Interaction of Huntingtin-Associated Protein with Dynactin P150Glued

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Huntingtin is the protein product of the gene for Huntington's disease (HD) and carries a polyglutamine repeat that is expanded in HD (>36 units). Huntingtin-associated protein (HAP1) is a neuronal protein and binds to huntingtin in association with the polyglutamine repeat. Like huntingtin, HAP1 has been found to be a cytoplasmic protein associated with membranous organelles, suggesting the existence of a protein complex including HAP1, huntingtin, and other proteins. Using the yeast two-hybrid system, we found that HAP1 also binds to dynactin P150Glued (P150), an accessory protein for cytoplasmic dynein that participates in microtubule-dependent retrograde transport of membranous organelles. An in vitro binding assay showed that both huntingtin and P150 selectively bound to a glutathione transferase (GST)–HAP1 fusion protein. An immunoprecipitation assay demonstrated that P150 and huntingtin coprecipitated with HAP1 from rat brain cytosol. Western blot analysis revealed that HAP1 was enriched in rat brain microtubules and colocalized with P150 and huntingtin in sucrose gradients. Immunofluorescence showed that transfected HAP1 colocalized with P150 and huntingtin in human embryonic kidney (HEK) 293 cells. We propose that HAP1, P150, and huntingtin are present in a protein complex that may participate in dynein–dynactin-associated intracellular transport.

Key words: Huntington's disease; huntingtin; dynactin; microtubule; intracellular transport; targeting

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MATERIALS AND METHODS

Yeast two-hybrid system. All experiments were performed with the yeast strain Y190. The yeast two-hybrid screen was conducted as described
previously (Fields and Song, 1989; Li et al., 1995) to isolate HAP1-associated proteins (HAPs). Full-length HAP1-A, an isof orm of HAP1, fused to the GAL-4 DNA-binding domain was used as a bait to screen a rat brain cDNA library (Li et al., 1995). Transformed yeast cells were grown in an 500 mM 0.6 – 0.8. Five milliliters of culture was pelleted, washed once with 1 ml of resuspended pellets at 10 mM concentration, and the extraction was performed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). In some cases, the gel was stained to confirm the leaky expression of the His phenotype. Positive colonies were identified by filter assays of β-galactosidase (β-gal) activity (Li et al., 1995). cDNAs from these positive colonies appearing within 120 min were rescued for retransformation of fresh yeast cells and confirmation of the interactions of these cloned proteins with HAP1.

Three HAP1 constructs containing different fragments between the middle region and the C terminus (amino acids 278–599) were fused to the GAL-4 activation domain in pPC86 vector to examine their interactions with huntingtin, DRPLA, and C-Jun proteins in pPC97 vector (Chevray and Nathans, 1992). These HAP1 fragments were also fused to the GAL-4 DNA-binding domain in pPC97 vector to test their interactions with P150 and other cloned HAP1 proteins in pPC86 vector. The N-terminal fragment (amino acids 1–253) of huntingtin containing 23 glutamine repeats was used. DRPLA is a glutamine-repeat protein product of the gene for dentatorubral and pallidoluysian atrophy (Nagafuchi et al., 1994) with a 21 glutamine repeat and was used as a control in a parallel experiment (Li et al., 1995). The specificity of purified EM49 was found to be comparable with that of the well characterized anti-P150 antibody UP235 (provided by Dr. Holzbaur, University of Pennsylvania; Tokito et al., 1996). Affinity-purified anti-P150 antibody was used to synthesize [35S]methionine-labeled P150 with the binding buffer, proteins bound to the beads were resolved by 8% SDS-PAGE and visualized by autoradiography. Quantitative assessment of protein in the gel was performed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). In some cases, the gel was stained with Coomassie brilliant blue to visualize the GST fusion proteins present in each track.

**Antibody production.** Two isoforms of HAP1, termed HAP1-A and HAP1-B, have been isolated. They differ in the sequences at their C termini; HAP1-A has 21 amino acids at the C terminus that differ from the 51 amino acids at the C terminus of HAP1-B (Li et al., 1995). Anti-peptide antibodies for HAP1 (EM1 and EM2) were raised against the C-terminal sequences of these two rat HAP1 isoforms (Li et al., 1995). SRPQHPAPSAGSYRSLT for HAP1-A and ATHTSPAR-EEEGPSGAT for HAP1-B. These peptides were conjugated with BSA to serve as immunogens for Covance Inc. (Denver, PA) to produce rabbit antisera. Anti-peptide antibodies were purified using affinity columns linked with immunogen (Li and Snyder, 1995). A fragment of rat P150 (amino acids 1023–1223) was used to produce GST–P150 fusion protein that served as immunogen to produce rabbit antibodies (EM49). The anti-P150 antibody was purified by incubation of the whole serum with a nitrocellulose strip containing electrophoretically purified GST–P150. After multiple washes, antibodies were eluted with 0.2 mM Gly, pH 2.15, for 10 min and immediately neutralized by addition of 1.5 mM Tris-HCl, pH 8.8. The specificity of purified EM49 was found to be comparable with that of the well characterized anti-P150 antibody UP235 (provided by Dr. Holzbaur, University of Pennsylvania; Tokito et al., 1996). Affinity-purified anti-P150 antibody was used to synthesize [35S]methionine-labeled P150 with the binding buffer, proteins bound to the beads were resolved by 8% SDS-PAGE and visualized by autoradiography. Quantitative assessment of protein in the gel was performed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). In some cases, the gel was stained with Coomassie brilliant blue to visualize the GST fusion proteins present in each track.

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rocked for 15 min at room temperature. The control was the microtubule pellets without ATP extraction. The extracted microtubules were then pelleted again, and the pellets were resuspended in 2 ml of PIPES buffer. The supernatant (100 μl) and pellet suspension (40 μl) were analyzed by 7.5% SDS-PAGE.

Coexpression and double labeling of transfected cells. Human embryonic kidney (HEK) 293 cells were used for cotransfection studies. To double label HAP1 and huntingtin that were coexpressed in HEK 293 cells, we tagged huntingtin (amino acids 1-253) containing 23 glutamine repeats with an HA epitope (YPYDVPDYA) at its C terminus so the mouse anti-HA antibody 12CA5 could recognize the transfected huntingtin, whereas rabbit polyclonal antibody to HAP1 could recognize transfected HAP1-A. To double label coexpressed HAP1-A and full-length P150, we tagged the C terminus of HAP1-A with the HA epitope to allow recognition by mouse antibody 12CA5. The addition of the HA epitope to HAP1-A did not alter the subcellular localization of the expressed HAP1-A in transfected cells. HEK 293 cells in chamber slides (Nalge Nunc., Naperville, IL) were cotransfected with HAP1-A and huntingtin or P150 (1–2 μg of cDNA for each) using lipofectin for 24–36 hr. The cells were then fixed with 4% paraformaldehyde in PBS for 15 min, permeabilized with 0.4% Triton X-100 in PBS for 30 min, preincubated with PBS containing 5% normal goat serum for 1 hr, and then incubated with specific primary antibodies in PBS containing 2% normal goat serum overnight. In general, rabbit polyclonal antibodies were used at 1:1000 dilution, and the mouse monoclonal antibody 12CA5 was used at 1:100 dilution for incubation with transfected cells. After the cells were washed with PBS three times, fluorescent FITC- or rhodamine-conjugated secondary antibodies (1:200 dilution; Jackson ImmunoResearch, West Grove, PA) were then added to the cells, and the expressed proteins were localized using fluorescence microscopy.

RESULTS

Interaction of HAP1 with P150 and huntingtin in yeast

To investigate whether HAP1 binds to proteins of known function, we fused full-length HAP1-A to the GAL-4 DNA-binding domain and screened a rat brain cDNA library using the yeast two-hybrid system. HAP1-A is a HAP1 isoform and has 21 amino acids at the C terminus that differ from those (51 amino acids) of HAP1-B (Li et al., 1995; Martin et al., 1996; Sharpe et al., 1997), the interaction between HAP1 and P150 was chosen for further characterization. The yeast two-hybrid screen suggests that HAP1 binds to several proteins. Analysis of the HAP1 protein using the Coils program (Lupas et al., 1991) predicted a coiled coil structure in

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Filter assay of β-galactosidase activities of yeast Y190 containing respective HAP1 and other protein constructs. For plasmid constructs, see Materials and Methods. (+) indicates a positive reaction, whereas (–) indicates a negative reaction. Numbers in parentheses indicate amino acid positions.

HAP1 between amino acids 120 and 380. The coated coil is also present in various microtubule-binding proteins including P150 from amino acids 200 to 550 and from 920 to 1020. To verify the specificity of the interactions between HAP1 and associated proteins in yeast, we examined the interactions of different HAPI fragments (between amino acids 278 and 599) with human huntingtin (amino acids 1–253), P150 (amino acids 1023–1223), HAPA-10, c-Jun (amino acids 250–334), and DRPLA (amino acids 450–712) (Table 1). The N-terminal huntingtin contained 23 glutamine repeats. c-Jun is a leucine zipper protein containing a coiled coil, and DRPLA is a polyglutamine (21 glutamine) protein that also contains a coiled coil. The result showed that all HAPI fragments interacted with huntingtin, suggesting that the shortest HAPI fragment (amino acids 278–370) contains the site for binding to huntingtin. P150 did not interact with the shortest fragment but did bind to two other HAPI fragments (amino acids 278–445 and 278–599). Thus, P150 seems to bind to a different region of HAPI, which may be between amino acids 370 and 445.

HAPI-10 only interacted with the longest HAPI fragment that included the C terminus of HAP1-A. In contrast, we observed no interaction of any of these HAPI fragments with c-Jun or DRPLA. These results suggest that the binding of HAP1 to huntingtin and P150 is selective.

Previous studies using HAP1 (1–445) demonstrated that HAP1 bound more huntingtin 44Q than 23Q (Li et al., 1995; Kalchman et al., 1997). As the shorter fragment of HAPI (278–370) was sufficient to bind to huntingtin, we quantitatively tested the binding of this fragment to huntingtin containing 23 or 44 glutamine repeats using a liquid assay. We observed that the interaction of HAPI (278–370) with huntingtin 44Q yielded more β-galactosidase activity (237.2 units/min/mg of protein) than did the interaction with huntingtin 23Q (119.6 units/min/mg of protein) (Table 2). Western blot analysis showed that huntingtin 44Q and 23Q were expressed at similar levels in yeast (data not shown). In the controls, the liquid assay showed background levels (8 and 12 units/min/mg of protein) of β-galactosidase activities for the interaction of HAPI with DRPLA and c-Jun.
In vitro binding of HAP1 to P150 and huntingtin

Because yeast two-hybrid assays are prone to artifacts, we performed an in vitro binding assay with GST–HAP1 (amino acids 278-599) (Fig. 1a). We used [35S]methionine-labeled full-length P150 in the binding assay so we could test the direct interaction between these proteins. To examine whether huntingtin and P150 could simultaneously bind to HAP1, we also generated 35S-labeled, N-terminal huntingtin containing 23Q or 44Q (Fig. 1b) and used them in the binding assay along with P150. P150 alone or mixed with huntingtin 23Q or 44Q was incubated with GST–HAP1 or GST (Fig. 1c). By autoradiography, we observed that both P150 and huntingtin bound to GST–HAP1 but not to GST alone. Quantitative assessment of the amounts of proteins bound to GST–HAP1 was then performed using a phosphorimager (Fig. 1d). In comparison with the input of proteins before the binding, more huntingtin 44Q (14.2% of input) appeared to bind to GST–HAP1. The amount of P150 bound to the GST–HAP1 (17.6% of input) seemed to be slightly higher than that of huntingtin. However, this binding was decreased in the presence of huntingtin 23Q (15.1% of input) and huntingtin 44Q (11.2% of input). Equal amounts of GST–HAP1 were used in each reaction. Thus the results suggest that these three proteins could form a protein complex in vitro and perhaps huntingtin, particularly with 44 glutamine repeats, may inhibit the binding of GST–HAP1 to P150.

Comigration of HAP1, huntingtin, and P150

To examine whether HAP1 associates with P150 and huntingtin in vivo, we conducted immunoprecipitations with antibodies to HAP1 and P150. Because the middle portion of HAP1 was found to bind to both huntingtin and P150, we generated anti-peptide antibodies against the C-terminal regions of HAP1 and expected that these antibodies might more efficiently precipitate the HAP1 complex. Western blots showed that these antibodies specifically reacted with both HAP1 isoforms (75 kDa for HAP1-A and 85 kDa for HAP1-B) in rat brain and in transfected cells (Fig. 2a). A rabbit polyclonal antibody (EM49) to P150 was also produced and found to react specifically with transfected P150 in HEK 293 cells and with a doublet at ~150/135 kDa in rat brain (Fig. 2b). The doublet represented isoforms of P150 that have been revealed by other anti-P150 antibodies (Tokito et al., 1996). Immunoprecipitation of rat brain cytosol with anti-HAP1 antibodies was then conducted, and the precipitated proteins were separated by SDS-PAGE. By Coomassie blue staining of the gel, we observed two weak bands of molecular weights corresponding to those of HAP1-A and HAP1-B in the sample precipitated by anti-HAP1 antibodies (Fig. 2c). However, it is difficult to define the other weak bands in the HAP1 precipitate. We then performed immunoblots and demonstrated the coprecipitation of HAP1, P150, and huntingtin by anti-HAP1 antibodies (Fig. 2c). Precipitation of P150 and huntingtin apparently depended on the presence of HAP1 because preincubation of these anti-peptide antibodies with the peptides (20 μg/ml) eliminated the precipitation for HAP1 as well as P150 and huntingtin. Controls using protein A-Sepharose beads alone or beads linked with rabbit IgG did not show any significant precipitation of HAP1, P150, or huntingtin. We also probed the blot with antibodies to other proteins, including nNOS, GTP-binding protein Rab2, and ubiquitin, but did not find these proteins in the immunoprecipitates. These results indicate a specificity of the coprecipitation of HAP1, P150, and huntingtin.

Comigration of HAP1, huntingtin, and P150 in sucrose gradients

Immunoprecipitation suggested that soluble forms of these proteins are involved in a protein complex in the cytosol. We therefore performed sucrose gradient fractionation of rat brain cytosol. This method separates protein complexes based on their deni-
ties. Rat brain cytosol was fractionated on a linear sucrose gradient (5–20%) and analyzed by Western blotting (Fig. 3). P150 has been found to peak at 20S (from fraction 13 to 15) in a 5–20% sucrose gradient (Paschal et al., 1993). We found that both P150 and HAP1 peaked in fractions 13–15 with nearly identical profiles. The distribution of huntingtin covered a relatively broad region from fraction 9 to 16. However, the highest concentration of huntingtin was also found between fractions 12 and 15. The comigration of these proteins in the gradient supports the idea that these proteins may be involved in the same protein complex in the cytosol.

Sediment of HAP1 in microtubule pellets

Because the dynactin complex can be purified by ATP extraction of microtubules and because the binding of dynactin P150 to microtubules is decreased by ATP (Waterman-Storer et al., 1995; Tokito et al., 1996), we examined whether HAP1 is associated with microtubules and whether this association is also regulated by ATP. We prepared microtubule pellets by sedimentation of polymerized microtubules from rat brain cytosol. Both HAP1 isoforms (HAP1-A and HAP1-B) cosedimented with endogenous rat brain microtubules (Fig. 4a,b). HAP1 and P150 were enriched in microtubules compared with huntingtin that was more concentrated in the eluate than in the pellets. Of two major isoforms of dynactin (150 and 135 kDa), only the 150 kDa polypeptide was preferentially associated with microtubules, consistent with a recent report (Tokito et al., 1996). However, much less HAP1 than P150 was released into the eluate after extraction of microtubule pellets with 10 mM ATP (Fig. 4). Thus, although HAP1 also associates with microtubules, this association seemed to be less sensitive to ATP extraction than that of P150.

Figure 2. Immunoprecipitation of rat brain proteins with anti-HAP1 antibodies. a, Anti-HAP1 antibodies specifically recognized transfected HAP1 (HAP1-A and HAP1-B) in HEK 293 cells. The control was untransfected cells. The antibodies also reacted with two bands (75 and 85 kDa) in rat brain that corresponded to HAP1-A and HAP1-B, respectively. b, Rabbit polyclonal antibody to P150 specifically reacted with transfected P150 in HEK 293 cells and with polypeptides of 150 and 135 kDa in rat brain. The control was untransfected HEK 293 cells. c, Rat brain cytosolic extracts were immunoprecipitated using anti-HAP1 antibodies (anti-HAP1). A Coomassie blue-stained SDS-polyacrylamide gel (8%) containing immunoprecipitates is shown. The same immunoprecipitates were also resolved by 6% SDS-PAGE, and the blot was cut to strips that were probed with antibodies to huntingtin, P150, and HAP1. Lysates were brain extracts before immunoprecipitation. The controls were immunoprecipitations with immunogen-preadsorbed HAP1 antibody (peptide), rabbit IgG (anti-IgG), and protein A-agarose beads alone (+ beads).

Figure 3. Comigration of HAP1 with P150 in a sucrose gradient. Fractionation of rat brain cytosolic fraction (S3) through a 5–20% sucrose density gradient. Sixty microliter samples from each of 16 fractions were resolved by 7.5% SDS-PAGE. The blot with transferred proteins was probed with antibodies to huntingtin, P150, and HAP1. H is rat brain cytosolic extract before the gradient fractionation.
subcellular localization was changed, and it was colocalized with other proteins in transfected cells.

Similarly, the N-terminal huntingtin or full-length huntingtin protein association in vivo allowed examination of the colocalization of HAP1 and its associated proteins in transfected cells. Because the antibodies we used were all rabbit polyclonal antibodies, we could not examine the colocalization of these proteins in the brain. Therefore we expressed HA-tagged HAP1 or huntingtin in HEK 293 cells so a mouse monoclonal antibody to the HA epitope could be used for immunofluorescent double labeling. Expressed N-terminal huntingtin (Fig. 5a) or full-length P150 (Fig. 5b) were diffusely distributed in the cytoplasm in transfected HEK 293 cells. In addition, transfection of P150 into the cells produced P150 decoration on thick, wavy bundle-like structures similar to those observed by Waterman-Storer et al. (1995) in the Rat-2 cell line. These bundle-like structures are thought to be formed by grouped microtubules (Waterman-Storer et al., 1995). Transfection of HAP1-A isoform alone into HEK 293 cells, however, resulted in HAP1-immunoreactive granular structures in the cytoplasm (Fig. 5c). Overexpression of HAP1-B or other proteins under the same conditions did not display such structures (data not shown). Similar HAP1 immunoreactive structures (0.5–5 μm in diameter) in the rat brain were also observed and appeared to be cytoplasmic inclusions (C. A. Gutekunst, S.-H. Li, X.-J. Li, S.M. Hersch, unpublished observations). While the nature of these structures is being studied, the unique and granular shapes of these structures allowed examination of the colocalization of HAP1 and its associated proteins in transfected cells.

We cotransfected HAP1-A with HA-tagged huntingtin into HEK 293 cells. The expressed huntingtin was precisely colocalized with HAP1-A to the granular or punctate-like structures (Fig. 5e). To confirm the specificity of these colocalizations, we cotransfected HAP1-A with HA-tagged DRPLA, another glutamine-repeat protein that does not interact with HAP1 (Li et al., 1995). DRPLA did not colocalize with HAP1-A in the cytoplasmic structures (Fig. 5f). We also coexpressed HAP1-A with GST protein or with another polyglutamine-repeat protein, ataxin-1 (Orr et al., 1993). None of them was found to localize with HAP1-A to these granules (data not shown).

**DISCUSSION**

The present study demonstrates that HAP1 interacts with dynactin P150 and huntingtin, thus implying the presence of a protein complex that includes huntingtin, HAP1, and P150. This conclusion is supported by the following findings: (1) P150 and huntingtin specifically interact with HAP1 in yeast; (2) P150 and huntingtin simultaneously bind to GST–HAP1 in vitro; (3) a protein complex containing these three proteins is precipitated by anti-HAP1 antibody and is also present in the same fractions of sucrose gradients; and (4) HAP1 colocalizes with P150 and huntingtin in transfected cells, suggesting that they do associate in vivo. Because dynactin P150 participates in dynein-mediated intracellular organelle or vesicle transport, we propose that this protein complex is involved in the coupling of the dynein–dynactin complex to intracellular organelles or structures and that the function of huntingtin may be associated with intracellular trafficking.

Consistent with the above idea, huntingtin has been found to associate with a variety of membranous organelles and synaptic vesicles (DiFiglia et al., 1995; Gutekunst et al., 1995; Sharp et al., 1995). Huntingtin has also been found to bind to various proteins including glyceraldehyde phosphate dehydrogenase (GAPDH) (Burke et al., 1996), an unidentified calmodulin-associated protein (Bao et al., 1996), a ubiquitin-conjugating protein (HIP2) (Kalchman et al., 1996), epidermal growth factor (EGF) receptor-signaling complexes (Liu et al., 1997), and a protein homologous to the yeast cytoskeleton-associated protein Sla2p (HIP1) (Kalchman et al., 1997; Wanker et al., 1997). Therefore,
huntingtin may be involved in various protein complexes. The cellular and subcellular localization of HAP1 has been found to be similar to that of huntingtin (Gutekunst, Li, Hersch, unpublished observations). Immunogold electron microscopy showed that HAP1 is associated with microtubules and many types of membranous organelles, including mitochondria, endoplasmic reticulum, tubulovesicles, endosomal/lysosomal organelles, and synaptic vesicles (Gutekunst, Li, Hersch, unpublished observations). HAP1 has also been found to associate with the mitotic spindle apparatus (Martin et al., 1997) and large dense-core vesicles in pheochromocytoma (PC12) cells (Sharp et al., 1997). Yeast two-hybrid assays suggest that HAP1 interacts with P150 and other unknown proteins. The interaction of HAP1 with dynactin has also been observed by other investigators (Engelender et al., 1997). Binding of HAP1 to various proteins may be necessary for HAP1 to associate with intracellular organelles such as microtubules and the granular structures seen in transfected cells. However, the HAP1 construct used in the binding assay contains a partial α-helical coiled coil that could also mediate nonspecific protein interactions in yeast. By testing the binding of HAP1 to various proteins that contain coiled coil structures, we observed that HAP1 did not interact with c-Jun and DRPLA that also contain a coiled coil structure (Li et al., 1995). Moreover, different regions of HAP1 mediated the binding of HAP1 to huntingtin, P150, or other proteins in yeast. Therefore, it is unlikely that the binding
of HAP1 to P150 results from a nonspecific interaction of the coiled coils. Instead, HAP1 may be a multifunctional polypeptide with distinct domains for interacting with various proteins.

Because yeast two-hybrid assays suggest that N-terminal huntingtin (amino acids 1–253) binds to the region of HAP1 (amino acids 278–370) that was unable to interact with P150, huntingtin and P150 may bind to different regions of HAP1 and thus form a stable protein complex. The N terminus of huntingtin was used to characterize its binding to HAP1 because it contains the polyglutamine repeat and could be expressed in yeast and in vitro. Moreover, the N-terminal human huntingtin containing an expanded glutamine repeat (>115 units) was sufficient to induce a progressive neurological phenotype in transgenic mice (Mangiarini et al., 1996). An in vitro binding assay showed that the N terminus of huntingtin and P150 could be precipitated by GST-HAP1. It is interesting to note that huntingtin, especially the huntingtin with 44 glutamine repeats, seemed to decrease the binding of P150 to HAP1. It remains to be shown whether an expanded polyglutamine repeat alters the association of HAP1 with P150 in vivo.

A protein complex containing HAP1, P150, and huntingtin in vivo is suggested by several lines of evidence. Immunoprecipitation showed that huntingtin and P150 were coprecipitated with HAP1 from rat brain. The nearly identical migrations of cytosolic HAP1 and P150 in a sucrose gradient further supports this suggestion. Because P150 associates with a dynein protein complex that can be isolated by ATP extraction of microtubules (Gill et al., 1991; Paschal et al., 1993; Schafer et al., 1994), we also examined the association of HAP1 with huntingtin and microtubules in the absence and presence of ATP. Although HAP1 was found to be as enriched as P150 in microtubules, ATP extraction of microtubules liberated less HAP1 than P150 in the ATP eluate. Therefore, unlike other substoichiometric components in the ATP-released dynactin complex (Gill et al., 1991; Paschal et al., 1993; Schafer et al., 1994), HAP1 may be a minor form in this complex. The association of HAP1 and P150 could mainly occur on membranous organelles, microtubules, and/or in the cytosol. The lower sensitivity of HAP1 to ATP extraction suggests that HAP1 may not directly or tightly bind to the dynein protein complex under these conditions. The enrichment of HAP1 in microtubules may be because of some direct binding of HAP1 to microtubules or to other molecules associated with microtubules.

Previous studies using immunocytochemistry demonstrated that huntingtin is associated with microtubules (DiFiggia et al., 1995; Gutekunst et al., 1995; Bhide et al., 1996). However, we found that huntingtin was not as enriched as HAP1 in microtubule pellets. It is possible that the association of huntingtin with microtubules is via its binding to HAP1 and is therefore not as stable as the binding of HAP1 and P150 to microtubules in vitro. Huntingtin may also be involved in protein complexes other than the HAP1–dynactin complex, as suggested by its relatively wide distribution in the sucrose gradient.

The distinct localization of P150 and huntingtin on HAP1 immunoreactive granular structures in transfected cells further suggests that these proteins may associate in vivo. Their colocalization was selective because another polyglutamine-repeat protein, DRPLA, did not colocalize with HAP1 on these structures. Overexpressed proteins in transfected cells may not display the same subcellular localization as they do in vivo; however, these HAP1-A-induced cytoplasmic structures in transfected cells enabled us to examine the protein colocalization in living cells. Although the nature of these structures remains to be defined, imaging the protein colocalization on these structures is especially helpful for confirming the binding results obtained from the yeast two-hybrid screen, in vitro binding, and immunoprecipitation. Because the definitive subcellular distribution of P150 in vivo has not been obtained, extensive studies are required to confirm whether HAP1 and P150 also colocalize in the brain.

The association of HAP1 with P150 and huntingtin provides a possible link between intracellular transport and the function of normal huntingtin. This is because the dynactin complex is required for dynein-mediated vesicle movement in vitro and the function of P150 is thought to be the targeting of dynein motor proteins to intracellular organelles (Gill et al., 1991; Schroer and Sheetz, 1991). Targeting of microtubule motor proteins to membranous organelles may involve a number of different proteins that dynamically associate with a variety of vesicles or organelles. In addition, huntingtin could be intracellularly transported by the HAP1–dynactin complex. Given that the dynactin P150 protein complex plays a role in the targeting or transporting of intracellular organelles or molecules, it is possible that HAP1 and normal huntingtin may have a role in intracellular trafficking.

REFERENCES


