; 5’-CGGCCGACGACTGTGAATAC-3’), HIP-1 was amplified using the mutagenesis primers and an anti-sense primer containing the HIP-1 Sapl restriction enzyme site (5’-TCCGAGGACGATATCCCT-3’). The mutagenesis destroyed an EcoRI site, facilitating screening of the clones. Positive clones were digested with SapI and BglI and ligated as a cassette into HIP-1. Each construct was sequenced across the mutation and ligation sites to verify sequence integrity.

Protein Expression Analysis—The expression of HIP-1, the DED, and the HIP-1 DED mutants was verified in transfected cells by Western blotting and immunofluorescence, as described previously (20). An anti-hip-1 monoclonal antisera, HIP1–526fp, was made against a glutathione S-transferase fusion protein encompassing amino acids 130–526 in HIP-1. The DED protein was detected using the anti-c-Myc antibody HIP-1. The DED expression construct, corresponding to amino acids 376–457 of HIP-1, was detected using the anti-c-Myc antibody MAB2166 (Chemicon), as described (20). The HIP-1 and HIP-1 DED construct were sequenced across the mutation and ligation sites to verify sequence integrity.

Huntingtin-containing aggregates were identified in transfected HEK 293T cells at 24 h post-transfection by immunofluorescence using the anti-huntingtin monoclonal antibody AMB1866 (Chemicon), as described previously (20). The cells were viewed with an Axioscope microscope (Carl Zeiss, Inc.), digitally captured with a CCD camera (Princeton Instrument Inc.), and the images were colorized and overlapped using the Eclipse software program (Empix Imaging Inc.). The percentage of cells with aggregates represents the percentage of all cells expressing huntingtin that contained aggregates.

RESULTS

HIP-1 Is a Proapoptotic Protein

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Subsequently, a 9 sequence, initiating methionine, and XbaI co-transfection of the expression constructs with a plasmid containing the Kozak consensus sequence and ATG start site of splice variant HIP1-2 (28), was used in combination with the antisense primer 5’-GACATGTCCAGGGATGCAAGATG-3’ to amplify this 533-base pair fragment that was then cloned into the SnaI site of pBluescript SKII (Stratagene). The Ndel–HpaI fragment of HIP-1 (3), containing the 5’-end of HIP-1, was isolated and ligated adjacent to the 5’-end using EcoRI–HpaI digestion. Subsequently, a NotI–KpnI fragment containing full-length HIP-1 was cloned into the mammalian expression vector pCRI (Promega) for in vitro studies.

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The cell death induced by HIP-1 was unlikely due to protein overload, since overexpression of other huntingtin interacting proteins, HIP-2, HIP-3, and HIP-12 (28), did not result in apoptosis by morphological assessment or MTT assay. Additionally, we have previously described that huntingtin overexpression is not directly toxic but increases the susceptibility to apoptotic stress in 293T cells (20). The comparative toxicities of HIP-1 and huntingtin are shown in Fig. 1d. HIP-1 expression is more toxic than huntingtin expression in 293T cells. Huntingtin fragment (771-128) with 128 repeats is toxic only in the presence of an apoptotic stress (tamoxifen, black), whereas HIP-1 is toxic without tamoxifen (white). *, viability of HIP-1 transfected cells is below the level of detection at 48 h post-transfection; ***, p < 0.001, comparing HIP-1 with lacZ and huntingtin.

Identification of a Death Effector Domain in HIP-1—Sequence similarity between HIP-1, HIP-1 family members, and the yeast homologue Sla2p has suggested functions for HIP-1 involving the actin cytoskeleton. Although Sla2p is not known to have any cell death-inducing activity, the morphological changes resulting from HIP-1 expression suggest that HIP-1 activates an apoptotic pathway. Since the HIP-1 sequence in the predicted coiled-coil region differs substantially from Sla2p, we wished to determine whether this region contains additional domains that may form the basis for the toxicity of HIP-1.

Mediators of apoptosis frequently have discrete domains that are necessary for their proapoptotic activity, including caspase recruitment domains, death domains, and DEDs. Searches for proteins homologous to HIP-1 and subsequent sequence alignments using the GCG software program revealed a novel domain not previously identified in HIP-1 (Fig. 2). The HIP-1 protein sequence, from residue 376 to 457, has homology to
**HIP-1 Is a Proapoptotic Protein**

DEDs found in caspase 10, caspase 8, FADD, usurpin (c-FLIP), and several viral DED-containing proteins (25, 29–33). DEDs act as protein interaction modules that link adapter proteins such as FADD to DED-containing caspases such as caspase 8, into a complex with activated membrane-bound receptors for Fas or tumor necrosis factor, called the death-inducing signaling complex (DISC) (30, 31, 34). The formation of the DISC protein complex is a crucial step in the initiation and signaling of apoptosis induced by multiple agents (35, 36).

Pairwise comparisons of the full-length protein sequences indicated that HIP-1 contains only one DED, in contrast to the proapoptotic caspase proteins, which have two DEDs (Fig. 2). Conserved residues are located throughout the HIP-1 DED. However, the average percentage of similarity between HIP-1 and other DED-containing proteins is 33%, which, although significant, is lower than the average similarity among caspase DEDs (41.7%). The HIP-1 DED is predicted to have a structure composed of six α-helices, based on its similarity to the protein FADD (Fig. 2). Thus, the presence of a DED in a huntingtin interacting protein suggests a direct link between huntingtin, HIP-1, and the apoptotic pathway.

**The DED Is Essential for HIP-1 Toxicity and Is Dependent on Amino Acid Phe**

Sequence analysis predicted a DED in HIP-1. However, to establish its functionality, we determined whether the HIP-1 DED is active and involved in HIP-1-mediated toxicity. To do this, a construct was created that encodes just the HIP-1 DED, epitope-tagged for detection. Transfection of the altered protein, HIP-1/F398G, resulted in levels of cell death equivalent to the control proteins (Fig. 3b). In contrast, substitution to a different hydrophobic residue (tyrosine; HIP-1/F398Y) or alteration of the adjacent amino acid to a glycine (HIP-1/E397G), did not change its toxicity from wild-type HIP-1 (n = 5) (Fig. 3b). Western blotting demonstrated the equivalent expression of each protein (Fig. 3c). Therefore, alteration of a single amino acid, at position 398, was remarkably effective at eliminating HIP-1-induced cell death. These results indicate that the DED in HIP-1 (and, in particular, a specific residue within the DED) is crucial for cell death. These mutagenesis data are also consistent with data from the identification of a HIP-1 family member, HIP-12 (28), which is nonapoptotic and has a glutamine residue instead of a phenylalanine at the analogous position. Furthermore, these results suggest that hydrophobicity at this location in the HIP-1 DED is important for function, similar to the protein FADD.

**HIP-1 Toxicity Requires Caspases and Involves Intrinsic Cell Death Pathways**—To further explore how HIP-1 overexpression induces a DED-dependent cell death, we examined the apoptotic pathways involved in HIP-1 toxicity. Two major pathways leading to apoptosis initiation have been described (reviewed in Refs. 38 and 39). The extrinsic pathway is activated by ligation of tumor necrosis factor family receptors, such as the tumor necrosis factor and Fas ligands, followed by recruitment of death domain- and DED-containing proteins into the oligomeric DISC protein complex, which leads to activation of DED-containing procaspases. By comparison, the intrinsic pathway depends on mitochondria-mediated events and Bcl-2 family members, resulting in cytochrome c release from the mitochondria, Apaf-1 binding and caspase 9 activation. Although these apoptosis pathways are usually considered to be independent processes, they may intersect at various points, as suggested recently by Zhang et al. (40). Huntingtin has been shown to play a role in the activation of the caspases, similar to the intracellular domain of FADD (29), a component of the death-inducing signaling complex. In support of this, overexpression of HIP-1 in HEK 293T cells induced activation of caspase 3 and 6, and inhibition of caspase cleavage of huntingtin by peptide inhibitors and mutagenesis has been shown to reduce cell death and aggregate formation in an in vitro model of HD (19). As shown in Fig. 4a, overexpression of HIP-1 in HEK 293T cells induced activation of caspase 3, which was significantly greater than the lacZ control (p < 0.001, n = 4). Increased caspase 3 activity was evident at the two time points tested: 24 h (Fig. 4a) and 18 h (data not shown). Therefore, the ap-

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**Fig. 2. HIP-1 contains a DED.** Alignment of the DED in HIP-1 with the DEDs in proteins known to mediate apoptosis. The α-helices are indicated by brackets below the alignment, as defined in FADD by Eberstadt et al. (37). Most of the proteins listed contain two DEDs (DED-A and B); MC159, E8, and KSORFK13 are viral DED-containing proteins. Casp8, caspase 8; casp10, caspase 10. Boxed letters indicate highly conserved residues. The arrow points to the phenylalanine residue mutated in this study within the predicted α-helix.
expression levels.

Alteration of the adjacent residue (E397G) did not alter toxicity (**, p < 0.01 compared with vector control, n = 5). b, HIP-1 mutants were generated by site-directed mutagenesis, and cell death was quantified in NT2 cells. The phenylalanine residue essential for HIP-1 toxicity in the DED was identified at position 398. Mutation to a nonhydrophobic residue (F398G) eliminated HIP-1 toxicity, whereas mutation to a different hydrophobic residue (F398Y) or co-expression of the nontoxic DED mutant F398G with caspase 8 did not reduce caspase 8-dependent toxicity, compared with the control transfection (n = 3). c, equivalent amounts of lysates from HIP-1 and HIP-1 DED mutant transfections were Western blotted and probed using the anti-HIP-1 antibody HIP-1fp to demonstrate expression levels. NT, nontransfected. Protein samples were analyzed on the same gel, and the relevant lanes were joined together for clarity using graphics software.

To confirm this result, 293T cells transfected with HIP-1 were incubated with the caspase inhibitor DEVD-CHO, which is conjugated to an internalization peptide to increase cell permeability. The inhibitor is directed to caspases 3 and 7 but is conjugated to an internalization peptide to increase cell permeability. The inhibitor is directed to caspases 3 and 7 but is conjugated to an internalization peptide to increase cell permeability. The inhibitor is directed to caspases 3 and 7 but is conjugated to an internalization peptide to increase cell permeability. The inhibitor is directed to caspases 3 and 7 but is conjugated to an internalization peptide to increase cell permeability.

To further confirm the involvement of caspase 3 in HIP-1 toxicity, we used the human breast carcinoma cell line MCF7, which is deficient in caspase 3 (47, 48). Western blotting using anti-caspase 3 antibodies demonstrated that the MCF7 cells used in these experiments did not express caspase 3 (n = 2) (Fig. 4d). In contrast to NT2 and 293T cell lines, transfection of HIP-1 into MCF7 cells did not result in increased cell death over control transfections at 24 and 48 h post-transfection, although the base-line level of cell death in MCF7 cells was high (Fig. 4c). Immunofluorescence staining indicated that the transfection efficiency of HIP-1 was equivalent to the control protein pyruvate kinase (data not shown). These data indicate that HIP-1 toxicity in 293T and NT2 cells is dependent on caspases, particularly caspase 3-like proteases.

The proximal events triggered by certain apoptotic stimuli occur by formation of the DISC (extrinsic apoptotic pathway), through recruitment and activation of procaspases such as caspase 8, by their interaction with adaptor proteins containing DEDs. Overexpression of DED-containing adaptor proteins in cell culture has been shown to induce cell death by recruiting procaspases to the DISC (29, 41, 42). Expression of DED-containing apoptosis inhibitor proteins, such as usurpin (c-FLIP), are known to prevent active DISC formation and thereby prevent cell death. To determine the involvement of DISC formation in HIP-1-induced cell death, HIP-1 was co-expressed with caspase 8 and usurpin, proteins known to be involved in DISC-mediated apoptosis in multiple cell types.

Co-expression of usurpin with caspase 8 in NT2 cells prevented caspase 8-mediated toxicity (n = 6, Fig. 5a), as previously reported (25). In contrast, expression of usurpin with HIP-1 did not reduce cell death induced by HIP-1 in NT2 cells (Fig. 5a) or caspase 3 activation in 293T cells (Fig. 5c). The inability of usurpin to inhibit HIP-1 is consistent with the observation that endogenous HIP-1 does not co-immunoprecipitate with usurpin or caspase 8 in 293T cells (n = 4, Fig. 5b), HeLa, or NT2 cells (data not shown), although usurpin and caspase 8 do co-immunoprecipitate in these cell types (data not shown).

To further assess the roles of caspase 8 and usurpin in HIP-1-mediated toxicity, we utilized a catalytically inactive “dominant negative” caspase 8 (caspase 8DN) that had been shown to reduce endogenous caspase 8 activity (25) and was effective at reducing caspase 3 activation induced by Fas ligand in 293T cells (data not shown). However, inhibiting endogenous caspase 8 by expression of the dominant-negative caspase 8 protein had no effect on HIP-1 toxicity, as shown in Fig. 5c, co-expression of caspase 8DN with HIP-1 in 293T cells did not reduce HIP-1-induced caspase 3 activation (n = 4).

As further evidence for HIP-1 toxicity occurring independently of caspase 8, co-expression of caspase 8 and HIP-1 in NT2 cells was shown to have no influence on the severity of cell death. This was evident by its equivalent toxicity to co-expression of caspase 8 and pyruvate kinase (n = 3, Fig. 5d). Additionally, co-expression of the nontoxic DED mutant F398G with caspase 8 did not reduce caspase 8-dependent toxicity, compared with the control transfection (n = 3, Fig. 5d). Together, these data show that direct cellular toxicity due to HIP-1 overexpression is independent of caspase 8 in the cell types tested.
and suggest that the pathway induced by HIP-1 is downstream from DISC formation. Caspase 8-mediated events have also been shown to be important to polyglutamine toxicity (17), suggesting that these events may occur in a different pathway than HIP-1-induced cell death.

To determine whether intrinsic apoptotic pathways are activated in HIP-1 induced cell death, HIP-1 was co-transfected in NT2 cells with the antiapoptotic protein Bcl-xL and with the control protein pyruvate kinase. Antiapoptotic Bcl family members are located on internal membranes and, at the mitochondrial membrane, act to prevent cytochrome c release (43, 44). In contrast to usurpin, co-expression of Bcl-xL dramatically reduced cell death due to HIP-1 (Fig. 5e), indicating that HIP-1-mediated toxicity may be mediated by the intrinsic apoptotic pathway.

**HIP-1 Toxicity Is Influenced by Length of the Polyglutamine Tract in Huntingtin**—Overexpression of mutant huntingtin has been shown to be toxic in vitro and in vivo (19, 45, 46). However, the proapoptotic nature of HIP-1 raises interesting possibilities for the involvement of HIP-1 in HD. Measurements of huntingtin/HIP-1 interaction by the yeast two-hybrid assays predicted that there would be excess unbound HIP-1 in the presence of mutant huntingtin, when compared with wild-type huntingtin (3). To further explore the altered huntingtin/HIP-1 interaction, we expressed HIP-1 in HEK 293 cell lines that also stably expressed large huntingtin fragments (nucleotides 1–1955; 548 amino acids) (14) containing 15 or 128 glutamines. This N-terminal 548-amino acid fragment was used originally to isolate HIP-1 (3).

The effects of HIP-1 transfection into cell lines stably expressing the 548-amino acid fragment with 15 (htt15 cells) and 128 polyglutamines (htt128 cells) were compared with, and normalized to, transfection of the lacZ controls. Viability was assessed at three time points post-transfection. As shown in Fig. 6a, HIP-1 toxicity was influenced by polyglutamine expansion at all three time points. HIP-1 resulted in significantly lower cell viability when expressed in the htt128 cells compared with expression in htt15 cells (n = 6). The slight increase in viability observed in htt128 at 24 h is probably due to recovery from transfection-related stress. The increased toxicity of HIP-1 in the presence of mutant huntingtin compared with wild-type huntingtin was observed in two different clones of both of the huntingtin stable cell lines, and transfection efficiencies and expression levels were equivalent in each cell line (Fig. 6b).

Based on the yeast two-hybrid data and the differential toxicity of the huntingtin stable cell lines (Fig. 6b), we hypothesized that when normal huntingtin is replaced by mutant huntingtin there would be excess “free” HIP-1 available for interaction with proteins that mediate the apoptotic pathway. Therefore, in our in vitro system, increasing expression of wild-type huntingtin by transfection would be expected to bind...
HIP-1 and reduce cell toxicity. Indeed, transient co-expression of the huntingtin fragment 1955-15 and HIP-1 in NT2 cells resulted in lower cell death than co-expression of a control protein with HIP-1 (p < 0.01, n = 6). In contrast, co-expression of huntingtin with expanded polyglutamine, 1955-128, with HIP-1 did not reduce the level of cell death, compared with the control (HIP-1 + PK) transfection. The interaction between mutant huntingtin and HIP-1, even when both proteins are overexpressed, may not be strong or stable enough to sequester HIP-1.

In the in vivo situation, wild-type and mutant huntingtin are both expressed. In order to model this in vitro, HIP-1 was co-transfected with both 1955-15 and 1955-128 into NT2 cells (Fig. 6d). There was no difference in toxicity when all three proteins were co-expressed, compared with the control co-transfection (PK + 1955-15 + 1955-128) (n = 4), as would be expected if HIP-1 was sequestered by 1955-15. The observed cell death in this experiment may be attributed to 1955-128 toxicity, since expression of mutant huntingtin in neuronal cells has been shown in previous studies to be cytotoxic (19, 45).

Therefore, our results indicate that mutant huntingtin toxicity may occur through at least two pathways: direct polyglutamine-mediated toxicity and release of HIP-1.

HIP-1 Expression Increases the Formation of Huntingtin Aggregates—Since cell death in vitro was influenced by HIP-1 and huntingtin co-expression, we reasoned that HIP-1 may have an effect on other features of HD that can be modeled in cell culture. The formation of aggregates containing mutant truncated huntingtin protein is a marker associated with disease in

![Image of Fig. 6](https://example.com/fig6.png)
patient brains, transgenic mice, and cellular models of HD. Although huntingtin aggregates are not the primary cause of neuronal cell death (49, 50), they may contribute to the progression of disease. Factors previously implicated in polyglutamine diseases, including chaperones (51), caspase activation (19), and nuclear localization (45), have been demonstrated to have an effect on aggregation. To determine whether HIP-1 expression influences aggregate formation, HIP-1 was co-transfected with the 1955-base pair huntingtin fragments in HEK 293T cells. This cell line was previously shown to form cytoplasmic aggregates from full-length or large truncated fragments of transfected huntingtin only when apoptosis was induced by an exogenous agent, such as tamoxifen (20).

Co-transfection of 1955-128 and the control protein β-galactosidase into 293T cells resulted in 1.8 ± 0.7% of huntingtin-expressing cells forming cytoplasmic aggregates composed of mutant huntingtin. In sharp contrast, 1955-128 and HIP-1 co-transfection resulted in 14.0 ± 1.2% of the cells forming aggregates (n = 5), when assessed at 24 h post-transfection. HIP-1 transfected into two different HD1955-128 HEK 293 cell lines resulted in aggregates in 15.0 ± 3.6% of the cells (n = 3), also measured at 24 h post-transfection. The difference in aggregate formation between HIP-1-transfected cells and control transfections is significant at p < 0.01. An example of a HIP-1-induced aggregate is shown in Fig. 7a. Note that HIP-1 does not appear to be co-localized or incorporated into the aggregate. As expected, HIP-1 did not form aggregates in cells that expressed the 1955-15 protein (n = 8). As described in Fig. 1, HIP-1 toxicity is clearly evident at 24 h post-transfection, coincident with the observation of increased aggregates. Therefore, the increased toxicity in 1955-128-expressing cells was associated with the ability of HIP-1 to promote huntingtin aggregate formation.

In order to address the issue of HIP-1 localization in vivo, we performed immunohistochemistry on huntingtin transgenic mice (exon 1, line R6/2) (46). These mice were chosen because they have a high number of intraneuronal aggregates as a well-documented characteristic of their phenotype. As shown in Fig. 1, HIP-1 toxicity is clearly evident at 24 h post-transfection, coincident with the observation of increased aggregates. Therefore, the increased toxicity in 1955-128-expressing cells was associated with the ability of HIP-1 to promote huntingtin aggregate formation.

In this paper, we have defined a novel function of HIP-1. We show by sequence and functional analyses that HIP-1 acts as a...
HIP-1 Is a Proapoptotic Protein

One early step in the pathogenesis of HD may be disturbed molecular events in the cytoplasm, occurring prior to aggregate formation or translocation of huntingtin into the nucleus (50). One early step in the pathogenesis of HD may be disturbed associations with cytoplasmic interacting proteins. HIP-1 is an attractive candidate protein as it is predominantly expressed in the CNS, although not exclusively in the striatum (28), and colocalizes with nonaggregated huntingtin in the cytoplasm of neuronal cells. Altered HIP-1/huntingtin interaction and HIP-1-mediated cell death may occur in regions of the brain that die in HD. Proteins that interact with HIP-1, such as HIPPI, could modulate the degree of HIP-1-mediated cell death, sparing neurons elsewhere. Finally, the hypothesis of HIP-1 toxicity can be considered in light of a recent report suggesting a "one-hit model" for diseases with neuronal death over an extended period, such as HD (56). Neurons in HD patient brains are predicted to be in an abnormal state in which there is increased probability that a single "catastrophic" event leads to cell death. The expanded polyglutamine tract induces the abnormal state, and the release of HIP-1, which may be influenced by additional interactions modulating accessibility and by turnover of the normal huntingtin protein, may be thought of as an example of a catastrophic event leading to neurodegeneration.

Additionally, there are several lines of evidence to suggest that overexpression of normal huntingtin provides resistance to cell death, as would be predicted if wild-type huntingtin sequesters HIP-1 and prevents it from inducing the apoptotic pathway. Rigamonti et al. (57) demonstrated that expression of wild-type huntingtin protected clonal striatal derived cells from several apoptotic stimuli. The protective effect occurred downstream of Bcl-2 and upstream from caspase 3 activation, which is consistent with the pathways implicated in HIP-1 toxicity. Furthermore, there is in vivo evidence that increasing the expression of wild-type huntingtin in transgenic mice protects against the toxic effects of mutant huntingtin, potentially by binding to HIP-1. A comparison of YAC transgenic mice expressing full-length huntingtin with 72 polyglutamines that also have varying copies of wild-type mouse huntingtin showed a dramatic differential cellular apoptotic phenotype (58). YAC72 mice with no wild-type huntingtin exhibited significant cell death at an early age, in contrast to minimal cell death observed in YAC72 mice with two copies of the normal allele and intermediate cell death in the YAC72 heterozygous mice. Future experiments to alter HIP-1 expression in vivo will determine the nature of HIP-1's involvement in apoptosis-related cytoskeletal changes and will assess whether HIP-1 is a molecular accomplice in the pathogenesis of HD.

Acknowledgments—We are grateful to Dr. Sophie Roy, Dita Rasper, and Dr. Michael Kalchman for helpful discussions, to Keith Fichter, Krista McCutcheon, and Carlos Saavedra for excellent technical expertise, and to Dr. Gordon Shore (Department of Biochemistry, McGill University) for the caspase 8DN construct.

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HIP-1 is a proapoptotic protein that is involved in various cellular processes, including cell death and survival. Its expression and function are regulated by multiple factors, and its role in neurodegenerative diseases is under investigation.