Perinuclear localization of huntingtin as a consequence of its binding to microtubules through an interaction with β-tubulin: relevance to Huntington's disease

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Summary

Huntington's disease results from an expansion of a series of glutamine repeats in the protein huntingtin. We have discovered from immunopurification studies that huntingtin combines specifically with the β subunit of tubulin. This binding explains why huntingtin can be shown on assembled microtubules by electron microscopy. Immunostaining shows that most of the huntingtin in the cytoplasm is associated with microtubules. Huntingtin is particularly abundant in the perinuclear region, where it is also associated with microtubules and in the centrosomal region, where it co-localizes with γ-tubulin. In Huntington's disease, inclusions are often nuclear or perinuclear. Since the perinuclear concentration of huntingtin does not depend on the number of its glutamine repeats, we propose that inclusions are found in perinuclear and intranuclear locations because the β-tubulin binding property of huntingtin brings it to the perinuclear region, from which it readily gains access to the nucleus. The mutational glutamine expansion then promotes insolubility and results in an inclusion.

Key words: Polyglutamine, Huntington's disease, Inclusions, Centrosomes, Cytoskeleton, Perikaryon

Introduction

Huntingtin bears a series of glutamine repeats close to its N-terminus and expansion of the polyglutamine sequence beyond 35-40 repeats results in Huntington's disease (Huntington's disease collaborative research group, 1993). Eight other diseases of the central nervous system, each associated with a different protein containing an expanded polyglutamine sequence are known (David et al., 1997; Imbert et al., 1996; Kawaguchi et al., 1994; Koide et al., 1994; La Spada et al., 1991; Nielsen et al., 1997; Orr et al., 1993; Pulst et al., 1996; Sanpei et al., 1996; Zhuchenko et al., 1997). The expanded polyglutamine produces a dominant gain of function in neuronal proteins and results in cell lethality. This gain of function is probably the formation of aggregates or inclusions containing the protein with expanded polyglutamine, such as have been described in regions of the brain affected by Huntington's disease (Davies et al., 1997; DiFiglia et al., 1997; Paulson et al., 1997; Skinner et al., 1997). Inclusions are often nuclear or perinuclear (DiFiglia et al., 1997; Meriin et al., 2001; Waelter et al., 2001). Although it has been difficult to establish with any precision the number and location of inclusions sufficient to kill a cell, it appears likely that aggregates have a causative role in neuronal death (Scherzinger et al., 1999; Perutz, 1999).

We show that huntingtin specifically interacts with β-tubulin and binds to microtubules. This explains why immunoelectron microscopy of neurons had shown that huntingtin appeared to be colocalized with microtubules (Gutekunst et al., 1995). Huntingtin is concentrated in the perinuclear region, where it associates with microtubules and in the centrosome. The perinuclear distribution of huntingtin is likely to explain why the inclusions characteristic of Huntington's disease are mostly nuclear or perinuclear.

Materials and Methods

Antibodies

For immunopurifications we used a monoclonal anti-huntingtin antibody (MAB 2166, Chemicon) to a fragment of the protein (amino acid residues 181-810) located outside the polyglutamine (Trottier et al., 1995) and a monoclonal anti-β-tubulin antibody (3F3G2, ICN). For western blots the following antibodies were used: anti-huntingtin (MAB 2166), anti-β-tubulin (3F3G2), anti-α-tubulin (B-5-1-2, Sigma), anti-MAP-2 (MAB 364, Chemicon), anti-NFM (MAB 1621, Chemicon), anti-NFL (MAB 1615, Chemicon), anti-kinesin (MAB 1614, Chemicon), anti-actin (MAB 1501, Chemicon) and anti-caspase-1 (Upstate biotechnology).

For immunocytochemistry we used five anti-huntingtin antibodies: two polyclonal antibodies prepared by immunizing rabbits against the first 17 amino acid residues of human huntingtin and affinity purified using the antigen (QCB, Hopkinton, MA), MAB 2166 and two polyclonal antibodies (HF1 and HP1) generated by Persichetti et al. (Persichetti et al., 1995). One monoclonal anti-β-tubulin (3F3G2) and one monoclonal anti-γ-tubulin (GTU 88, Sigma) were also used.

Preparation of extracts from lymphoblasts and brain

Protein extracts containing normal (Q10) and expanded (Q80) huntingtin were prepared from lymphoblastoid line SALVAL 2050013. Cell suspension (50 ml) was centrifuged at 800 ***g*** for 5 minutes at 4°C. The pellet was washed twice with 30 ml of cold phosphate buffer saline (PBS) and resuspended in 300 µl of hypotonic buffer containing 50 mM Tris-HC1 pH 7.8, 10% glycerol, 10 mM EDTA, 5 mM KC1, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 0.1 mM leupeptin. After a 10 minute incubation on ice, cells were disrupted by sonication.

For preparation of brain extracts, cortex (50 mg) was homogenized in 500 µl of the buffer described above, using a motor-driven Teflon pestle (13 strokes at 900 rpm). The protein concentration was determined with a Bio-Rad protein assay kit using IgG as standard.

Immunopurification with magnetic beads

The anti-huntingtin (MAB 2166, Chemicon) or the anti-β-tubulin (3F3G2, ICN) were crosslinked to an anti-IgG antibody, itself covalently coupled to magnetic beads by the manufacturer (Dynal). Chemical crosslinking of the anti-huntingtin or the anti-β-tubulin antibody to the anti-IgG antibody was necessary. In the absence of such crosslinking, the two antibodies used for immunopurification elutes from the magnetic beads together with huntingtin and are stained by the secondary anti-mouse antibody that is used in conjunction with the monoclonal anti-tubulin antibody during the western blot. Because the size of the IgG heavy chain is practically identical to that of tubulin, the two proteins cannot be resolved by the electrophoresis.

Magnetic beads ($2.67.10^7$) were incubated overnight at 4°C in the presence of 16 µl of specific antibody (8-40 µg IgG/µl) in PBS containing 0.1 mM leupeptin, 1 mM PMSF and 0.1% Tween 20. The excess of specific antibody was removed by washing the beads six times in the same buffer using a magnetic particle concentrator (Dynal).

For crosslinking of the specific antibody to the anti-mouse IgG, the beads were washed twice in 0.2 M triethanolamine (TEA) pH 9.0, then incubated in the same buffer containing 20 mM dimethylpimelimidate dihydrochloride (Sigma) for 45 minutes at room temperature. Crosslinking was stopped by removing the beads from the buffer containing the crosslinker. The beads were then incubated for 10 minutes at 4°C in TEA buffer containing 1% Triton, and washed three times in PBS-Tween buffer.

For immunopurification, the lymphoblast or brain extracts (100 µg of protein) were incubated in the presence of the beads for 2 hours 30 minutes at 4°C. After six washes in the PBS-Tween buffer, the immunopurified product was eluted from the beads by vortexing in 35 µl of a solution containing 2 M NaI and 50 mM Tris-HCl pH 9 for 1 minute. The magnetic beads were concentrated with a magnet, the eluate was recovered and the few remaining beads were removed with the magnetic particle concentrator. The entire eluate derived from 100 µg of extract was then analyzed by western blotting.

Western blots

For analysis of huntingtin, proteins (100 µg) were subjected to electrophoresis according to Laemmli (Laemmli, 1970) using a 4% acrylamide plus bisacrylamide stacking and resolving gels (ratio 29:1 for both gels). Electrophoresis was at 18.2 V/cm for 2 hours. For analysis of tubulins, proteins (50 µg) were subjected to electrophoresis using an 8% polyacrylamide gel. Immunoblotting was carried out exactly as described (Kahlem et al., 1998). All antibodies used were absolutely specific as they detected a single protein with the appropriate molecular weight. Bands on films were quantitated with an AGFA digital light sensing scanner and the Fotolook version 2.07 program; images were analyzed with the NIH IMAGE version 1.61 program.

Association of huntingtin with paclitaxel-stabilized microtubules

Rat brain (500 mg) was homogenized in 350 µl of cold PEM buffer (0.1 M PIPES-NaOH (pH 6.6), 1 mM EGTA, 1 mM $MgSO_4$, 1 mM PMSF and 0.1 mM leupeptin), using a motor-driven teflon pestle (10 strokes at 1200 rpm). The homogenate was clarified by centrifugation at 150,000 ***g*** for 1 hour at 4°C and the supernatant (S_1) containing soluble

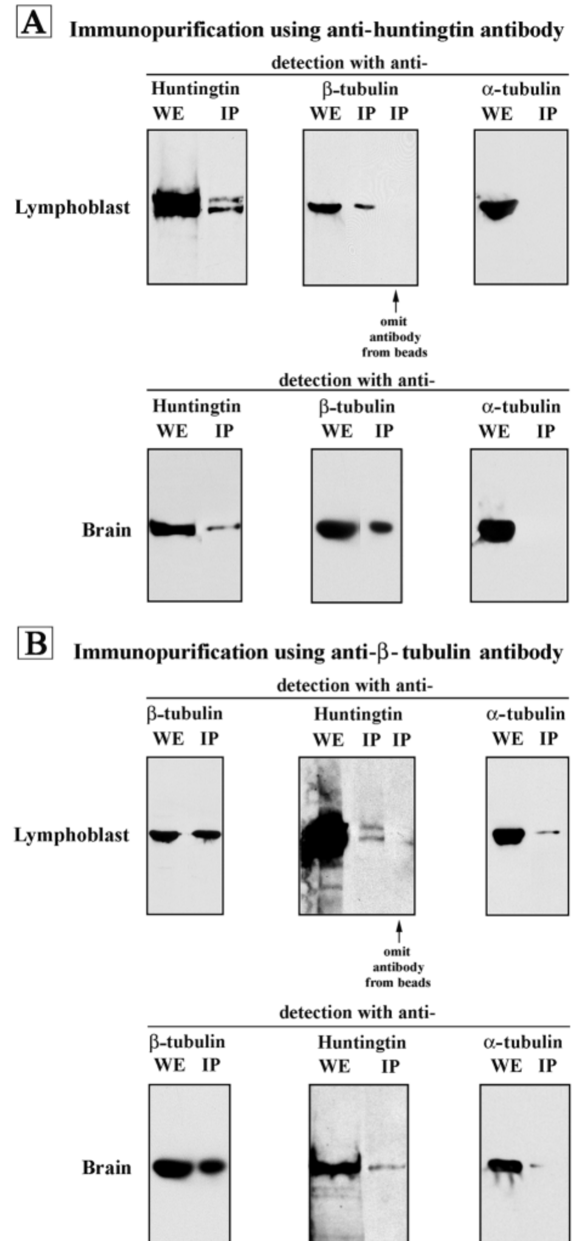

Fig. 1. Co-purification of huntingtin and β-tubulin from lymphoblast and brain extracts. A whole lymphoblast extract from a patient with juvenile Huntington's disease and a rat brain extract were incubated in the presence of either the MAB 2166 anti-huntingtin (A) or the 3F3G2 anti-β-tubulin (B) antibody coupled to magnetic beads. After several washes, the bound material was eluted. Equal amounts of the whole extract (WE) and of the immunopurified material (IP) were then examined by immunoblotting, using either an anti-huntingtin, an anti-β-tubulin or an anti-α-tubulin antibody. In both lymphoblasts and brain the anti-huntingtin antibody purified specifically huntingtin and β-tubulin. Reciprocally the anti-β-tubulin antibody purified β-tubulin and huntingtin. Neither β-tubulin nor huntingtin were immunopurified when the corresponding specific antibodies were omitted from beads.

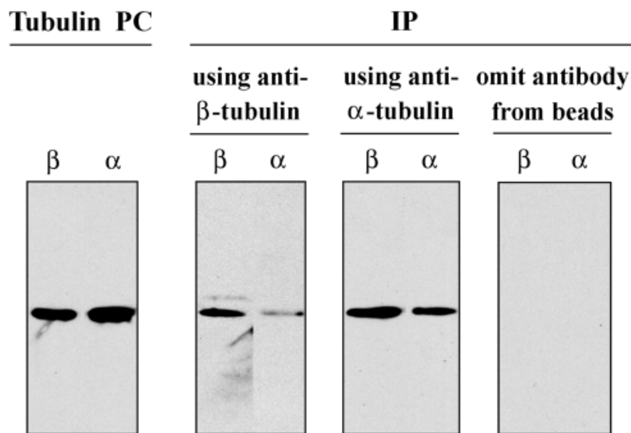

Fig. 2. Dissociation of the α/β tubulin heterodimer by the anti-β-tubulin antibody. Tubulin heterodimer purified from mouse brain by chromatography on phosphocellulose columns (Tubulin PC) and consisting of equal amounts of α- and β-tubulin was submitted to immunopurification by an anti-β-tubulin antibody (3F3G2), as described for Fig. 1. The anti-β-tubulin antibody purified almost exclusively β-tubulin because it caused dissociation of the heterodimer.

tubulin was collected. Half of S_1 was then incubated in the presence of 20 μM paclitaxel (Sigma) and 1 mM GTP for 30 minutes at 37°C to allow microtubules to form. Microtubules were then purified by centrifugation (at 48,000 ***g*** for 30 minutes at 37°C) in a 50 Ti rotor through an equal volume of PEM buffer containing 10% sucrose. The resulting supernatant (S_2) was collected, whereas the pellet (P_2) was washed and resuspended in PEM buffer supplemented with paclitaxel and GTP. The remaining half of S_1 was sedimented by centrifugation at 48,000 ***g*** for 30 minutes at 4°C (depolymerization conditions); the resulting supernatant (S_2') and pellet (P_2') were collected. The presence of huntingtin and β-tubulin in S_2, P_2, S_2' and P_2' was then determined by western blotting. Equal fractions of the pellets and supernatants were loaded on the gels for western blot analysis.

Immunocytochemistry

For immunocytochemistry, Swiss 3T3 cells (Todaro and Green, 1963) were inoculated on glass coverslips. The next day, cells were washed twice with PBS, then fixed in acetone-methanol (1:1) for 20 minutes at –20°C and air dried. To block nonspecific binding, the coverslips were incubated in PBS containing 5% bovine serum albumin (BSA) for 1 hour. For tubulin and huntingtin staining, cells were incubated in 5% BSA/PBS containing both the rabbit anti-huntingtin (1:100 dilution) and a monoclonal anti-tubulin (Sigma) antibody (1:1000 dilution), then washed three times for 5 minutes with PBS containing the nonionic detergent IGEPAL CA 630 (Sigma). Cells were then incubated for 1 hour at room temperature in the presence of 5% BSA/PBS containing both a goat anti-rabbit IgG linked to the fluorescent dye Alexa 488 (Molecular Probes) and a goat anti-mouse IgG linked to biotin (Jackson Immunoresearch). The anti-rabbit IgG antibody used to detect huntingtin was added at a 1:100 dilution and the anti-mouse IgG used to detect tubulin was added at a 1:1000 dilution. At the end of the incubation, coverslips were washed three times in PBS containing 0.1% IGEPAL and incubated for 1 hour with streptavidin linked to cyanin-3 at a 1:1000 dilution to stain tubulin. Coverslips were then washed at room temperature with PBS/IGEPAL before being incubated for 5 minutes in the presence of PBS containing bisbenzimide H 33258 (1 μg/ml) and 0.1% IGEPAL. Coverslips were then washed once in PBS/IGEPAL, then in water and mounted onto slides in mowiol containing 2.5% 1-4,diazobicyclo-(2.2.2.)-octane (DABCO). Cells were viewed using a Nikon E600 fluorescent microscope equipped with a Nikon digital camera. The images were displayed with the accompanying software and Adobe Photoshop V5.5. For confocal laser microscopy either a Zeiss or a Leica microscope were used. In all immunocytochemistry experiments it was verified that there was no detectable staining when the specific antibodies were omitted and that there was no overlapping of either color (red or green) onto the opposite filter in single-labeled cells.

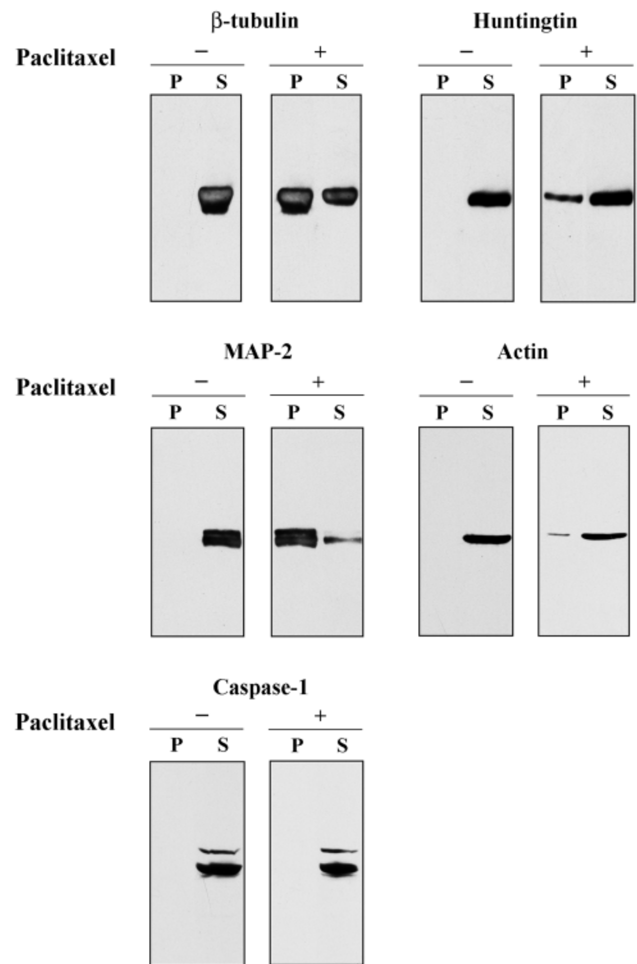

Fig. 3. Huntingtin coassembles with purified microtubules. A rat brain homogenate was clarified by high-speed centrifugation and the soluble tubulin present in the supernatant was allowed to polymerize in the presence of paclitaxel and GTP. Microtubules were then sedimented through a sucrose cushion. The pellet (P) containing microtubules and the supernatant (S) were examined by immunoblotting using monoclonal antibodies directed against either β-tubulin, huntingtin, MAP-2, actin or caspase-1. Huntingtin and MAP-2 cosediment with microtubules, whereas actin and caspase-1, two proteins not known to be associated with microtubules, remained virtually entirely in the supernatant. In the absence of paclitaxel, no microtubules were formed and both huntingtin and MAP-2 remained in the supernatant.

Results

Huntingtin interacts specifically with β-tubulin in lymphoblasts and brain

To establish whether huntingtin was associated with tubulin, we set out to determine whether huntingtin and tubulin could be co-immunopurified. A lymphoblast extract from a patient with

juvenile Huntington's disease containing both normal and expanded huntingtin, and a rat brain extract containing normal huntingtin, were incubated in the presence of a monoclonal anti-huntingtin antibody (MAB 2166) bound to an anti-IgG antibody covalently coupled to magnetic beads. Proteins bound to the anti-huntingtin antibody were then eluted, resolved by electrophoresis on polyacrylamide gels, transferred to nitrocellulose and stained with either an anti-α-tubulin or an anti-β-tubulin antibody. The western blot shows that about 20% of the β-tubulin present in the lymphoblast extract was copurified with the huntingtin, whereas no detectable α-tubulin was copurified. When the anti-huntingtin antibody was omitted from the beads, no β-tubulin was bound. Similar results were obtained with a brain extract (Fig. 1A). Release of both β-tubulin and huntingtin after immunopurification with the anti-huntingtin antibody must result from the purification of a β-tubulin/huntingtin complex and its subsequent disruption by the denaturing buffer used in the electrophoresis.

Because the anti-huntingtin antibody used for immunopurification was monoclonal and was therefore directed against a single huntingtin epitope, it was necessary to verify that this epitope was not also present in β-tubulin. We therefore submitted tubulin purified to homogeneity by phosphocellulose chromatography (Weingarten et al., 1975; Boucher et al., 1994) to immunopurification by the anti-huntingtin antibody. This tubulin preparation, which was confirmed to be free of huntingtin by western blotting, could not bind the anti-huntingtin antibody. This result confirmed that the anti-huntingtin antibody purified β-tubulin in cell and tissue extracts because of its interaction with huntingtin.

We then carried out the reciprocal experiment of immunopurifying huntingtin with the anti-β-tubulin antibody. Lymphoblast and brain extracts were incubated in the presence of an anti-β-tubulin antibody (3F3G2), itself crosslinked to the anti-IgG antibody coupled to magnetic beads. Western blot analysis of the eluate revealed that equal amounts of normal and expanded huntingtin were recovered from the lymphoblast extract by the anti-β-tubulin antibody. A small amount of α-tubulin was also recovered. In control experiments performed in the absence of anti-β-tubulin antibody, no huntingtin was bound. In rat brain also, the anti-β-tubulin antibody copurified β-tubulin and huntingtin (Fig. 1B). The yield of huntingtin purification by the anti-β-tubulin antibody was relatively low because of the large excess of competing β-tubulin, particularly in brain.

To show further the specificity of the immunopurification, several abundant brain proteins were tested: MAP2, kinesin, neurofilaments M and H. None of these proteins was purified by the anti-huntingtin antibody: no signal was detected even when the blots were largely overexposed.

In summary, our results clearly establish with the use of two unrelated antibodies (one anti-huntingtin and

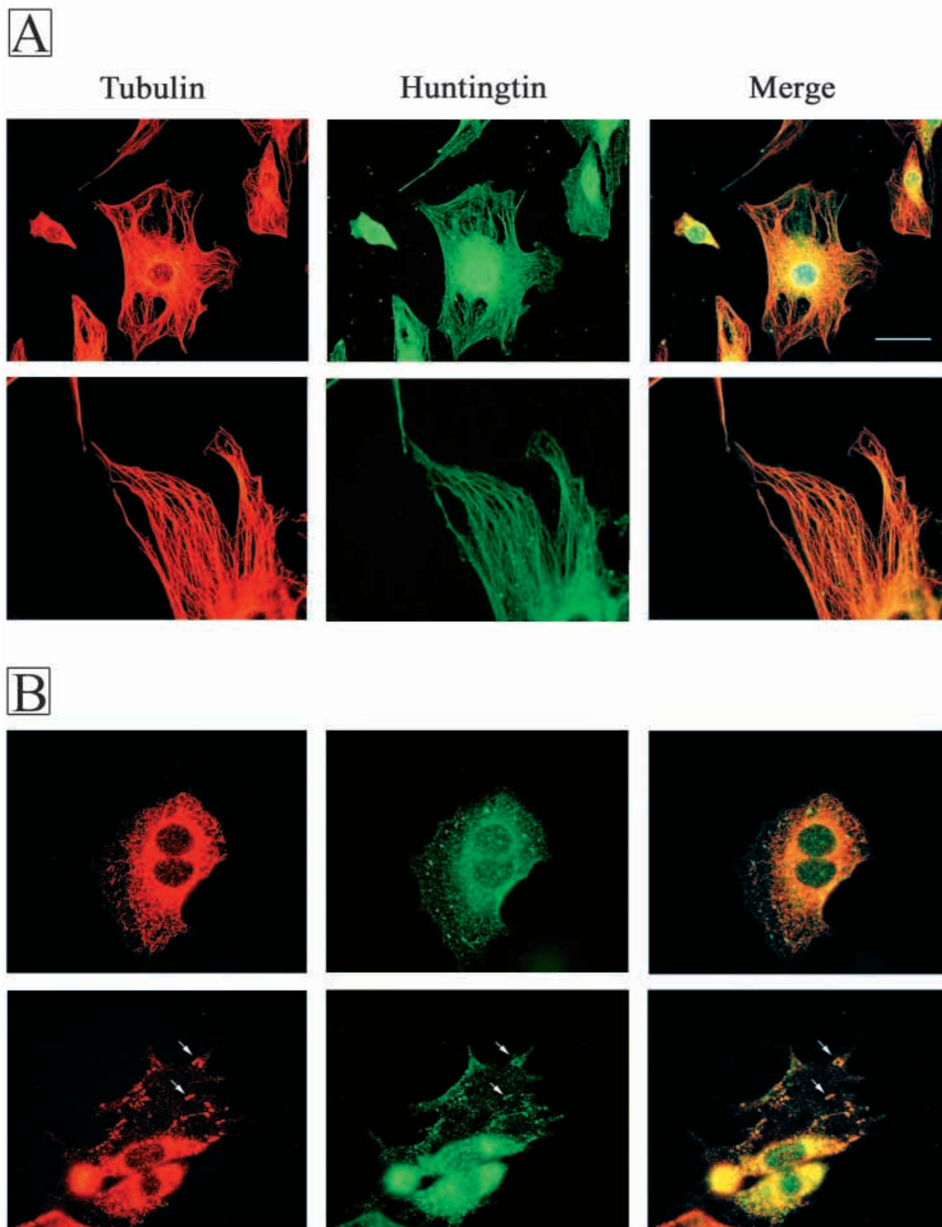

Fig. 4. Association of huntingtin with microtubules in 3T3 cells. 3T3 cells were double stained with an antibody raised against the first 17 amino acid residues of huntingtin (green) and the monoclonal 3F3G2 anti-β-tubulin antibody (red). Nuclei were counterstained with Hoechst (blue). (A) Before treatment with nocodazole huntingtin and tubulin immunoreactivity largely overlaped. (B) After addition of nocodazole to the culture medium, microtubules were disrupted and both huntingtin and tubulin acquired a patchy and diffuse distribution. Arrows show fragments of microtubules to which huntingtin has remained attached. Bar, 10 µm.

one anti-β-tubulin) that there exists a specific interaction between huntingtin and β-tubulin.

In the cell, most β-tubulin forms a heterodimer with α-tubulin. The purification of monomeric β-tubulin but not α-tubulin by the anti-β-tubulin antibody (Fig. 1) could result either from dissociation of the tubulin heterodimer by the antibody or binding of huntingtin to some free β-tubulin that might exist within the cell. To determine whether the anti-β-tubulin caused dissociation of the tubulin heterodimer, we incubated the tubulin heterodimer purified by phosphocellulose chromatography and known to contain no free monomeric β-tubulin (Weingarten et al., 1975) in the presence of the anti-β-tubulin antibody bound to beads. The fact that the anti-β-tubulin antibody purified mostly monomeric β-tubulin and little α-tubulin confirmed dissociation of the tubulin heterodimer during immunopurification (Fig. 2).

Huntingtin binds to microtubules

Because huntingtin interacts with β-tubulin in brain, it should be associated with purified microtubules prepared from this tissue. Irreversibly polymerized microtubules were prepared from rat brain in the presence of paclitaxel and purified by centrifugation through a sucrose cushion (Vallee, 1986). Proteins present in the pellet and in the supernatant were analyzed by western blotting. About 25-30% of huntingtin and 75% of MAP2 were reproducibly found to be associated with the microtubules. Two proteins not known to be associated with microtubules, actin and caspase-1, remained in the supernatant (Fig. 3).

These experiments demonstrate the association of huntingtin with microtubules formed in vitro, but they do not show whether such an association exist in the cell. It has been very difficult to observe clear colocalization of huntingtin with microtubules by immunofluorescence microscopy. The density of the microtubular network in most cells and the fact that some huntingtin is not associated with microtubules render the staining rather diffuse. To circumvent these problems, we used Swiss 3T3 cells, which, because of their large size and flat morphology, have a sparse microtubular network at their periphery. 3T3 cells were double stained with a polyclonal anti-huntingtin and a monoclonal anti-tubulin antibody. Colocalization of huntingtin with individual microtubules was evident at the periphery of the cell. Some of the huntingtin staining was also diffuse within the cytoplasm (Fig. 4A). After exposure to nocodazole (10 μg/ml) for 20 hours, microtubules were disrupted and huntingtin also acquired a diffuse and patchy distribution. However, some huntingtin was still colocalized with fragments of microtubules that persisted after treament with the drug (Fig. 4B).

Huntingtin has a perinuclear distribution and is found at the centrosome

Microtubules converge around the nucleus and ultimately to the centrosome where their minus-ends bind γ-tubulin (Oakley and Oakley, 1989; Stearns et al., 1991; Li and Joshi, 1995). We had observed that most of the huntingtin staining was perinuclear and we attributed this to the binding of the protein to microtubules (Fig. 4A). However, this apparent perinuclear localization could simply reflect the fact that in cultured cells the cytoplasm is much thicker around the nucleus than at the periphery of the cell. We therefore decided to use confocal microscopy in order to analyze a thin section of the cell (0.8 μm). Staining with both a polyclonal anti-huntingtin and a monoclonal anti-β-tubulin antibody revealed that most of huntingtin was indeed found around the nucleus where it colocalized with the denser part of the microtubular network (Fig. 5). Perinuclear distribution overlapping with the microtubules was also observed with the monoclonal anti-huntingtin antibody MAB 2166.

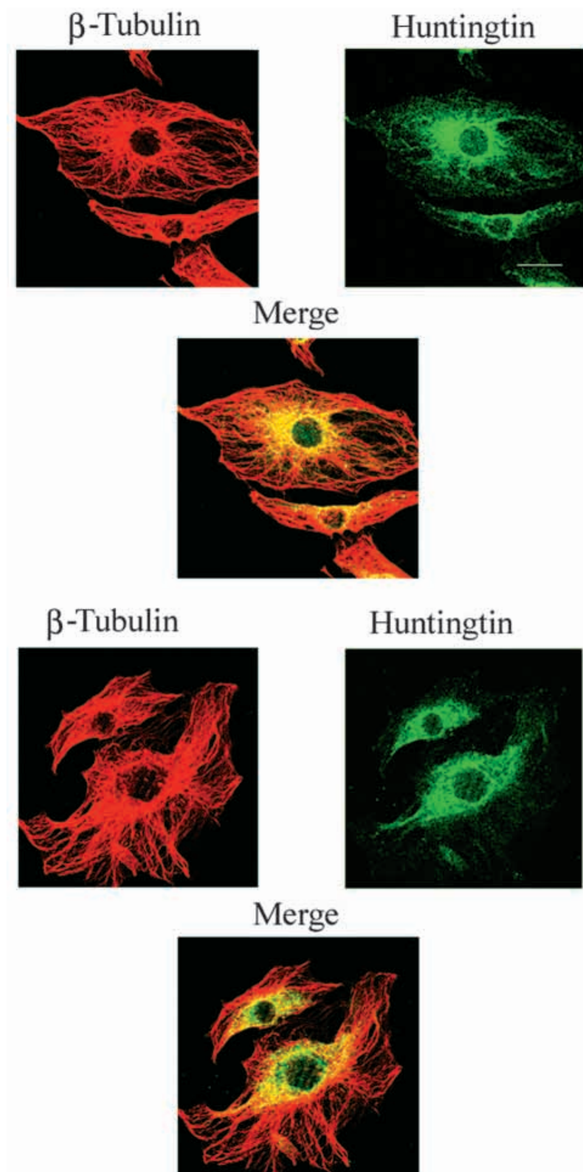

Fig. 5. Perinuclear distribution of huntingtin. 3T3 cells were stained simultaneously with the anti-huntingtin (green) and the anti-β-tubulin (red) antibodies used in Fig. 4. Thin sections of the cells were then examined by confocal microscopy. In these little-exposed photomicrographs, huntingtin staining is mostly perinuclear, where it overlaps with the denser part of the microtubular network.

To determine whether huntingtin was also present at the centrosome, 3T3 cells were stained with both a polyclonal anti-huntingtin and a monoclonal anti-γ-tubulin antibody. The anti-γ-tubulin antibody strongly stained the centrosome in which

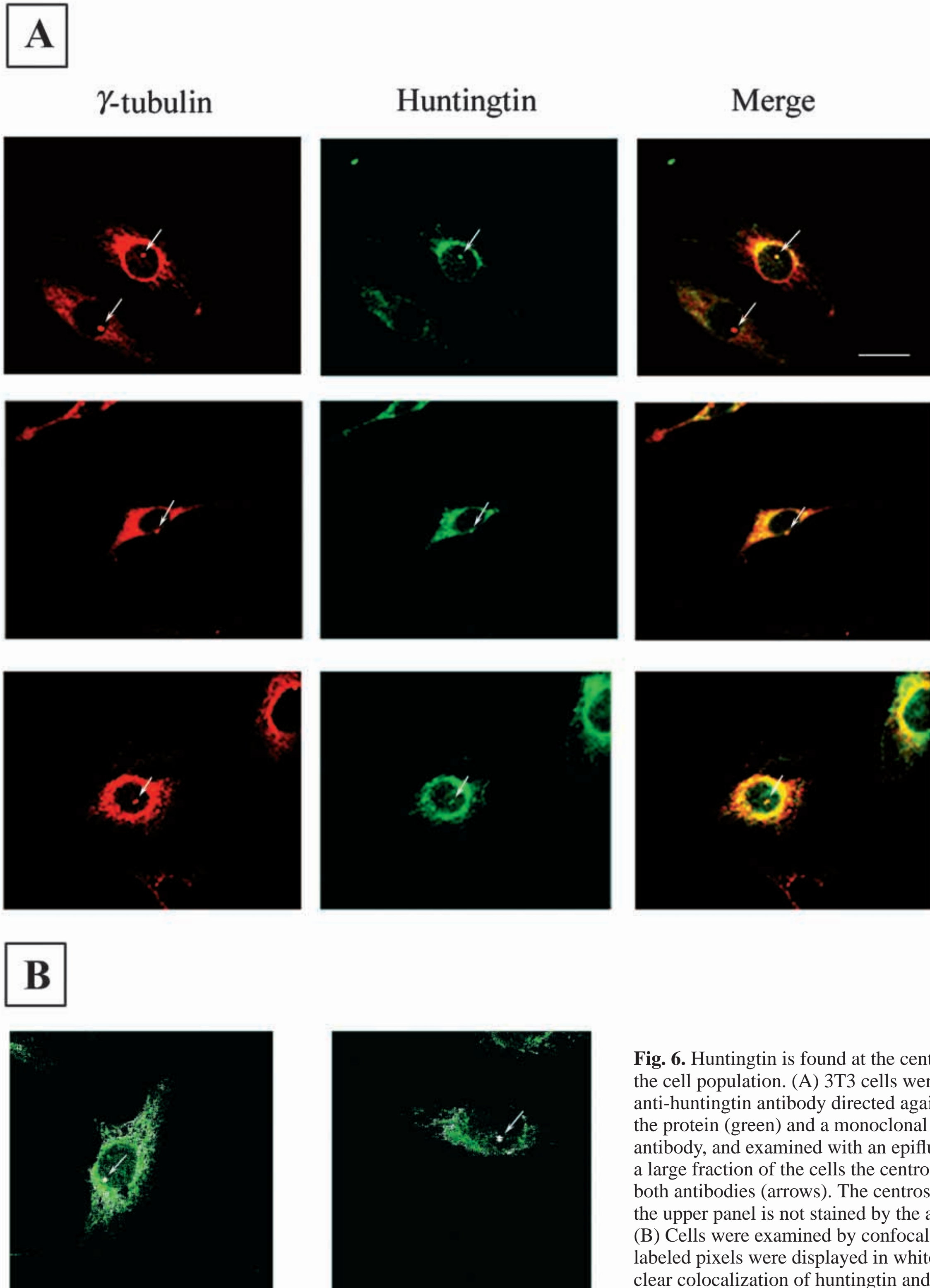

Fig. 6. Huntingtin is found at the centrosome in a fraction of the cell population. (A) 3T3 cells were stained with both the anti-huntingtin antibody directed against the first 17 residues of the protein (green) and a monoclonal anti-γ-tubulin (red) antibody, and examined with an epifluorescence microscope. In a large fraction of the cells the centrosome is clearly labeled by both antibodies (arrows). The centrosome of the lower cell of the upper panel is not stained by the anti-huntingtin antibody. (B) Cells were examined by confocal microscopy and double-labeled pixels were displayed in white. The centrosome shows clear colocalization of huntingtin and γ-tubulin.

the two centrioles could often be distinguished. It also stained the perinuclear region, probably because of the existence of a large pool of soluble γ-tubulin in this region (Murphy et al., 1998). In a fraction of the cells (30-40%), the anti-huntingtin antibody stained the centrosome and often the two centrioles (Fig. 6A). The presence of huntingtin at the centrosome was confirmed by laser confocal microscopy and analysis of local image correlation with the appropriate software (Demandolx and Davoust, 1997) (Fig. 6B). The presence of huntingtin in the centrosomal region could also be detected with another anti-N-terminal antibody (G.H. and P.D., unpublished), as well as with the HP1 and HF1 antibodies (Persichetti et al., 1995).

Discussion

We show here that both in lymphoblasts and brain, huntingtin is specifically complexed with β-tubulin, coassembles with paclitaxel-stabilized microtubules and colocalizes with microtubules in cultured cells. In earlier work by immunoelectron microscopy of neurons, Gutekunst et al. (Gutekunst et al., 1995) had observed that huntingtin immunoreactivity was most highly related to microtubules. The interaction of huntingtin to β-tubulin explains why huntingtin is bound to microtubules.

Under our conditions of immunopurification, both the anti-huntingtin and the anti-β-tubulin antibody caused dissociation of the tubulin heterodimer (Figs 1, 2). This is indeed why we could show that huntingtin was specifically associated with β-tubulin. Dissociation of the tubulin heterodimer under nondenaturing conditions is far from unprecedented. Reversible dissociation of the tubulin dimer with a K_d of about 10^{-6} M has been shown by a variety of techniques (Detrich and Williams, 1978; Mejillano and Himes, 1989; Sackett and Lippoldt, 1991; Panda et al., 1992; Shearwin et al., 1994). Dissociation of the heterodimer also occurs after treatment with lactoperoxidase, which forms complexes with the tubulin monomers (Wolff and Knipling, 1995) and during immunopurification in Tris buffer (Giraudel et al., 1998). At least three proteins, Rbl2p-β/cofactorA, Cin1p/cofactorD and the inner centromere protein have been shown by immunoprecipitation to interact with monomeric β-tubulin, and not with α-tubulin (Archer et al., 1995; Archer et al., 1998; Fleming et al., 2000; Wheatley et al., 2001).

The fact that huntingtin appeared to associate with microtubules and vesicles had suggested that huntingtin participated in vesicle trafficking and that expansion of the polyglutamine sequence of huntingtin could lead to disease by decreasing transport of vesicles (Gutekunst et al., 1995; Di Figlia et al., 1995; Gutekunst et al., 1998). However, this idea is not supported by the finding that expansion of the polyglutamine sequence did not alter the affinity of huntingtin for β-tubulin (Fig. 1B).

Several proteins interacting with huntingtin have been found by affinity methods: calmodulin, ubiquitin-conjugating enzyme (Kalchman et al., 1996), glyceraldehyde phosphate dehydrogenase (Burke et al., 1996), HAP 1 (Li et al., 1995) and HIP 1 (Wanker et al., 1997; Kalchman et al., 1997). HAP 1 and glyceraldehyde phosphate dehydrogenase are thought to associate with microtubules (Martin et al., 1999; Kumagai and Sakai, 1983). These proteins could therefore mediate the interaction of huntingtin with β-tubulin. However, the binding of huntingtin to these two proteins has been shown to increase with the length of the polyglutamine sequence (Burke et al., 1996; Li et al., 1995), whereas normal and expanded huntingtin bind β-tubulin with no obvious difference (Fig. 1B). The fraction of huntingtin that is free in the cytoplasm of neurons could interact with proteins such as HIP 1 or calmodulin independently of microtubules.

Although β-tubulin is a ubiquitous protein, it is much more abundant in neurons than in other cell types. Its participation in the aggregates formed by the huntingtin bearing an expanded polyglutamine could therefore lead to the more rapid formation of inclusions in neurons than in other cell types. It would be of interest to determine by immunocytochemistry whether β-tubulin is present in the inclusions characteristic of Huntington's disease (Davies et al., 1997). Such experiments would require purification of the inclusions, as the large amount of β-tubulin present in neurons would render very difficult the detection of β-tubulin in inclusions by direct staining of tissue sections.

The inclusions characteristic of Huntington's disease are mostly nuclear or perinuclear (Di Figlia et al., 1997; Meriin et al., 2001; Waelter et al., 2001). The high concentration of huntingtin around the nucleus and at the centrosome would readily explain why aggregation is more likely to develop in the perinuclear region than in the rest of the cytoplasm. An N-terminal fragment of huntingtin with a molecular weight of about 40 kDa has been identified in the nuclear fraction of the cerebral cortex of patients with Huntington's disease (DiFiglia et al., 1997). The perinuclear distribution of huntingtin shown in Fig. 5 would also explain why a small fragment would tend to diffuse into the nucleus, although it lacks a functional nuclear localization signal (Hackam et al., 1999). The presence of an excessively long polyglutamine sequence would then promote its aggregation in the nucleus.

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