Huntington's disease (HD) is caused by the expansion of a glutamine repeat in the protein huntingtin. The expanded glutamine repeat is thought to mediate a gain of function by causing huntingtin to abnormally interact with other proteins. We previously identified a rat huntingtin-associated protein (HAP1) that binds to huntingtin; HAP1 binds more tightly to huntingtin with an expanded glutamine repeat than to wild type huntingtin. Identification of the human homologue of HAP1 is necessary for investigation of the potential role of HAP1 in HD pathology. Here, we report the cloning of a human HAP1 homologue (hHAP) that shares 62% identity with rat HAP1 over its entire sequence and 82% amino acid identity in the putative huntingtin-binding region. The hHAP gene encodes a 4.1-kilobase transcript and a 75-kDa protein which are specifically expressed in human brain tissues. Its expression in Huntington's disease brains is reduced in parallel with a decreased expression of huntingtin. While two isoforms of rat HAP1 are expressed at similar levels in rat brain, only a single major form of hHAP is found in primate brains. In vitro binding, immunoprecipitation, and coexpression studies confirm the interaction of hHAP with huntingtin. The in vitro binding of hHAP to huntingtin is enhanced by lengthening the glutamine repeat. Despite similar binding properties of rat HAP1 and hHAP, differences in the sequences and expression of hHAP may contribute to a specific role for its interaction with huntingtin in humans.

Huntingtin is the protein product of the gene for Huntington's disease (HD). The N terminus of human huntingtin contains a glutamine stretch encoded by a CAG repeat (1). Expansion of the CAG/glutamine repeat (>37 units) causes HD (1) and the length of the CAG repeat is inversely correlated with the age of onset of HD (2–4).

Although the genetic basis of HD has been identified, its pathogenesis remains unclear. The widespread expression of huntingtin does not explain the selective neuropathology of HD, which is characterized by massive neuronal loss specifically in the basal ganglia (5–7). Since mice that lack huntingtin or express reduced levels of huntingtin display aberrant brain development and perinatal lethality (8), huntingtin is thought to be important for neurogenesis. However, loss of huntingtin activity is unlikely the cause of HD because heterozygous HD inactivation by translocation in man (9) or by targeted mutagenesis of the mouse HD gene (10, 11) does not cause any HD phenotype. A gain of function model for the disease is thus widely accepted. It has been proposed that the expanded glutamine repeat may induce an abnormal interaction between the mutant protein and other cellular proteins (12–14). Consistent with this idea, studies to date have shown that huntingtin interacts with several proteins including HAP1 (15), glyceraldehyde phosphate dehydrogenase (GAPDH) (16), an unidentified calmodulin-associated protein (17), and a protein homologous to the yeast cytoskeleton-associated protein Sla2p (HIP1) (18, 19). These huntingtin-associated proteins are likely involved in HD pathology because their interactions with huntingtin are affected by an expanded glutamine repeat.

HAP1 was first identified in rat brain and was found to bind to the N-terminal region of human huntingtin (15). It binds more tightly to mutant huntingtin than to normal huntingtin (15). Furthermore, rat HAP1 is specifically expressed in neurons (20), making it a good candidate to be involved in the neuropathology of HD. Recent studies also show that rat HAP1 associates with a number of intracellular organelles (20–22), moves with huntingtin in nerve fibers (23), and interacts with dynactin P150Glued (24, 25) which participates in dynein-mediated retrograde transport. It remains to be shown whether human brain also contains a HAP1 homologue with similar binding properties. Since mice with normal levels of mutant huntingtin do not display any abnormal phenotype (8), specific properties of the proteins which associate with human huntingtin may account for the HD phenotype that occurs in man. Therefore, it will be interesting to identify human proteins that associate with huntingtin.

In this study, we isolated a human HAP1 homologue (hHAP) by molecular cloning. hHAP shares 62% amino acid identity with rat HAP1A. Although its binding to huntingtin is comparable to that of rat HAP1, hHAP only has a single major form expressed in primate brains, in contrast to two isoforms of HAP1 in rat brain. The different sequences and expression of hHAP could contribute to a specific role in the interaction of hHAP and huntingtin in human brain. Identification of the human HAP1 homologue will facilitate the study of the relevance of this interaction.
vance of the interaction between HAP1 and huntingtin in the pathology of HD.

**EXPERIMENTAL PROCEDURES**

cDNA Cloning—PCR products (previously named hHAP1 and hHLP1) that encode sequences with homology to rat HAP1 (15) were used as probes to screen human brain cDNA libraries made from human brain stem, striatum, and cerebral cortex (Stratagene). Sixteen clones were obtained from screening 10 million recombinants with these probes using high stringency hybridization (50% formamide, 5 × SSC, 5 × Denhardt’s, 25 mM sodium phosphate, 0.1% sodium pyrophosphate, 100 μg/ml yeast tRNA, and 1% SDS at 42 °C) and washing (0.2 × SSC at 55 °C). Sequencing revealed that they all contained partial sequences with about 80% amino acid identity to HAP1. We thus renamed them hHAP (human huntingtin-associated protein). To obtain the full-length hHAP cDNA, we used 5′-RACE with an antisense primer (5′-CACTGATGCTGTTTTCGTTG-3′) that corresponds to rat HAP1’s amino acids 243 to 249. The sense primer was a 5′-RACE anchor primer (5′-ggccacagctgcatagtcggagggngggngg-3′) from the RACE kit (Life Technologies, Inc.). First strand cDNA was generated from RNA of human caudate tissue (CLONTECH). PCR products were electrophoresed on a 1% agarose gel. PCR product of about 900 base pairs was observed and subcloned from the gel for subcloning and sequencing. The full-length cDNA was then assembled by ligation of this PCR product with a cDNA clone that contains the rest of the coding region of hHAP using a DraIII site. A cDNA with a full coding region of hHAP was subcloned into the expression vector pcIS (15) for sequencing and expression in human kidney embryonic 293 (HEK 293) cells.

**Northern Blot Analysis**—Nitrocellulose membranes containing poly(A)+ RNAs of human brain and peripheral tissues were obtained from CLONTECH. A [32P]dCTP-labeled hHAP cDNA fragment (1057–1440 nucleotides) that encodes the putative huntingtin-binding region was used as a probe. The GAPDH cDNA probe was obtained from CLONTECH. The blots were hybridized in 50% formamide and 5 × SSC at 65 °C. The blots were then exposed to x-ray film for 2 days at −70 °C.

A full-length hHAP cDNA fragment containing amino acids 323–455 was fused in-frame to the GST fusion protein vector pGEX4T (Pharmacia Biotech) to produce GST-hHAP fusion proteins in bacteria BL21. GST-hHAP fusion proteins were purified with glutathione-agarose beads (Sigma). The purified GST-hHAP protein served as immunogen for Covance Inc. (Denver, PA) to produce rabbit antiserum. The antibody (EM39) was purified by incubating whole serum with a nitrocellulose strip containing GST-hHAP overnight. After multiple washes of the strip with 10 mM Tris-HCl, pH 7.5, antibodies were eluted with 0.2 M glycine, pH 2.1, for 5 min and immediately neutralized by adding 0.1 volume of 2 × Tris-Cl, pH 8.8. The eluted antibody was then dialyzed in PBS overnight. A truncated human huntingtin cDNA that encodes the first 256 amino acids with 2 glutamine repeats was obtained by RT-PCR with an in vitro Translation kit (Promega). The constructs from 23 to 44 CAG repeats were obtained previously (15). The construct with 73 CAG repeats was the result of repeat length instability during propagation of a phage clone containing exon 1 of the HD gene with 150 CAG repeats (provided by Dr. Gillian Bates (31)). Lysates containing radiolabeled huntingtin were incubated with 300 ng of GST-hHAP fusion protein or GST protein. After incubation for 1 h at 4 °C, the beads were washed with the lysis buffer (0.4% Triton in PBS and protease inhibitors: 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin A) three times. Proteins bound to the beads were then eluted with SDS sample buffer, resolved by SDS-PAGE, and visualized by autoradiography. The beads containing GST-hHAP (300 ng) were also incubated with 1 ml of cell lysates of HEK 293 cells from a 10-cm culture dish for binding to native huntingtin. Huntingtin bound to the beads was detected by Western blotting with anti-hHAP antibody (26).

**Immunoprecipitation**—Immunoprecipitation was conducted using monkey brain tissues that were obtained from Yerkes Primate Center at Emory University. Fresh monkey tissues (1 g/2 ml) were homogenized in PBS buffer containing 0.4% Triton X-100 and protease inhibitors. Protein homogenates were centrifuged at 18,000 × g for 15 min at 4 °C. The supernatant was then centrifuged again at 120,000 × g for 30 min at 4 °C. The clarified supernatant (600 μl) was preincubated with protein A-Sepharose beads (50 μl of 1:1 slurry, Pharmacia) for 30 min at 4 °C. The beads were pelleted and the supernatant was then incubated with 50 μl of protein A-Sepharose beads linked with 5 μl of affinity purified anti-hHAP antibodies or rabbit anti-huntingtin antibody (26) in 0.05 M Tris, pH 7.5. Controls were run without immunoprecipitations with protein A-Sepharose beads alone or beads linked with an anti-rat HAP1 antibody that does not cross-react with hHAP on Western blot. The beads containing immunocomplexes were precipitated and washed twice with 1 ml of PBS containing 0.4% Triton X-100. The precipitated proteins were resuspended in 100 μl of SDS sample buffer and boiled for 5 min. The samples (50 μl) were resolved by SDS-PAGE and detected by Western blotting.

**Coexpression and Double Labeling of Transfected Cells**—To assemble a full-length huntingtin construct, we ligated a partial cDNA containing 44 CAG repeats (15) with cDNAs encoding the middle and C-terminal regions of huntingtin. These cDNAs were isolated from screening human brain cDNA libraries and RT-PCR using primers derived from published sequences (1). The full-length cDNA of huntingtin was inserted into the pE BHs vector (Invitrogen) for transfection of cells. Cells were transfected using LipofectAMINE (Life Technologies, Inc.) for 16–72 h, fixed in PBS containing 4% (w/v) paraformaldehyde for 15 min, permeabilized in 0.4% Triton X-100 in PBS, and preincubated with PBS containing 5% normal goat serum and 0.2% Triton X-100 for 1 h. After three washes with PBS, the cells were incubated in antibody cocktail overnight. After washing the cells, secondary antibody conjugated with either fluorescent fluorescein isothiocyanate or rhodamine (Jackson Immunoresearch) was added to the cells for 1 h at room temperature. The subcellular localization of expressed proteins was examined using fluorescence microscopy. The expressed huntingtin was detected by rat monoclonal antibody to huntingtin (1:100 dilution) (26). The transfected hHAP was detected by the rabbit antibody EM39 (1:500 dilution). For
labeling the expressed DRPLA (amino acids 450–712) in HEK 293 cells, DRPLA was tagged with a HA (human influenza hemagglutinin YPY-DVPDYA) epitope on its C terminus so it could be detected by mouse antibody 12CA5.

RESULTS

Cloning of hHAP cDNA—We previously identified two human PCR products (hHAP1 and hHLP1) that encode amino acid sequences with 96 and 80% identity to rat HAP1, respectively (15). Although genomic Southern blot analysis suggests the existence of distinct human genes for these two PCR products, Northern blot analysis and screening of cDNA libraries only identified the expression of hHLP1 (data not shown). Whether the previously isolated hHAP1 cDNA is expressed at a very low level or is the result of PCR artifact remains to be studied. In this study we have obtained 16 hHLP1 clones which we have analyzed by enzymatic digestion and sequencing. All 16 clones contain a region encoding 93 amino acids (amino acids 323–416) which has 82% identity to the huntingtin-binding region of rat HAP1. We renamed these cDNA clones hHAP (human huntingtin-associated protein) based on their homology to rat HAP1, their expression on Northern blots (see below), and their binding to huntingtin. When compared with the rat HAP1 sequence, 12 of the 16 clones have one or more insertion or deletion after the 93 amino acids. The insertion or deletion introduces sequences without any homology to rat HAP1 or shifts the reading frame and gives rise to premature stop codons. These divergent sequences may be the result of alternative splicing or incomplete splicing of introns.

All 16 clones lacked sequences homologous to the N-terminal region of rat HAP1. We thus used 5’-RACE PCR to identify the sequences encoding the N terminus of hHAP. By RACE-PCR we cloned a 5’-terminal end similar to rat HAP1 and contains a likely initiating methionine (Fig. 1). By splicing together a 5’-end fragment obtained from RACE and a cDNA clone containing the rest of the coding region, we assembled a full-length cDNA sequence. This sequence contains an open reading frame with 619 amino acids having 62% identity to rat HAP1 (Fig. 1 b) and a predicted molecular mass of 68.8 kDa. Like rat HAP1, hHAP also has a region rich in acidic amino acids (Fig. 1). Some gaps and insertions also exist in hHAP’s sequences when the sequences of hHAP and rat HAP1 are aligned. Rat HAP1 is expressed as two alternatively spliced isoforms, HAP1-A and HAP1-B, which differ at their C termini (15). The C-terminal sequence of hHAP is highly homologous to that of rat HAP1-A.
We have not identified any human cDNA encoding the amino acid sequences homologous to the C terminus of rat HAP1-B during cDNA library screening and RT-PCR.

Expression of hHAP mRNA—Northern blot analysis shows a prominent transcript of approximately 4.1 kb in human brain (Fig. 2). Its size is equivalent to that of rat HAP1 (15) and its expression is also restricted to brain tissues, suggesting that it encodes a brain-enriched protein (Fig. 2). The hHAP transcript is most abundant in subthalamic nucleus and is also enriched in amygdala, thalamus, and substantia nigra. An intermediate level of this transcript was found in caudate nucleus and hippocampus. A lower level of hHAP transcript was found in corpus callosum. Several other minor bands were also found in human brain regions, including two upper bands (between 4.4 and 7.5 kb) that were visible in the subthalamic nucleus and a lower band of 3 kb that was present in all brain regions examined. These minor bands were also seen in human heart and skeletal muscle (Fig. 2). These minor bands could represent transcript variants, homologues of hHAP, or cross-hybridization to unrelated transcripts. The same blots were probed with GAPDH cDNA probe. Considering the amounts of RNA used for the blots and the ratio of the expression level of hHAP to that of GAPDH, hHAP mRNA appears to be expressed at a level similar to that of rat HAP1 mRNA (15).

Expression of hHAP Protein—To examine the protein expression of hHAP, we developed a rabbit antibody against GST-hHAP that contains the middle portion of hHAP (amino acids 323–455). We then examined the immunoreactivity of the purified antibody in transfected cells and brain tissues. Transfected proteins were produced by transfection of HEK 293 cells with hHAP, rat HAP1-A, or HAP1-B cDNA and brain regional tissues were obtained from monkey and human brains. Western blotting demonstrated that the antibody reacted with the transfected hHAP (75 kDa) in HEK 293 cells (Fig. 3a). The antibody also recognized transfected rat HAP1-A (75 kDa) and HAP1-B (85 kDa). In addition, the antibody cross-reacted with the doublet (75 and 85 kDa) in rat brain that corresponds to rat HAP1-A and HAP1-B. More important, the antibody recognized a single major band (75 kDa) in monkey brain. This brain protein and transfected hHAP have an equivalent molecular weight, suggesting that our cloned hHAP encodes the same hHAP seen in brain (Fig. 3a). Both transfected and native hHAP migrate a little slowly in SDS gels, appearing as a 75-kDa protein rather than the predicted 68.8-kDa protein. Similarly, rat HAP1-A is observed as a band at 75 kDa instead of 66.6 kDa as predicted by its amino acid sequences. hHAP and rat HAP1 may share the same structural properties that slow their migration in SDS gels. A weak immunoreactive product of 68 kDa in monkey brain was sometimes seen and this could be an isoform or a homologue of hHAP. Preabsorption of the antibody with GST-HAP1 fusion protein could eliminate these immunoreactive bands whereas preabsorption with GST alone did not (data not shown).

We then performed Western blot analysis of monkey and human tissues to examine the regional brain distribution of hHAP. The result shows that hHAP is expressed in the cerebral cortex, striatum, cerebellum, and hippocampus (Fig. 3b). Like huntingtin, hHAP is expressed in the striatum at a level not significantly different from that in other brain regions. A weak band of 98 kDa was also seen in human brain (Fig. 3b). In some brain samples, we observed a weak immunoreactive band of 68 kDa (data not shown). Examination of seven different peripheral tissues of monkey did not reveal the expression of these proteins, although some other faint bands were occasionally seen (data not shown).

Expression of hHAP in HD Brain—Since the most affected areas in HD brain are the striatum and deep layers of the
from two normal individuals (m)ission of GAPDH was not significantly altered. GAPDH, respectively. Note that the expression of huntingtin and hHAP were immunoprobed with antibodies to huntingtin, hHAP, and huntingtin constructs containing the first 230 amino acids plus a normal repeat (23 glutamines) or expanded repeats (44 or 73 glutamines) were used to test the interaction of hHAP and huntingtin constructs containing the first 230 amino acids plus normal repeat (23 glutamines) or expanded repeats (44 or 73 glutamines) were used to test the interaction of hHAP and huntingtin. The protein interactions were examined using two different approaches: the yeast two-hybrid system and in vitro binding assays. A previous study using a PCR product that encodes hHAP amino acids 255–579 failed to show any interaction between hHAP and huntingtin in yeast. As huntingtin 44Q yielded more interaction with hHAP, the result also suggests that the interaction of hHAP with huntingtin was increased by an expanded glutamine repeat.

To confirm the above result, we performed in vitro binding assays using a GST-hHAP fusion protein containing amino acids 323–455. 35S-Labeled huntingtin was synthesized by in vitro translation of N-terminal huntingtin containing 23, 44, or 73 glutamine repeats. This assay allowed us to use equivalent amounts of these huntingtins to react with GST-hHAP or GST so that we could observe any differences in their binding associated with different lengths of the glutamine repeat. Autoradiography demonstrated that 35S-labeled huntingtin bound to GST-hHAP but not GST (Fig. 5c). We then used a PhosphorImager to quantify the amount of huntingtin bound to GST-hHAP in comparison with the input used for the binding. We observed that the binding of huntingtin to hHAP depended on the number of glutamine repeats: huntingtin with 23 glutamines (23Q) bound least GST-hHAP (11.1% of input), huntingtin with 44 glutamines (44Q) bound more GST-hHAP (17.2% of input), and huntingtin with 73 glutamine repeats (73Q) bound most GST-hHAP (22.5% of input) (Fig. 5d). This result is consistent with the data of the yeast two-hybrid assay showing that more huntingtin 44Q than 23Q bound to hHAP.

Interaction of hHAP with Full-length Huntingtin—To test the binding of hHAP to full-length huntingtin, we conducted an in vitro binding assay using HEK 293 cells that express native human huntingtin. The in vitro binding assay showed that GST-hHAP but not GST bound to native huntingtin (350 kDa) in HEK 293 cells (Fig. 6a).

The in vivo association of hHAP with full-length huntingtin was examined by immunoprecipitation of monkey cerebral cortex tissues. The anti-hHAP antibody was able to precipitate hHAP and huntingtin from monkey brain lysates (Fig. 6b). In addition, anti-huntingtin antibody also precipitated both huntingtin and hHAP. The precipitation depended on the presence of the antibodies because protein A-linked beads alone did not give any precipitation. We also used rabbit IgG and anti-hHAP antibody preadsorbed with antigen as controls. Neither of them precipitated any hHAP or huntingtin under the same conditions (data not shown). The antibody to rat HAP1 failed to precipitate hHAP or huntingtin under the same conditions (data not shown). The antibody to rat HAP1 failed to precipitate hHAP or huntingtin from monkey brain tissues (Fig. 6b). Because this antibody did not recognize the 75-kDa band in monkey or human brain on Western blots (data not shown), we believe that it has little or no cross-reactivity with HAP and thus failed to pull down the hHAP-huntingtin complex. Although we could precipitate monkey HAP and hunting-
The interaction of hHAP with N-terminal huntingtin. a, Western blot analysis of the expression of N-terminal huntingtin with 23 (23Q) or 44 (44Q) glutamine repeats in yeast. C is the control of nontransformed yeast Y190. b, liquid assay showing β-galactosidase activity of yeast which had been co-transformed with hHAP and huntingtin containing 23 or 44 glutamines. DRPLA is a polyglutamine repeat control protein and vector is the GAL4-binding domain vector. Numbers indicate amino acid positions. Values obtained from three experiments are expressed as units/min/mg of yeast protein ± S.D. c, in vitro binding of GST-hHAP to 35S-labeled huntingtin. Western blot shows equal amounts of GST-hHAP and GST used for in vitro binding. The same blot was analyzed by autoradiography showing that 35S-radio-labeled huntingtin with 23, 44, or 73 glutamine repeats bind to GST-hHAP but not GST. d, quantitative assessment by PhosphorImaging of huntingtin bound to GST-hHAP in comparison with the input of huntingtin. The data were obtained from three experiments and represented as % of input ± S.D. on the x axis.

Co-localization of hHAP and Huntingtin in Transfected Cells—Rat HAP1-A was found to co-localize with huntingtin in transfected cells (24). Thus, we co-transfected hHAP and huntingtin into HEK 293 cells and imaged the localization of hHAP and huntingtin in the transfected cells. We found that transfection of hHAP into HEK 293 cells resulted in perinuclear inclusions in many transfected cells. The inclusions were intensely labeled by anti-hHAP antibody (Fig. 7). hHAP immunoreactive inclusions vary in their size (Fig. 7a). We examined the cells that had been transfected for 16, 24, or 72 h. Although cells transfected for longer times usually have larger inclusions, not every cell overexpressing hHAP has the inclusion (Fig. 7b).

Only a single inclusion occurred in a transfected cell. In contrast, transfection of rat HAP1-A into HEK 293 cells resulted in multiple cytoplasmic and perinuclear inclusions in a cell (Fig. 7c), suggesting that the subcellular localization of rat HAP1-A and hHAP may not be identical in vivo. These inclusions have not been found in cells overexpressing other transfected proteins. In fact, overexpression of full-length huntingtin that contains 44 glutamine repeats results in diffused distribution of the transfected protein (Fig. 7d). Despite the presence of hHAP immunoreactive inclusions, most HEK 293 cells that have been transfected less than 24 h preserve normal structures of endoplasmic reticulum, Golgi, mitochondria, and other cytoplasmic organelles. Antibodies specific for the lysosomal enzyme, cathepsin D or membrane protein LAMP, and for proteins of Golgi (anti-M6-P-R), endosomes (anti-M6-P-R), or endoplasmic reticulum (anti-OPD) label the respective organelles but not hHAP immunoreactive inclusions in the transfected cells (data not shown).

While the nature of these structures remains to be identified, localization of proteins to these distinct structures provides an assay for the association of hHAP with huntingtin in living cells. A rat monoclonal antibody against human huntingtin (26) allowed us to conduct double immunofluorescent labeling with the rabbit anti-hHAP antibody. However, the affinity of this antibody to endogenous huntingtin in HEK 293 cells is not high enough to reveal the subcellular localization of native huntingtin. We thus detect the localization of transfected huntingtin and hHAP. When coexpressed with hHAP, huntingtin’s localization was altered and it was co-

![Image](https://example.com/image.png)
FIG. 7. Co-localization of hHAP and full-length huntingtin with 44 glutamine repeats in transfected HEK 293 cells. a–b, transfection of hHAP alone (16 or 72 h) resulted in a single, perinuclear inclusion per cell (arrows) while c transfection of rat HAP1 produce multiple cytoplasmic or perinuclear inclusions. These inclusions were labeled by antibodies to hHAP or rat HAP1. d, transfected full-length huntingtin (24 h) was diffusely distributed in the cytoplasm. e and f, coexpression of huntingtin and hHAP resulted in co-localization of both proteins on the hHAP immunoreactive inclusions (arrows). e, a rabbit antibody to hHAP, and f, a rat monoclonal antibody to huntingtin were used in immunofluorescent double labeling. g and h, coexpression of HA-tagged DRPLA and hHAP (24 h) did not show co-localization on the inclusions. g, the rabbit antibody to hHAP, and h, a mouse antibody (12CA5) to the HA epitope were used to recognize the transfected proteins. Scale bars, 10 μm.

localized with hHAP on the perinuclear inclusions (Fig. 7, e–f). We also observed that the N-terminal fragment of human huntingtin (amino acids 1–253 with 23Q) co-localized with hHAP in transfected HEK 293 cells (data not shown). As a control, another glutamine repeat protein DRPLA was also co-transfected with hHAP into HEK 293 cells. The overexpressed DRPLA did not co-localize with hHAP on this distinct structure (Fig. 7, g–h), suggesting that the co-localization of huntingtin and hHAP in transfected cells is specific.

DISCUSSION

Several lines of evidence in the present study demonstrate that hHAP is a human counterpart of rat HAP1. First, the sequence of hHAP shows a high degree of homology to that of rat HAP1. Second, like rat HAP1 (15, 20), primate HAP1 is enriched in monkey and human brains. In addition, hHAP appears to be expressed with huntingtin in the neurons that are vulnerable in HD. Third, in vitro binding, immunoprecipitation, and coexpression studies consistently show that hHAP specifically interacts with huntingtin, and its in vitro binding seems to be enhanced by an expanded glutamine repeat. Rat HAP1 has been also found to bind more tightly to huntingtin (15, 24). The similarity of hHAP and rat HAP1 in their sequences and binding to huntingtin suggests that hHAP and rat HAP1 bind to huntingtin in the same manner.

There are also differences between hHAP and rat HAP1. The amino acid sequences of hHAP display a number of insertions or deletions when compared with the sequences of rat HAP1. Unlike huntingtin which is highly conserved between humans and rodents (91% amino acid identity), hHAP and rat HAP1 share 62% amino acid identity overall. However, amino acids in the huntingtin-binding regions of both proteins are well conserved (82% identity), suggesting that their binding properties are similar while other functional aspects may not be identical. In addition, unlike rat HAP1 which is expressed as two isoforms (HAP1-A and HAP1-B), hHAP only displays a single major form in primate brains. The expression pattern of hHAP in transfected cells is also different from that of rat HAP1-A. These differences may reflect unique properties of hHAP that could confer a specific role of the interaction of HAP1 and huntingtin in human brain.

Several hHAP cDNA variants were found during cDNA screening. Most of them have insertions or deletions after the putative huntingtin-binding region. This may explain why no one has succeeded in isolating hHAP using yeast two-hybrid screening. However, the hHAP amino acid sequence presented in Fig. 1 is likely to represent the major protein product of the hHAP gene. This is because the cDNA probe and the antibody to the middle region of cloned hHAP consistently detect a major band on Northern and Western blots, respectively. Also, the mass of transfected hHAP is the same as that of native hHAP on Western blots. Several other weak bands are also seen. These weak bands could represent alternatively spliced products or other homologues of hHAP. Whether other hHAP cDNA variants are also expressed remains to be studied using antibodies specifically to their encoding amino acids.

Identification of hHAP further suggests a potential role of HAP1 in the pathology of HD. First, the parallel reduction in the expression of huntingtin and hHAP in the HD brains suggests that both proteins are expressed in the same neurons that are vulnerable in HD. However, the expression of hHAP is not restricted to the brain regions that can be affected in HD. The selective neurodegeneration in HD may also be associated with heterogeneous expression of huntingtin in the striatum (32) and/or other specific neuronal factors. Second, like rat HAP1, hHAP binds to the N-terminal region of huntingtin in vitro. Proteins that bind to the N-terminal region of huntingtin are especially interesting because the N-terminal fragment of huntingtin (amino acids 1–67) has been found to cause neurological disorders in transgenic mice (31, 33). We observed that the binding of the N-terminal huntingtin (amino acids 1–230) with the glutamine repeat (23Q, 44Q, or 73Q) to GST-hHAP is increased by lengthening the repeat. Although the increase is not dramatic in vitro, it could contribute to cumulative effects of huntingtin mutation or late onset of pathophysiology if it also occurs in vivo. The interaction of hHAP and huntingtin is also supported by their co-localization in hHAP immunoreactive inclusions in transfected cells. Their co-localization is specific because DRPLA, another glutamine repeat protein, does not co-localize with hHAP on these distinct structures. Rat HAP1 has been found to associate with similar cytoplasmic structures in the rat brain.2

2 C. A. Gutekunst, S-H. Li, X-J. Li, and S. M. Hersch, unpublished observations.
Whether human brain also contains hHAP immunoreactive cytoplasmic inclusions remains to be studied.

Unique components or specific properties of human huntingtin complexes may account for the neuropathology of HD in man. This is suggested by recent findings that transgenic mice expressing mutant human huntingtin do not display the graded loss of neurons in brain (8, 31, 33) that is the hallmark of human HD disease (5–7). It is possible that expansion of the huntingtin glutamine repeat may alter specific properties of human HD disease (5–7). It is possible that expansion of the huntingtin repeat may alter specific properties of human huntingtin. This is suggested by recent findings that transgenic mice containing exon 1 of the human HD gene with 150 CAG repeats. We thank Dr. Richard Kahn for providing antibodies and Dr. Nathan H. Canadian-American Society for Human Molecular Genetics.

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