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Co-chaperone CHIP Associates with Expanded Polyglutamine Protein and Promotes Their Degradation by Proteasomes*

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Nihar Ranjan Jana‡§¶, Priyanka Dikshit‡¶, Anand Goswami‡, Svetlana Kotliarova‖, Shigeo Murata, Keiji Tanaka**, and Nobuyuki Nukina‖‡‡**

*From the ‡Cellular and Molecular Neuroscience Laboratory, National Brain Research Centre, Manesar, Gurgaon 122-050, India, the ‖Laboratory for Structural Neuropathology, RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan, and the **Department of Molecular Oncology, Tokyo Metropoliton Institute of Medical Science, Bankyo-ku, Tokyo 113-8613, Japan*

A major hallmark of the polyglutamine diseases is the formation of neuronal intranuclear inclusions of the disease proteins that are ubiquitinated and often associated with various chaperones and proteasome components. But, how the polyglutamine proteins are ubiquitinated and degraded by the proteasomes are not known. Here, we demonstrate that CHIP (C terminus of Hsp70-interacting protein) co-immunoprecipitates with the polyglutamine-expanded huntingtin or ataxin-3 and associates with their aggregates. Transient overexpression of CHIP increases the ubiquitination and the rate of degradation of polyglutamine-expanded huntingtin or ataxin-3. Finally, we show that overexpression of CHIP suppresses the aggregation and cell death mediated by expanded polyglutamine proteins and the suppressive effect is more prominent when CHIP is overexpressed along with Hsc70.

The pathological expansion of unstable trinucleotide repeats has been found to cause 15 neurological diseases, 9 of which are neurodegenerative diseases (also referred to as polyglutamine diseases) resulting from the expansion of CAG repeats within the coding region of the responsible genes. Those nine include Huntington's disease (HD),[1] dentatorubral pallidoluysian atrophy, X-linked spinal bulbar muscular atrophy (SBMA), and several spinocerebellar ataxias (SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17). All nine disorders are progressive, dominantly inherited (except spinal bulbar muscular atrophy), typically begin in midlife, and result in severe neuronal dysfunction and neuronal cell death. Increasing length of glutamine repeats in the affected individual strongly correlates with earlier age of onset and disease severity (1).

Evidence suggests a toxic gain-of-function effect of the polyglutamine expansion on the protein, and this novel neurotoxic property most likely involves an increased propensity for the disease protein to aggregate (2). In human disease tissue, transgenic animal models, and transfected cells expanded polyglutamine proteins have been shown to undergo intracellular aggregation, in most cases forming neuronal intranuclear inclusions (3). However, the discovery of ubiquitinated aggregates or the neuronal intranuclear inclusions and the association of various chaperones and proteasome components with the aggregates suggest that the cells recognize the aggregated disease protein as abnormal and may represent an appropriate cellular response to refold or degrade aggregated mutant protein (4–9). Consistent with this idea, it has been experimentally demonstrated that overexpression of selective chaperones in the mammalian cell culture suppresses the aggregate formation and cell death (4, 6, 7, 9) and that the proteasome system is indeed involved in the degradation of polyglutamine proteins (5, 10, 11). However, very little is known about the delivery of the expanded-polyglutamine proteins to the ubiquitin proteasome pathway (UPP) for degradation.

In the present investigation, we studied the detail mechanism of ubiquitination of the expanded polyglutamine proteins using polyglutamine-expanded truncated N-terminal huntingtin (tNhtt) as well as truncated ataxin-3 as models. We found that CHIP, an ubiquitin ligase, associates with the expanded polyglutamine proteins and is responsible for their ubiquitination and degradation by proteasomes.

EXPERIMENTAL PROCEDURES

Materials—Lactacystin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dbcAMP, and all cell culture reagents were obtained from Sigma. Lipofectamine 2000, Zeocin, G418, ponasterone A, and mouse monoclonal anti-v5 were purchased from Invitrogen. Rabbit polyclonal anti-ubiquitin was from Dako, and mouse monoclonal anti-GFP was from Roche Applied Science. Goat anti-mouse IgG-Cy3 was purchased from Molecular Probes and horseradish peroxidase-conjugated anti-mouse and anti-rabbit IgG were from Amersham Biosciences.

Expression Plasmids and Stable Cell Lines—The enhanced green fluorescence protein (EGFP) and tNhtt expression constructs, pIND-tNhtt-EGFP-16Q, pIND-tNhtt-150Q, and the generation of the stable cell lines of these constructs have been described previously (12). The construction of plasmids, pEGFP-N1-MJD(f)-20CAG and pEGFP-N1-MJD(f)-130CAG, pEGFP-N1-MJD(t)-20CAG, and pEGFP-N1-MJD(t)-80CAG were described elsewhere (13). The full-length CHIP cDNA was isolated from the total RNA extracted from HeLa cells by reverse transcription-PCR. Construction of full-length and the U-box-deleted CHIP in pcDNA vector with v5 tag were made using PCR.

Cell Culture, Transfection, Cell Viability Assay, and Counting of Aggregates—The wild type mouse neuro2a cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum and the antibiotics penicillin/streptomycin. The stable cell lines (HD 16Q and HD 150Q) were maintained in the

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§ To whom correspondence may be addressed. Tel.: 91-124-2338922; Fax: 91-124-2338927; E-mail: nihar@nbrc.ac.in.

¶ Both authors contributed equally to this work.

‡‡ To whom correspondence may be addressed. E-mail: nukina@riken.brain.go.jp.

[1] The abbreviations used are: HD, Huntington's disease; UPP, ubiquitin proteasome pathway; tNhtt; truncated N-terminal huntingtin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; dbcAMP, N^6,2′-*O*-dibutyryl-adenosine-3′:5′-cyclic monophosphate; GFP, green fluorescent protein; EGFP, enhanced GFP; HSC, heat shock cognate.

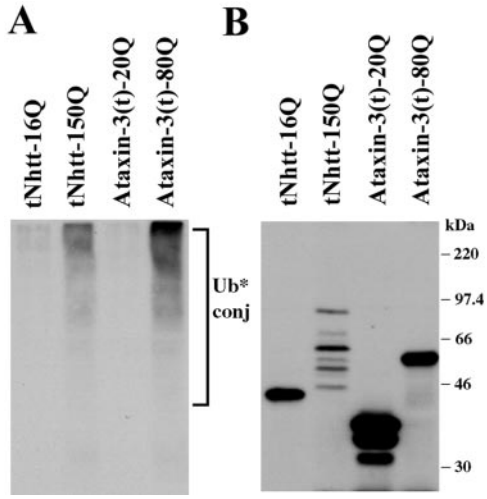

FIG. 1. **Ubiquitination of expanded polyglutamine proteins.** The HD 16Q and HD 150Q cell lines were induced with 1 μM ponasterone A, or the truncated ataxin-3-EGFP fusion constructs with 20Q and 80Q were transiently transfected (1 μg of each/well of 6-well tissue-cultured plate) to the neuro2a cells. Twenty-four hours after induction or transfection, cell lysate were made and subjected to immunoprecipitation as described under "Experimental Procedures." Blots were probed sequentially with ubiquitin antibody (*A*) and GFP antibody (*B*). *Ub * conj*, ubiquitin conjugates.

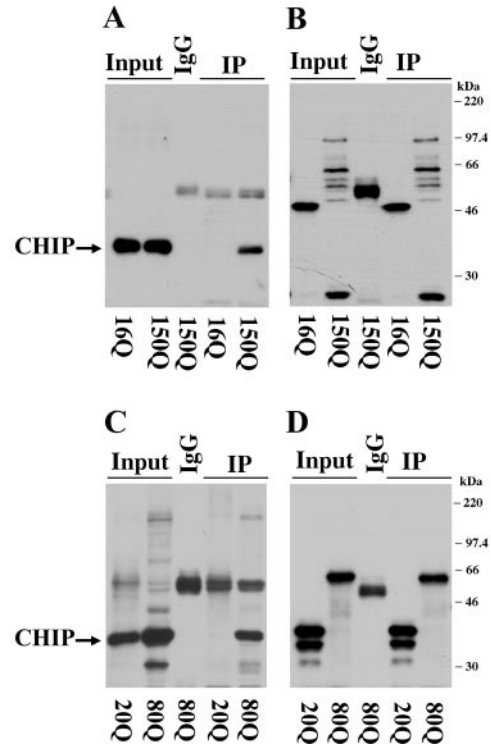

FIG. 2. **Interaction of CHIP with the expanded polyglutamine proteins.** *A* and *B*, the HD 16Q and HD 150Q cell lines were transiently transfected with CHIP (2 μg/well of 6-well tissue-cultured plate), and 12 h after transfection, media were changed, and the cells were induced with 1 μM ponasterone A. Twenty-four hours after induction, cells were collected and processed for immunoprecipitation (*IP*) by anti-GFP. Blots were sequentially probed with anti-v5 (*A*) and anti-GFP (*B*). *C* and *D*, neuro2a cells were first transfected with CHIP (2 μg/well of 6-well tissue-cultured plate). Twelve hours after first transfection, the medium was changed, and the cells were transfected again with the truncated ataxin-3-EGFP fusion constructs (1 μg of each/well) containing 20Q and 80Q. Twenty-four hours after the transfection of ataxin-3 constructs, cell lysates were made and subjected to immunoprecipitation as described in *A* and *B*. The blots were sequentially probed with anti-v5 (*C*) and anti-GFP (*D*).

same medium containing 0.4 mg/ml Zeocin and 0.4 mg/ml G418. One day prior to transfection, cells were plated into 6-well tissue-cultured plates at a subconfluent density. Cells were transiently transfected with expression vectors using Lipofectamine 2000 reagent according to the manufacturer's instruction. Transfection efficiency was ~80–90%. After 24 or 48 h of transfection, cells were used for immunofluorescence staining, co-immunoprecipitation, and immunoblotting. For cell viability assay, cells were first transfected with different expression plasmids. Twelve hours later, cells were harvested and replated into 96-well plates (5×10^3 cells/well). The cells were then differentiated with 5 mM dbcAMP and induced with 1 μM ponasterone A for 3 days. Cell viability was measured by MTT assay as described previously (12). Statistical analysis was performed using Student's t test, and $p < 0.05$ was considered to indicate statistical significance. Aggregate formation was manually counted under a fluorescence microscope (~500 transfected cells in each case), and the cells containing more than one aggregate were considered to have a single aggregate.

Co-immunoprecipitation and Immunoblotting Experiment—After 24 or 48 h of transfection, cells were washed with cold phosphate-buffered saline, scraped, pelleted by centrifugation, and lysed on ice for 30 min with radioimmune precipitation assay buffer (10 mM Hepes (pH 7.4), 150 mM NaCl, 10 mM EDTA, 2.5 mM EGTA, 1% Triton X-100, 0.1% SDS, 1% sodium deoxycholate, 10 mM NaF, 5 mM $Na_4P_2O_7$, 0.1 mM Na_2VO_5, 1 mM phenylmethylsulfonyl fluoride, 0.1 mg/ml Aprotinin). Cell lysates were briefly sonicated, centrifuged for 10 min at 15,000 $\times$ *g* at 4 °C, and the supernatants (total soluble extract) were used for immunoprecipitation as described earlier (9). For each immunoprecipitation experiment, 200 μg of protein in 0.2 ml of radioimmune precipitation assay buffer was incubated either with 5 μl (2 μg) of GFP antibody or 4 μl (2 μg) of normal mouse IgG. Bound proteins were eluted from the beads with SDS (1×) sample buffer, vortexed, boiled for 5 min, and analyzed by immunoblotting according to the procedure described earlier (9). Blot detection was carried out with enhanced chemiluminescence reagent. All primary antibodies were used in 1:1000 dilutions for immunoblotting.

Immunofluorescence Techniques—Cells grown in chamber slides or in 6-well tissue cultured plates were transiently transfected with different constructs. Forty-eight hours after transfection, cells were washed twice with phosphate-buffered saline, fixed with 4% paraformaldehyde in phosphate-buffered saline for 20 min, permeabilized with 0.5% Triton X-100 in phosphate-buffered saline for 5 min, washed extensively, then blocked with 5% nonfat dried milk in TBST (50 mM Tris, pH 7.5, 0.15 M NaCl, 0.05% Tween) for 1 h. Primary antibody (anti-v5, 1:5000 dilutions) incubation was carried out overnight at 4 °C. After several washings with TBST, cells were incubated with Cy3-conjugated secondary antibody (1:500 dilutions) for 1 h, washed several times, and mounted in antifade solution. Samples were observed using a confocal microscope (Fluoview, Olympus), and digital images were assembled using Adobe Photoshop.

Degradation Assay—Neuro2a cells were plated in a 6-well tissue-cultured plate, and on the following day, cells were transiently transfected with full-length ataxin-3 with 20Q and 130Q with or without CHIP. Twenty-four hours post-transfection, cells were chased with 10 μg/ml of cycloheximide for different time periods. Cells collected at each time point were then processed for immunoblotting by anti-GFP.

RESULTS

Misfolded Truncated N-terminal Huntingtin or Ataxin-3 Are Ubiquitinated—We developed several stable neuro2a cell lines in an inducible system, that express tNhtt with normal (16Q) and expanded polyglutamine (150Q) (12). These cell lines were named HD 16Q and HD 150Q, and their corresponding expressed proteins were named tNhtt-16Q and tNhtt-150Q. The cell lines were induced for 1 day with ponasterone A (1 μM) and then processed for immunoprecipitation by anti-GFP. In another experiment, we transfected the truncated ataxin-3 constructs to the neuro2a cell, and after 1 day, cells were collected and processed for immunoprecipitation by GFP antibody. Blots were sequentially probed with anti-ubiquitin and anti-GFP. As shown in Fig. 1*A*, truncated huntingtin with 150Q proteins or the truncated ataxin-3 with 80Q proteins were ubiquitinated, whereas those truncated proteins with normal glutamine repeats were not ubiquitinated. Fig. 1*B* showed the same blot as those in *A* after probing with GFP antibody. The tNhtt-150Q

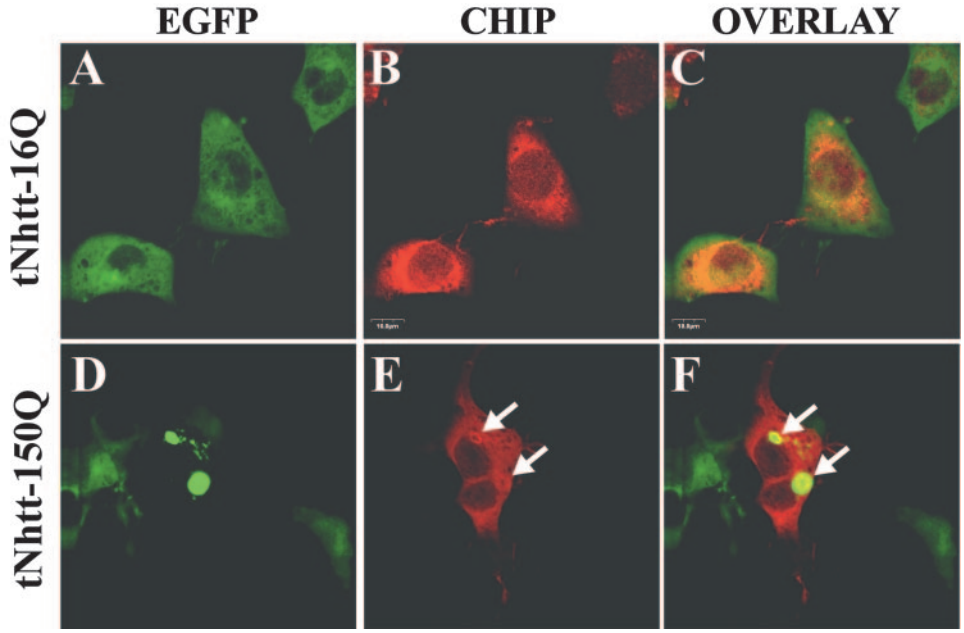

FIG. 3. **Recruitment of CHIP to the mutant huntingtin aggregates.** The HD 16Q (*A–C*) and HD 150Q (*D–F*) cells were transiently transfected with CHIP and induced in the similar way as described in the Fig. 2. Cells were then subjected to immunofluorescence staining with anti-v5. Cy3-conjugated secondary antibody was used to stain the CHIP. *Arrows* indicate the recruitment of CHIP to the huntingtin aggregates.

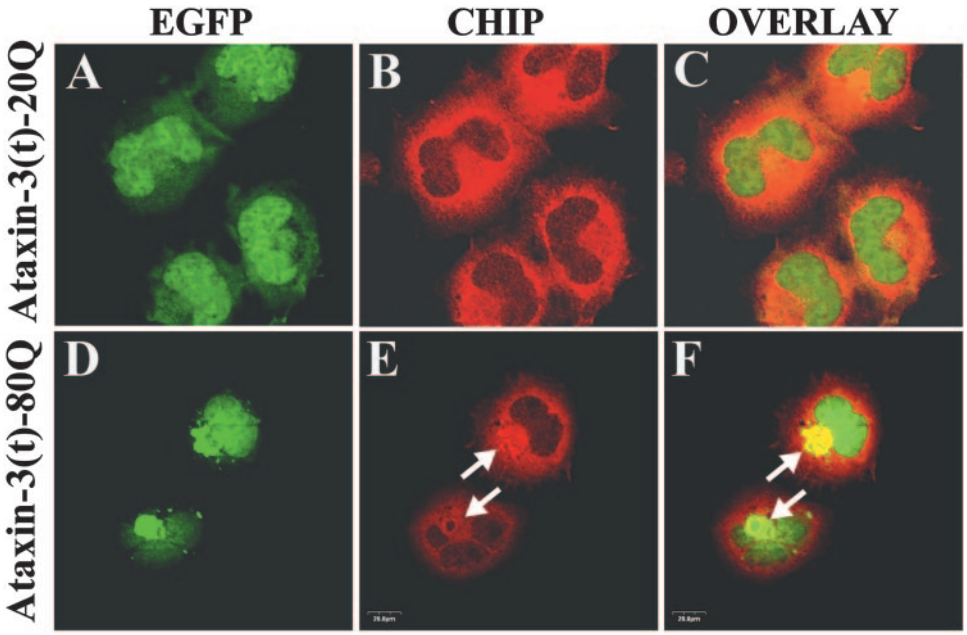

FIG. 4. **CHIP associates with the ataxin-3 aggregates.** The neuro2a cells were sequentially transfected with CHIP and truncated ataxin-3-EGFP fusion constructs containing 20Q (*A–C*) and 80Q (*D–F*). Forty-eight hours later, cells were processed for immunofluorescence staining using v5 antibody. Cy3-conjugated secondary antibody was used to stain the CHIP. *Arrows* indicate the recruitment of CHIP to the ataxin-3 aggregates.

appeared as multiple bands because of the instability of the CAG repeats.

CHIP Interacts with the Polyglutamine-expanded Truncated N-terminal Huntingtin or Ataxin-3—Because misfolding promotes the ubiquitination of the expanded polyglutamine proteins, we next wanted to know the identity of the ubiquitin ligase that is responsible for the misfolding-dependent ubiquitination. We first tested the possibility of CHIP ubiquitin ligase, because recently, CHIP has been shown to be responsible for the ubiquitination and degradation of the misfolded proteins. CHIP was transiently transfected into HD 16Q and HD 150Q cells, the cells were induced with ponasterone A for 1 day, and then the cell lysates were processed for immunoprecipitation by anti-GFP. In a similar experiment, CHIP was co-transfected along with a different truncated ataxin-3 construct, and then the cell lysates were processed for immunoprecipitation. In both experiments, blots were detected with anti-v5 antibody. As shown in Fig. 2, *A* and *C*, CHIP was immunoprecipitated with the truncated N-terminal huntingtin with 150Q and truncated ataxin-3 with 80Q but not the truncated N-terminal huntingtin with 16Q or truncated ataxin-3 with 20Q. Fig. 2, *B* and *D* showed the same blot as in Fig. 2, *A* and *C*, respectively, after detection with anti-GFP.

Association of CHIP with Polyglutamine Aggregates—Next

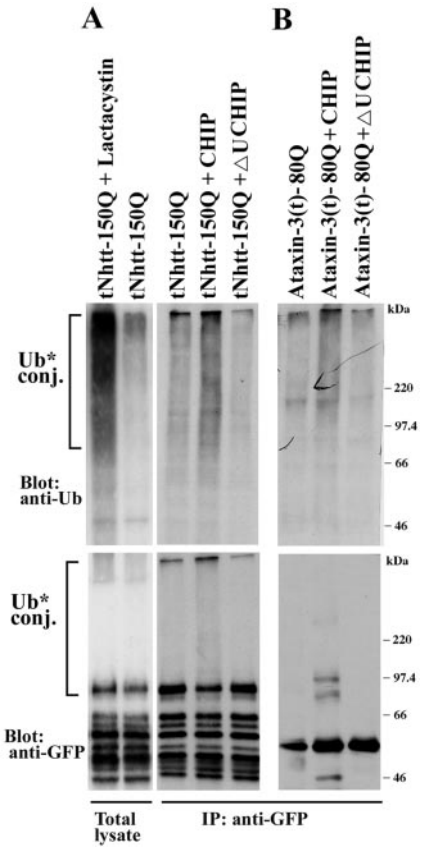

FIG. 5. **Involvement of CHIP in the ubiquitination of expanded polyglutamine proteins.** *A*, the HD 150Q cells were transiently transfected with full-length CHIP, U-box-deleted CHIP or the empty vectors (2 μg of each/well of 6-well tissue-cultured plate) and induced and processed for immunoprecipitation (*IP*) in the similar way as described in the Fig. 2. Blots were sequentially probed with anti-ubiquitin (*top blot*) and anti-GFP (*bottom blot*). In total lysate lanes, induced HD 150Q cells were left untreated or treated with 10 μM lactacystin for 8 h, and then the cell lysate were made and subjected to immunoblotting. *B*, neuro2a cells were first transfected with full-length CHIP, U-box-deleted CHIP, and empty vector (same amounts as used in *A*), and after 12 h, the cells were transfected again with truncated ataxin-3 constructs with 20Q and 80Q in the similar way as described in Fig. 2. The cell lysate were then processed for immunoprecipitation by anti-GFP followed by sequential immunoblotting with anti-ubiquitin (*top blot*) anti-GFP (*bottom blot*). *Ub* conj*, ubiquitin conjugates.

we checked the normal distribution and recruitment of CHIP to the polyglutamine aggregates. First we transiently transfected the CHIP into the HD 16Q and HD 150Q cells, and then the cells were induced to express the truncated huntingtin proteins. After 1 day of induction, cells were processed for immunofluorescence experiments using anti-v5 antibody. CHIP was normally localized into the cytosolic compartment in the wild type neuro2a cells or in the uninduced HD 16Q and HD 150Q cells (Fig. 3). Induction of the expression of the tNhtt-16Q protein did not alter the localization pattern of CHIP in the HD 16Q cell; however, the induction of tNhtt-150Q protein in the HD 150Q cell caused the recruitment of CHIP to the aggregates (Fig. 3). Next, we tested the similar redistribution of CHIP in the ataxin-3 aggregates. CHIP was co-transfected along with truncated ataxin-3 constructs and after 2 days of transfection, cells were processed for immunofluorescence experiments. As expected, CHIP was also recruited to truncated ataxin-3 aggregates (Fig. 4).

CHIP Enhances the Ubiquitination of Polyglutamine-expanded Truncated N-terminal Huntingtin or Ataxin-3—Because CHIP co-immunoprecipitates with expanded polyglutamine proteins and recruits the polyglutamine aggregates, we further tested its possible involvement in the ubiquitination of the expanded polyglutamine proteins. To test this hypothesis, we transfected CHIP (both full-length and U-box-deleted) to the HD 150Q cells, or co-transfected CHIP along with truncated ataxin-3 constructs. The cell lysate were then made and processed for immunoprecipitation by anti-GFP. Fig. 5 showed that CHIP enhanced the rate of ubiquitination of both truncated N-terminal huntingtin containing 150Q (Fig. 5*A*, *top blot*) as well as truncated ataxin-3 with 80Q (Fig. 5*B*, *top blot*). This enhanced rate of ubiquitination was prevented by the deletion of U-box of CHIP. The bottom blots of both Fig. 5, *A* and *B* are the same blots as the top blots, respectively, but probed with anti-GFP. Anti-GFP also detected smears of ubiquitinated derivatives of expanded polyglutamine proteins in the only CHIP-transfected cell lysate. The lactacystin-treated cell lysate was used as positive control to compare the CHIP-induced ubiquitination profile (Fig. 5*A*, *first two lanes*). Because the deletion of U-box of CHIP reduced the rate of ubiquitination, we further tested whether the U-box-deleted CHIP still associates with the polyglutamine aggregates. As shown in Fig. 6, U-box deleted CHIP also recruits to the huntingtin aggregates. We have also observed the association of U-box-deleted CHIP with the

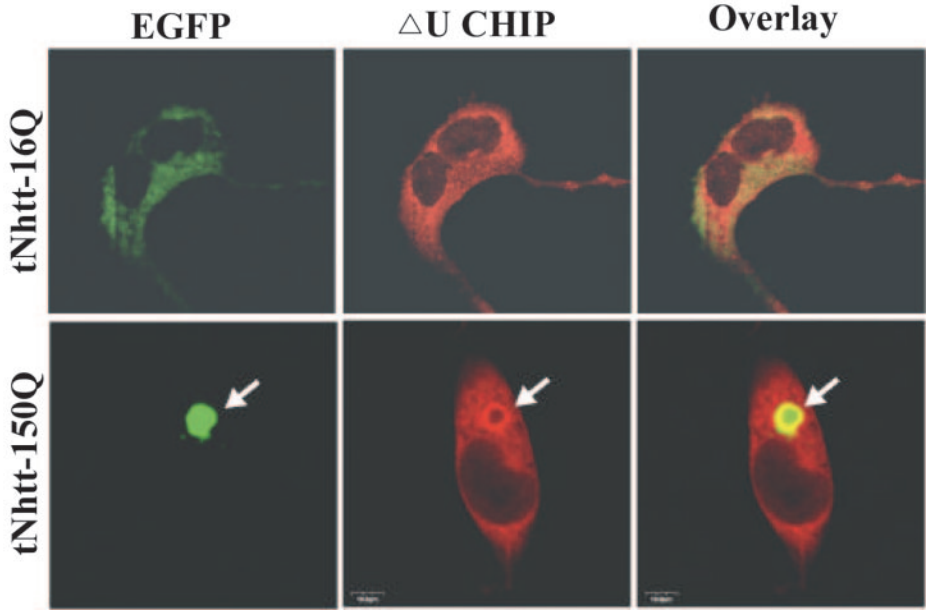

FIG. 6. **Association of U-box-deleted CHIP with the huntingtin aggregates.** The HD 16Q and HD 150Q cells were transiently transfected with U-box-deleted CHIP and induced in a similar way as described in the Fig. 2. Cells were then subjected to immunofluorescence staining with anti-v5. Cy3-conjugated secondary antibody was used to stain the CHIP. *Arrows* indicate the recruitment of U-box deleted CHIP to the huntingtin aggregates.

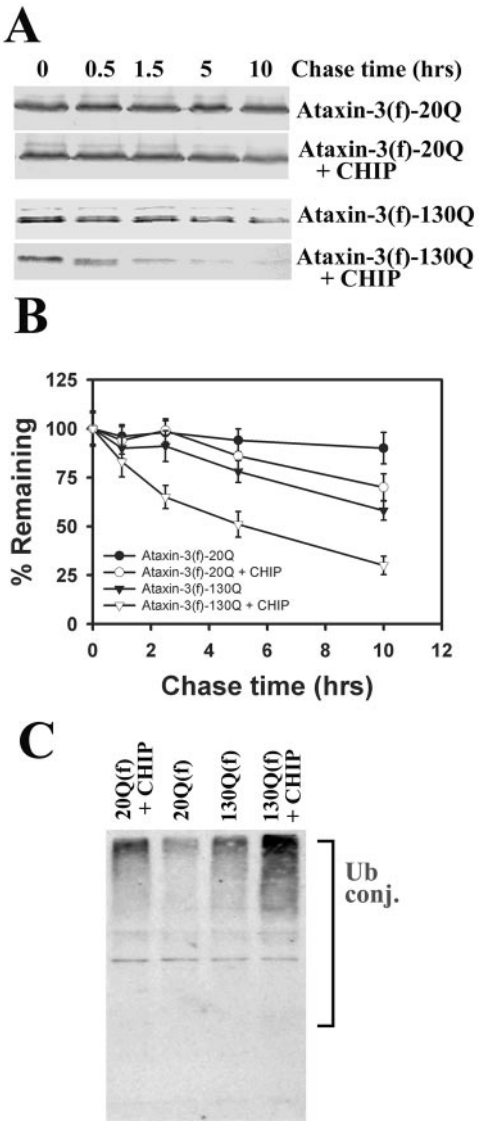

FIG. 7. **CHIP promotes degradation of polyglutamine-expanded proteins.** *A*, mouse neuro2a cells were transiently transfected with full-length ataxin-3 constructs (20Q and 130Q) either alone or along with CHIP. Twenty-four hours later, cells were chased in the presence of 10 μg/ml cycloheximide for different time periods as indicated in the figure. Cells were then collected and processed for immunoblotting using anti-GFP. *B*, quantitation of the band intensities of the blots collected from three independent experiments were performed using NIH Image analysis software. Values are means ± S.D. *C*, cells were transfected as described in *A*. Cells were collected and subjected to immunoprecipitation using anti-GFP. Blot was detected with anti-ubiquitin. *Ub* conj*, ubiquitin conjugates.

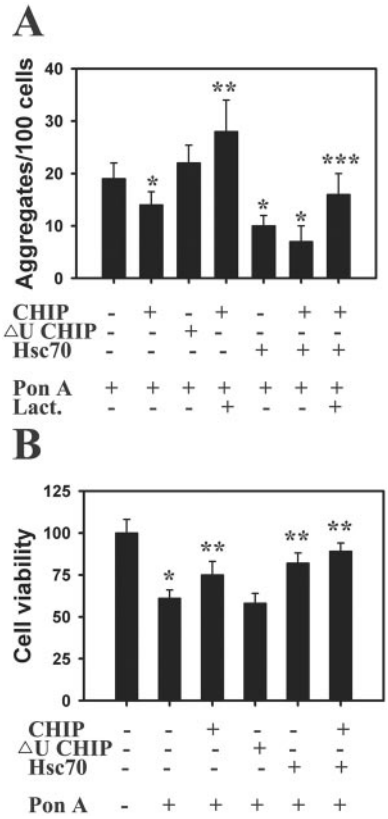

FIG. 8. **CHIP reduces the aggregate formation (*A*) and cell death (*B*) caused by expanded polyglutamine protein.** *A*, HD 150Q cells were transiently transfected with CHIP and U-box-deleted (ΔU) CHIP independently or together with Hsc70 (2 μg of each/well of 6-well tissue-cultured plate). Transfected DNA was equalized by using empty pcDNA vector. Twelve hours later, the medium was replaced, and then the cells were induced with 0.5 μM ponasterone A (*Pon A*). Aggregate counting was monitored 24 h after ponasterone A treatment in the fluorescence microscope as described under "Experimental Procedures." Lactacystin (*Lact.*) was used at a dose of 2.5 μM. Results are means ± S.D. of three independent experiments each performed triplicate. *, $p < 0.05$ as compared with control; **, $p < 0.01$ as compared with CHIP-transfected experiment; ***, $p < 0.01$ as compared with CHIP plus Hsc70-transfected experiment. *B*, HD 150Q cells were transiently transfected with CHIP and U-box deleted CHIP independently or together with Hsc70 as described under *A*. Cells were harvested and replated in the 96-well tissue cultured plate. The cells were then differentiated with 5 mM dbcAMP and induced with 1 μM ponasterone A for 3 days. Cell viability was measured by MTT assay. Values are means ± S.D. of two independent experiments each performed triplicate. *, $p < 0.01$ as compared with control; **, $p < 0.01$ as compared with ponasterone A treated experiments.

ataxin-3 aggregates (data not shown). Result strongly indicates that the CHIP associates with the expanded polyglutamine protein through its interaction with Hsc70.

CHIP Enhances the Degradation of Polyglutamine-expanded Proteins—Because CHIP enhanced the ubiquitination of polyglutamine-expanded proteins; we further checked their rate of degradation upon CHIP overexpression. For this experiment, we used full-length ataxin-3 with 20Q and 130Q, because full-length ataxin-3 with 130Q forms very few aggregates (~5–10% cells form aggregates) after 48 h of transfection. Neuro2a cells were transiently transfected with ataxin-3 constructs either alone or along with CHIP. Twenty-four hours later, cells were chased with cycloheximide. As shown in Fig. 7, *A* and *B*, full-length ataxin-3 with 20Q is not degraded; however, full-length ataxin-3 with 130Q is degraded after 1.5, 5, and 10 h of chase. Overexpression of CHIP enhanced the degradation of full-length ataxin-3 with 130Q. Overexpression of CHIP also slightly enhanced the degradation of ataxin-3 with 20Q (Fig. 7, *A* and *B*). Fig. 7*C* demonstrated that full-length ataxin-3 with 130Q was ubiquitinated, and CHIP overexpression enhanced the rate of ubiquitination.

CHIP Decreases the Aggregation and Cell Death Mediated by the Expanded Polyglutamine Proteins—Because CHIP promotes the ubiquitination of expanded polyglutamine proteins, we expected that its overexpression would increase the rate of degradation of expanded polyglutamine proteins by proteasomes. If so, CHIP should decrease the aggregation of polyglutamine proteins. Therefore, we next checked the effect of CHIP on the rate of aggregate formation and cell viability in the HD 150Q cells after different days of transfection. As shown in Fig. 8*A*, overexpression of CHIP reduced the polyglutamine-expanded tNhtt aggregation, and the suppressive effect is more prominent when the CHIP is overexpressed along with Hsc70.

The deletion of the U-box of the CHIP abolished the suppressive effect on aggregation. CHIP overexpression also decreased the aggregation of truncated ataxin-3 with 80Q (data not shown). This inhibitory effect of CHIP on aggregate formation was prevented by the proteasome inhibitor lactacystin. CHIP was also able to protect the polyglutamine protein-induced cell death, and again, the protective effect was more when the CHIP was overexpressed along with Hsc70 (Fig. 8*B*).

DISCUSSION

Ubiquitin is a well known marker of polyglutamine aggregates, but how and when polyglutamine aggregates are ubiquitinated is not yet known. The most likely hypothesis is that the expanded polyglutamine proteins are misfolded, and failure to refold might cause their ubiquitination before they are degraded by proteasome. Here we first demonstrated that the expanded polyglutamine proteins that are misfolded became ubiquitinated. Secondly, we identified CHIP ubiquitin ligase that is responsible for the misfolding-dependent ubiquitination of the expanded polyglutamine proteins. Finally, we showed that overexpression of CHIP reduces the aggregate formation and cell death mediated by expanded polyglutamine proteins.

Ubiquitination begins with the ATP-dependent activation of ubiquitin by an activating enzyme (E1). The ligation of ubiquitin to the substrate is then carried out by a specific complex composed of an ubiquitin-conjugating enzyme (E2) and ubiquitin protein ligase (E3) (14). The question now is how the misfolded polyglutamine protein is recognized by the ubiquitination machine and whether chaperones play any role. The expanded polyglutamine protein has been shown earlier to specifically interact with Hsc70/Hsp70 chaperones (9), and now we have shown that CHIP associates and ubiquitinates expanded polyglutamine proteins. Results suggest that the Hsc70/Hsp70 and CHIP both play a critical role in the process of ubiquitination of polyglutamine proteins. CHIP was first identified as an interacting protein with the C terminus of Hsp70 and shown to negatively regulated Hsp70 chaperone activity (15). Subsequently, CHIP was demonstrated to be a ubiquitin ligase of the U-box family (16, 17). Recent reports also demonstrated that CHIP is responsible for the misfolding-dependent ubiquitination and degradation of cystic fibrosis transmembrane regulator (18), glucocorticoid receptor (19), mutant copper/zinc superoxide dismutase 1 (20, 21), and Tau protein (22, 23) and therefore could be a general ubiquitin ligase for the misfolded proteins (24, 25).

We have also observed that the overexpression of CHIP inhibits polyglutamine protein aggregation and cell death and that the inhibitory effects are more prominent when CHIP is expressed along with the Hsc70 chaperone. The results suggests that polyglutamine proteins are degraded by proteasomes after they are ubiquitinated by CHIP and that the removal of polyglutamine proteins protects cells from their toxic effect. Others have reported similar findings (20–23) where they have shown that overexpression of CHIP reduced the aggregation and cell death mediated by mutant copper/zinc superoxide dismutase 1 or Tau protein. However, there are reports suggesting that the expanded polyglutamine proteins are not degraded efficiently by the proteasome and that also there is proteasomal malfunction in the expanded polyglutamine protein-expressing cells (11, 26). In both the cases, there could be an increased accumulation of ubiquitinated derivatives of expanded polyglutamine proteins. CHIP along with Hsc70 might enhance the rate of degradation by increasing the ubiquitination rate. Overexpression of CHIP along with Hsc70 could also conceivably have recovered proteasomal malfunction by reducing the burden of aggregated polyglutamine proteins as well as other misfolded proteins. Altogether, our results demonstrate that the CHIP along with Hsc70 promotes the ubiquitination and degradation of expanded polyglutamine proteins that ultimately leads to the suppression of aggregation and cell death.

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Nihar Ranjan Jana, Priyanka Dikshit, Anand Goswami, Svetlana Kotliarova, Shigeo Murata, Keiji Tanaka and Nobuyuki Nukina

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