Hsp105α Suppresses the Aggregation of Truncated Androgen Receptor with Expanded CAG Repeats and Cell Toxicity*

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Spinal and bulbar muscular atrophy (SBMA) is a neurodegenerative disorder caused by the expansion of a polyglutamine tract in the androgen receptor (AR). The N-terminal fragment of AR containing the expanded polyglutamine tract aggregates in cytoplasm and/or in nucleus and induces cell death. Some chaperones such as Hsp40 and Hsp70 have been identified as important regulators of polyglutamine aggregation and/or cell death in neuronal cells. Recently, Hsp105α, expressed at especially high levels in mammalian brain, has been shown to suppress apoptosis in neuronal cells and prevent the aggregation of protein caused by heat shock in vitro. However, its role in polyglutamine-mediated cell death and toxicity has not been studied. In the present study, we examined the effects of Hsp105α on the aggregation and cell toxicity caused by expansion of the polyglutamine tract using a cellular model of SBMA. The transient expression of truncated ARs (tARs) containing an expanded polyglutamine tract caused aggregates to form in COS-7 and SK-N-SH cells and concomitantly apoptosis in the cells with the nuclear aggregates. When Hsp105α was overexpressed with tAR97 in the cells, Hsp105α was colocalized to aggregates of tAR97, and the aggregation and cell toxicity caused by expansion of the polyglutamine tract were markedly reduced. Both β-sheet and α-helix domains, but not the ATPase domain, of Hsp105α were necessary to suppress the formation of aggregates in vivo and in vitro. Furthermore, Hsp105α was found to localize in nuclear inclusions formed by ARs containing an expanded polyglutamine tract in tissues of patients and transgenic mice with SBMA. These findings suggest that overexpression of Hsp105α suppresses cell death caused by expansion of the polyglutamine tract without chaperone activity, and the enhanced expression of the essential domains of Hsp105α in brain may provide an effective therapeutic approach for CAG repeat diseases.

Spinal and bulbar muscular atrophy (SBMA) is an X-linked motor neuropathy characterized by proximal muscle atrophy, weakness, contraction fasciculation, and bulbar involvement (1, 2). In SBMA patients, a normally polymorphic CAG repeat (10–36 CAGs) in exon 1 of the androgen receptor (AR) gene expands to 40–62 CAGs (3), and nuclear inclusions containing mutant and truncated ARs with an expanded polyglutamine tract are characteristically found in the residual motor neurons in the brain stem and spinal cord (4) as well as in the skin, testis, and other visceral organs (5). In addition to SBMA, expansions of CAG repeats encoding polyglutamine tracts in unrelated proteins are responsible for at least another eight different neurodegenerative diseases including Huntington’s disease (6), dentatorubral pallidoluysian atrophy (7, 8), Machado-Joseph disease (9), and several types of spinocerebellar ataxia (10–15). All of these disorders show a late onset of neurological symptoms with progressive neuronal dysfunction and eventual neuronal loss, although the susceptible regions in the nervous system differ among the various disorders. The appearance of intranuclear aggregates/inclusions in neurons is associated with these neurodegenerative diseases. The intranuclear inclusions contain the insoluble protein aggregates of abnormal proteins or their fragments, heat shock proteins, and components of the ubiquitin-dependent proteasome degradation pathway (16, 17). Although the nature of the toxic insult of a polyglutamine mutation and its cell-biological consequences in each disease are unclear, it is possible that the polyglutamine expansion interferes with basic cellular processes such as transcription, protein degradation, and survival/death signaling (17). However, the exact role of these protein aggregates in polyglutamine pathology is still controversial because large polyglutamine aggregates may provide an advantage over small oligomers by exposing less potentially dangerous protein surfaces (18). The cellular components involved in protein folding and degradation are also associated with intracellular inclusions in other neurodegenerative diseases not caused by polyglutamine expansion, including Alzheimer’s, Parkinson’s, and the prion diseases (19), which suggests that common mechanistic principles may underlie these misfolding diseases in general.

A considerable effort has been made to find molecules that suppress polyglutamine aggregation and cell death/toxicity for therapeutic purposes (20–22). In general, the misfolding and aggregation of proteins are prevented by molecular chaperones (23, 24). Some molecular chaperones such as heat shock protein (Hsp) 70 and Hsp40 have recently been identified as important regulators of polyglutamine aggregation and/or cell death in in vitro cell studies. In neuronal cells, some heat shock protein family members such as Hsp40 and Hsp70 have been shown to suppress the formation and toxicity caused by expansion of the polyglutamine tract. However, their role in polyglutamine-mediated cell death and toxicity has not been studied. In the present study, we examined the effects of Hsp105α on the aggregation and cell toxicity caused by expansion of the polyglutamine tract using a cellular model of SBMA. The transient expression of truncated ARs (tARs) containing an expanded polyglutamine tract caused aggregates to form in COS-7 and SK-N-SH cells and concomitantly apoptosis in the cells with the nuclear aggregates. When Hsp105α was overexpressed with tAR97 in the cells, Hsp105α was colocalized to aggregates of tAR97, and the aggregation and cell toxicity caused by expansion of the polyglutamine tract were markedly reduced. Both β-sheet and α-helix domains, but not the ATPase domain, of Hsp105α were necessary to suppress the formation of aggregates in vivo and in vitro. Furthermore, Hsp105α was found to localize in nuclear inclusions formed by ARs containing an expanded polyglutamine tract in tissues of patients and transgenic mice with SBMA. These findings suggest that overexpression of Hsp105α suppresses cell death caused by expansion of the polyglutamine tract without chaperone activity, and the enhanced expression of the essential domains of Hsp105α in brain may provide an effective therapeutic approach for CAG repeat diseases.

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‡The abbreviations used are: SBMA, spinal and bulbar muscular atrophy; AR, androgen receptor; BSA, bovine serum albumin; GFP, green fluorescent protein; HA, hemagglutinin; PBS, phosphate-buffered saline; tAR, truncated androgen receptor; TUNEL, terminal nucleotidyl transferase-mediated UTP nick end labeling.

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Hsp105α Suppresses Polyglutamine Aggregation and Cytotoxicity

TABLE I

| Primers used for construction of expression plasmids for Hsp105α deletion mutants |
|---------------------------------|---------------------------------|
| Expression in mammalian cell    | Expression in bacterial cell    |
| Hsp105N1 (a.a. 1–392)           | Hsp105β                          |
| Sense: 5'-CCGGAGAAGATGTCACACTG-3' | Sense: 5'-CTCGAGGGCAATGGAATGTTCA-3' |
| Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' | Antisense: 5'-CTCGAGGGCAATGGAATGTTCA-3' |
| Hsp105N2 (a.a. 1–511)           | Hsp105L                          |
| Sense: 5'-GGGGATCCACCTAGCTGAGCAAGATGGAATGTTCA-3' | Sense: 5'-GGGGATCCACCTAGCTGAGCAAGATGGAATGTTCA-3' |
| Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' | Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' |
| Hsp105N3 (a.a. 1–606)           | Hsp105L                          |
| Sense: 5'-TAATCGGCGTACCAGAAGATGTTCA-3' | Sense: 5'-TAATCGGCGTACCAGAAGATGTTCA-3' |
| Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' | Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' |
| Hsp105C1 (a.a. 605–858)         | Hsp105L                          |
| Sense: 5'-GGGATCCACCTAGCTGAGCAAGATGGAATGTTCA-3' | Sense: 5'-GGGATCCACCTAGCTGAGCAAGATGGAATGTTCA-3' |
| Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' | Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' |
| Hsp105C2 (a.a. 511–858)         | Hsp105L                          |
| Sense: 5'-TAATCGGCGTACCAGAAGATGTTCA-3' | Sense: 5'-TAATCGGCGTACCAGAAGATGTTCA-3' |
| Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' | Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' |
| Hsp105C3 (a.a. 386–858)         | Hsp105L                          |
| Sense: 5'-GGGATCCACCTAGCTGAGCAAGATGGAATGTTCA-3' | Sense: 5'-GGGATCCACCTAGCTGAGCAAGATGGAATGTTCA-3' |
| Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' | Antisense: 5'-CTCGAGGGGGCTTGGCAACA-3' |
| Hsp105ΔL (a.a. 1–392 + a.a. 605–858) | Hsp105L                          |
| Sense: 5'-GGGAGAAGCTCTCTTTACACATGTATGG-3' | Sense: 5'-GGGAGAAGCTCTCTTTACACATGTATGG-3' |
| Antisense: 5'-CGAAGAAGATGGAATGTTCA-3' | Antisense: 5'-CGAAGAAGATGGAATGTTCA-3' |
| Hsp105ΔL (a.a. 1–511 + a.a. 605–858) | Hsp105L                          |
| Sense: 5'-GGGAGAAGCTCTCTTTACACATGTATGG-3' | Sense: 5'-GGGAGAAGCTCTCTTTACACATGTATGG-3' |
| Antisense: 5'-CGAAGAAGATGGAATGTTCA-3' | Antisense: 5'-CGAAGAAGATGGAATGTTCA-3' |
| Hsp105ΔL                          | Hsp105L                          |
| Sense: 5'-GGGAGAAGCTCTCTTTACACATGTATGG-3' | Sense: 5'-GGGAGAAGCTCTCTTTACACATGTATGG-3' |
| Antisense: 5'-CGAAGAAGATGGAATGTTCA-3' | Antisense: 5'-CGAAGAAGATGGAATGTTCA-3' |

vitro assays (25), in cultured mammalian cells (26–31), in a Drosophila model (32) and in transgenic mice (33, 34). Hsp27 was also identified as a suppressor of polyglutamine-mediated cell death using a cellular model of Huntington’s disease (35). However, because Hsp70/40 and Hsp27 suppressed polyglutamine-mediated death without suppressing polyglutamine aggregation in some experimental systems (35, 36), elucidation of the ways in which Hsps protect cells against polyglutamine mutations might be of relevance for other neurodegenerative conditions in which pathology is associated with protein deposition in neuronal cells.

Hsp105α is highly conserved in organisms from yeast to human (37–42) and is expressed in various tissues of mammals, but especially at high levels in brain (43). Recently, Hsp105α was demonstrated to have antiapoptotic properties for neuronal survival (44). Furthermore, Hsp105α prevents the aggregation of thermal denatured protein in vitro (45). However, its role in polyglutamine-mediated cell death/toxicity has not been studied. In the present study, we examined the role of Hsp105α in the context of polyglutamine aggregation and cell death using a cellular model of SBMA and demonstrate that Hsp105α without chaperone activity protects cells against polyglutamine-mediated cell death by reducing polyglutamine-protein aggregation. These findings suggest an important role for Hsp105α in preventing neurodegenerative diseases associated with polyglutamine expansions.

EXPERIMENTAL PROCEDURES

Plasmids—We used constructs expressing the N-terminal fragment of the AR fused to green fluorescence protein (GFP) containing 24, 65, or 97 CAG repeats (tAR24, tAR65, and tAR97, respectively) as a cellular model of SBMA (29), human Hsp70 (pCMV-Hsp70) (46), and Hsp40 (pRC-Hsp40) (29) in mammalian cells. The constructs expressing Hsp105α (pCDNA105α) and Myc-epitope/His-tagged Hsp105α (pCDNA105α-mycHis) in mammalian cells were generated by ligating a full-length mouse Hsp105α cDNA (38) into pCDNA3.1 (Invitrogen) at BamHI and EcoRI sites and pcDNA3.1(+)-mycHis vector (Invitrogen) at XbaI and Kpn1 sites, respectively. The constructs expressing Hsp105N1–N3, Δβ, ΔL, and ΔL, in mammalian cells were generated by self-ligation of DNA made by PCR using pCDNA105α-mycHis (Hsp105N1–N3) or pCDNA105α (Hsp105α; Δβ, ΔL, ΔL) and C1–C3) as template DNA and a specific set of primers (Table I). We used a construct expressing GST- and HA-tagged TαR65 (GST-TαR65-HA) in bacterial cells (47). The constructs expressing Hsp105α deletion mutants in bacterial cells were generated by ligating insert DNA made by PCR using pTrcHis105-1 (45) as a template DNA and a specific set of primers (Table I) into pTrcHisA vector (Invitrogen) at the Kpn1 site.
Cell Culture and Transfection—African green monkey kidney cells (COS-7) and human neuroblastoma cells (SK-N-SH) were supplied from Riken cell bank. COS-7 cells were maintained in Dulbecco’s modified Eagle’s medium (Nissui Pharmaceutical) supplemented with 10% fetal bovine serum. SK-N-SH cells were maintained in α-minimal essential medium (Invitrogen) with 10% fetal bovine serum. For transfection of plasmid DNA, cells were grown on coverslips to 70–80% confluence and incubated further for 48 or 72 h. Then cells were fixed and stained with Hoechst 33342. Rates of cells with aggregates in nuclei and/or cytoplasm were transfected with the expression plasmid for tAR97 and incubated further for 72 h. Rates of apoptotic cells versus cells with aggregates in nuclei and/or cytoplasms are presented. Values in A and B represent the mean ± S.D. of three independent experiments. C, fragmentation of DNA in nuclei was evaluated by TUNEL methods 72 h after the transfection of tAR97. DIC represents a difference interference contrast image of cells.

Detection of the Apoptotic Cells—The apoptotic cells were identified by their nuclear morphology and the terminal nucleotidyl transferase-mediated UTP nick end labeling (TUNEL) method (29). Nuclear morphology was examined by staining with Hoechst 33342. The TUNEL method was performed using a DeadEnd™ apoptosis detection kit (Promega) according to the manufacturer’s instructions. Briefly, cells were fixed with 4% paraformaldehyde at 72 h after transfection. Fixed cells were incubated with biotinylated deoxynucleotides, then stained with streptavidin-rhodamine conjugate (Molecular Probes) and Hoechst 33342. Cells were then observed by confocal laser scan microscopy.

Western Blotting Analysis—Cells were lysed with a solution containing 0.1% SDS at 72 h after transfection. The cellular proteins (15 μg) were separated by 7.5% SDS-PAGE and blotted onto a nitrocellulose membrane. The membrane was incubated with rabbit anti-human Hsp105 (37) or mouse anti-Hsp70 (Sigma) antibody, then incubated with horseradish peroxidase-conjugated anti-rabbit IgG (Santa Cruz) for Hsp105 or anti-mouse IgG (Santa Cruz) for Hsp70 and at a 1:2,000 dilution. These proteins were detected using the enhanced chemiluminescence (ECL) detection system (Amersham Biosciences).

Protein Purification—GST-tAR65-HA was expressed in BL21 bacterial cells on addition of 1 mM isopropyl-β-D-thiogalactopyranoside. Cells were collected and resuspended in ice-cold TEGM buffer (10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 10% glycerol, and 10 mM sodium molybdate). Cells were then sonicated for 1 min and centrifuged for 30 min at 10,000 × g. To purify GST-tagged proteins, the supernatants were mixed with glutathione-Sepharose 4B (Amersham Biosciences) and incubated at 4 °C for 1 h. The Sepharose beads were then washed with PBS(−) and eluted with 10 mM reduced glutathione. Wild-type Hsp105α and its mutants were purified as His-tagged proteins by successive Ni2+–agarose (Molecular Probes) and Mono Q anion exchange column (Amersham Biosciences) chromatographies, as described previously (45).

Detection of Aggregates of Truncated AR in Vitro—GST-tAR65-HA (1 μM) was incubated with Hsp105α, its mutants, or BSA in 20 μl of buffer A (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride) at 30 °C for 12 h in the presence of 2 mM ADP. The reaction was stopped by the addition of 20 μl of a solution containing 2% SDS and 100 mM dithiothreitol, and the mixtures were heated at 98 °C for 5 min. After the addition of 200 μl of a 1% SDS solution, the mixture was filtered through a 0.2-μm cellulose acetate membrane (Advantec). Aggregates on the membrane were incubated with anti-HA tag antibody (1:500, Santa Cruz) then with peroxidase-conjugated anti-mouse IgG antibody (1:2000) and detected with an ECL detection system (Santa Cruz).

Immunohistochemistry—We perfused 20 ml of a 4% paraformaldehyde fixative in 0.1 M phosphate buffer, pH 7.4, through the left cardiac ventricle of SBMA transgenic mice (49) deeply anesthetized with ketamine-xylazine, postfixed tissues overnight in 10% phosphate-buffered formalin, and processed tissues for paraffin embedding. Then, 4-μm thick tissue sections were deparaffinized, dehydrated with alcohol, and
treated in formic acid for 5 min at room temperature and with trypsin (Dako) for 20 min at 37 °C. The tissue sections were blocked with normal goat serum (1:20) and incubated with rabbit anti-mouse Hsp105 antibody (1:100). The sections were incubated with biotinylated goat anti-rabbit IgG (Vector Laboratories), and immune complexes were visualized using streptavidin-horseradish peroxidase (Dako) and 3,3′-diaminobenzidine (Dojindo) substrate and counterstained with methyl green. For the immunohistochemistry of tissues of SBMA patients, paraffin-embedded sections of the spinal cord and scrotal skin from nine patients with clinicopathologically and genetically confirmed SBMA (age 51–84 years, mean 64.3 years) were examined using rabbit anti-human Hsp105 antibody (1:100).

RESULTS

Aggregation of Truncated AR with an Expanded Polyglutamine Tract and Induction Apoptosis in COS-7 and SK-N-SH Cells—Truncated ARs containing 24, 65, or 97 polyglutamine repeats (tAR24, tAR65, or tAR97, respectively) were transiently expressed in non-neuronal (COS-7) and neuronal cells (SK-N-SH) (Fig. 1). Because these tAR constructs were connected with GFP at the C terminus, the cellular localization of these chimerical peptides was detected by fluorescence microscopy, tAR65 or tAR97, but not tAR24, aggregated in cytoplasm and/or nucleus in non-neuronal COS-7 cells as well as neuronal SK-N-SH cells, as shown previously in the neuronal Neuro2a cell line (29). Proportions of cells with aggregates increased depending on the incubation time after transfection and polyglutamine repeat length. Approximately 45 and 60% of GFP-positive cells had aggregates in the COS-7 and SK-N-SH cell lines, respectively, at 72 h after transfection of the expression plasmid for tAR97. Among the cells with aggregates of tAR97, intranuclear aggregates were detected in 40 and 8% of transfected COS-7 and SK-N-SH cells, respectively (data not shown).

Under these conditions, apoptotic cells with condensed chromatin were observed in 12 and 18% of all COS-7 cells expressing GFP at 48 and 72 h after transfection of the expression plasmid for tAR97, respectively (Fig. 2A). In contrast, cells expressing tAR24 showed little apoptotic morphology after the transfection (Fig. 2A). Most apoptotic cells had aggregates of tAR97 in the nucleus, whereas cells containing cytoplasmic aggregates exhibited few apoptotic features (Fig. 2B). Furthermore, when the presence of fragmented DNA was assessed by the TUNEL method, the cells with intranuclear aggregates, but not cells with cytoplasmic aggregates, were found to be positive (Fig. 2C). These findings suggested that the intranuclear aggregates induced apoptosis in COS-7 cells. Similar results were obtained with SK-N-SH cells (data not shown). Thus, although both non-neuronal and neuronal cells could be utilized as a cell model of SBMA, we used the COS-7 model for further study because the cells expressed the transfected plasmids markedly well.

Colocalization of Hsp105α and Hsp70 with Aggregates of Truncated AR—We next examined the cellular distribution of Hsp105α and Hsp70 in COS-7 cells by indirect immunofluorescence using anti-human Hsp105 and anti-Hsp70 antibodies. Under nonstressed conditions, endogenous Hsp105α and Hsp70 were localized mainly in the cytoplasm of cells (Fig. 3A). When tAR24 was transiently expressed in COS-7 cells, both endogenous Hsp105α and Hsp70 were also detected in the cytoplasm of the cells (Fig. 3, B and C). In contrast, when tAR97 was transiently expressed in COS-7 cells, endogenous Hsp70 was colocalized to the aggregates of tAR97, whereas endogenous Hsp105α was not. However, when overexpressed with tAR97 in cells, Hsp105α was colocalized to the aggregates of tAR97 (Fig. 3, D), whereas Hsp105α was localized to the cytoplasm of cells in which tAR97 was not expressed. Thus, the increased amounts of Hsp105α in cells seemed necessary for the interaction with and binding to the tAR containing an expanded polyglutamine tract.

Overexpression of Hsp105α Reduced Aggregation of tAR97—Hsp105α prevents the aggregation of denatured protein in vitro (45) and suppresses apoptotic cell death induced by various forms of stress in neuronal PC12 cells (44). Because the overexpressed Hsp105α colocalized to intracellular aggregates of tAR97 (Fig. 3), we next examined the effects of Hsp105α on the aggregation of tAR containing an expanded polyglutamine tract. When expression plasmids for Hsp105α and tAR97 were cotransfected into COS-7 cells, the proportion of cells with tAR97 aggregates was reduced to ~50% of that transfected without Hsp105α (Fig. 4, A and B). Overexpression of Hsp70 or Hsp40 also suppressed the formation of aggregates similarly to Hsp105α, and Hsp70 and Hsp40 in combination suppressed the formation strongly. However, the suppression of aggregation by Hsp70 and Hsp40 was not enhanced by the coexpression of Hsp105α.

When cellular toxicity was analyzed by examining the nuclear morphology of cells stained with Hoechst 33342, numbers of apoptotic cells with condensed chromatin were found to be markedly reduced by coexpression of Hsp105α with tAR97 (Fig. 4C). Overexpression of Hsp70 and/or Hsp40 also suppressed apoptotic cell death caused by expression of tAR97. Furthermore, when various amounts of Hsp105α were coexpressed
with tAR97, the aggregation of tAR97 and apoptosis were both suppressed depending on cellular levels of Hsp105/H9251 (Fig. 5). Under these conditions, cellular levels of endogenous Hsp70 were not changed by overexpression of Hsp105/H9251 (Fig. 5, lower panel). These findings strongly suggested that when overexpressed, Hsp105/H9251 suppressed effectively not only the formation of aggregates but also the expanded polyglutamine-mediated cellular toxicity.

Identification the Domain of Hsp105/H9251 Required for Suppression of tAR97 Aggregation—Hsp105/H9251 is composed of N-terminal ATP binding, central β-sheet, loop and C-terminal α-helix domains, similar to the Hsp70 family proteins. To determine the domain of Hsp105/H9251 essential to suppress the aggregation caused by expansion of the polyglutamine tract, we constructed expression plasmids for various deletion mutants of Hsp105/H9251, as shown in Fig. 6A. When coexpressed with tAR97 in COS-7 cells, the Hsp105/H9251 mutant C3 or L significantly suppressed the aggregation of tAR97 as did wild-type Hsp105/H9251 (Fig. 6B). However, other deletion mutants failed to suppress the aggregation of tAR97. Because the C3 and L mutants contain both β-sheet and α-helix domains, these domains seemed to be essential to suppress the aggregation caused by expansion of the polyglutamine tract.

Hsp105/H9251 Inhibits Aggregation of GST-tAR65 in Vitro—To examine further whether Hsp105/H9251 can directly suppress aggregation of the expanded polyglutamine tract, we analyzed the effects of Hsp105/H9251 on the aggregation of tAR65 in vitro (Fig. 7). GST-tAR65-HA was incubated with or without Hsp105/H9251 or its mutant, and insoluble aggregates that formed during the incubation were collected on cellulose acetate membranes. Hsp105/H9251 suppressed the aggregation of tAR65 in a dose-dependent manner (Fig. 7A). Furthermore, the aggregation was suppressed by wild-type Hsp105/H9251 and the mutants C3 and L but not by other deletion mutants (Fig. 7B). Thus, it was suggested that Hsp105/H9251 itself suppressed the aggregation of truncated AR containing an expanded polyglutamine without other cellular components and that both the β-sheet and
versus

However, although Hsp105 has been shown to have chaperone activity (45). Here, we showed in vitro suppression of aggregation of tAR97. A, schematic diagram of deletion mutants of Hsp105. B, COS-7 cells were cotransfected with expression plasmid for tAR97 and deletion mutant of Hsp105 and incubated further for 72 h. GFP fluorescence was observed using a confocal laser scan microscope. Rates of cells with aggregates versus GFP-positive cells are shown. Values represent the mean ± S.D. of three independent experiments. Statistical significance was determined using Student’s t test; *p < 0.01 versus control with vector.

α-helix domains of Hsp105a seemed necessary for the suppression in vitro as well as in vivo.

Immunohistochemistry of Hsp105a in Nuclear Inclusions in the Tissues of SBMA Patients and Transgenic Mice—Nuclear inclusions containing mutant and truncated AR with an expanded polyglutamine have been shown to occur in residual motor neurons in the brain stem and spinal cord (4) and also in the skin, testis, and some other visceral organs of SBMA patients (5). We next examined whether Hsp105a localizes in the nuclear inclusions in these tissues of SBMA patients. As shown in Fig. 8, A and B, Hsp105a staining was observed in nuclear inclusions in neurons of the spinal anterior horn and scrotal skin epidermal cells. Furthermore, when male transgenic mice carrying a full-length AR with an expanded polyglutamine (97 repeats) tract and showing neuropathologic changes equivalent to human SBMA (49) were examined immunohistochemically, Hsp105a was also detected in nuclear inclusions in neurons of the spinal anterior horn and muscle cells (Fig. 8, C and D). However, although Hsp105a was commonly observed in nuclear inclusions in scrotal skin epidermal cells of SBMA patients and in muscle cells of the transgenic mice, only a few Hsp105-immunoreactive nuclear inclusions were observed in neurons of the spinal anterior horn of either patients or mice.

**DISCUSSION**

Hsp105a is a stress protein expressed at an especially high level in mammalian brain (43) and has an antiapoptotic effect in neuronal cells (44). Hsp105a prevents the aggregation of denatured proteins caused by heat shock in vitro but has not been shown to have chaperone activity (45). Here, we showed that Hsp105a suppressed not only the formation of intracellular aggregates but also apoptosis caused by an expansion of the polyglutamine tract in a cellular model of SBMA. Hsp105a is composed of N-terminal ATP binding, central β-sheet, loop, and C-terminal α-helix domains, similar to the Hsp70 family proteins. For the suppression of aggregation of truncated AR containing an expanded polyglutamine tract, β-sheet and α-helix domains of Hsp105a were essential in vivo and in vitro. Hsp70 binds unfolded proteins at the β-sheet domain and prevents aggregation of denatured proteins (50), and the α-helix domain is essential for stable binding to the substrate protein (50). Recently, we found that the β-sheet domain of Hsp105a could bind to denatured proteins. Because Hsp105a mutants with β-sheet but not α-helix domains did not prevent the aggregation of truncated AR containing an expanded polyglutamine tract in vivo and in vitro, the α-helix domain may be necessary for stabilization of the Hsp105a-substrate complexes as is Hsp70.

Hsp70 and Hsp40 have recently been identified as important regulators of polyglutamine aggregation and/or cell death in cellular models of polyglutamine disease (29). Hsp70 promotes protein folding by an ATP-dependent process involving polypeptide segments enriched in hydrophobic residues (50, 51) and cooperates in this function with members of the Hsp40 family (52). The binding of Hsp70 to substrate proteins may prevent protein aggregation directly by shielding the interactive surfaces of nonnative polypeptides. Suppression of polyglutamine-induced neurotoxicity by expression of Hsp40 alone

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*N. Yamagishi, K. Ishihara, and T. Hatayama, unpublished data.*
is most likely caused by the ability to activate the endogenous Hsp70 for suppression of the neurotoxicity. Here, we showed that overexpression of Hsp105α alone suppressed the aggregation of truncated AR containing an expanded polyglutamine tract similarly to Hsp70 or Hsp40, whereas Hsp70 and Hsp40 in combination suppressed the aggregation much more markedly. However, because the suppression of aggregation by Hsp70 and Hsp40 was not enhanced by the coexpression of Hsp105α, Hsp70α, and Hsp70/Hsp40 seem to suppress the polyglutamine-induced neurotoxicity by similar mechanisms.

In polyglutamine diseases such as spinocerebellar ataxia type 3/Machado-Joseph disease, Hsp70 colocalizes in intracellular aggregates, whereas Hsp105α is not found in the aggregates (27). In the present study, Hsp105 was detected in intranuclear inclusions in the neurons of the spinal anterior horn and scrotal skin epidermal cells of SBMA patients and also in neurons of the spinal anterior horn and muscle cells of SBMA transgenic mice, although only a few Hsp105-immunoreactive nuclear inclusions were observed in neurons of the spinal cord of the patients and mice. On the other hand, in a cellular model of SBMA, endogenous Hsp70 but not endogenous Hsp105α localized to aggregates of TAR97, although the overexpressed Hsp105α localized to the aggregates of tAR97. Because Hsp70 exists in much larger amounts than Hsp105α in cells, Hsp70 may interact preferentially with truncated AR containing an expanded polyglutamine tract. However, when Hsp105α was overexpressed in the cells, reaching high levels, it seemed to bind and localize to truncated AR containing an expanded polyglutamine tract like Hsp70. Thus, the existence of molecular chaperones at high concentrations in cells may be essential to prevent the aggregation of truncated AR containing an expanded polyglutamine tract.

The intranuclear aggregation of truncated AR containing an expanded polyglutamine tract and apoptotic cell death coincided in the cellular model of SBMA, and both processes were suppressed by overexpression of Hsp105α. As to the mechanism by which Hsp105α suppresses the apoptosis caused by expansion of the polyglutamine tract, one possibility is that suppression of aggregation by Hsp105α mediates suppression of apoptosis. Key components of the transcription apparatus, such as cAMP response element binding protein-binding protein, p53, and TAF153, are sequestered in polyglutamine-containing inclusions, then the expanded polyglutamine tract causes altered gene transcription (30, 59–57). By preventing the formation of intranuclear aggregates, Hsp105α may suppress the alteration of gene transcription caused by an expanded polyglutamine tract and eventually apoptotic cell death.

Another possibility is that the abilities of Hsp105α to suppress aggregate formation and cellular toxicity caused by expansion of the polyglutamine tract are independent. Recently, molecular chaperones, such as Hsp70, Hsp40, and Hsp27, were shown to suppress an expanded polyglutamine-mediated cellular toxicity independently of suppression of aggregation (35, 36). Although the relationship between the aggregation and the induction of apoptosis remains unknown, the suppression of cellular toxicity by molecular chaperones may be caused by the ability to inhibit apoptosis. Hsp105α suppresses heat shock-induced apoptosis in neuronal cells by preventing the activation of c-Jun N-terminal kinase (44). Because c-Jun N-terminal kinase is activated by the expanded polyglutamine tract (58), Hsp105α may also suppress the cellular toxicity by its ability to inhibit apoptosis.

In conclusion, we identified Hsp105α as a novel molecule that reduces aggregation and cellular toxicity caused by an expansion of the polyglutamine tract. Molecular chaperones, such as Hsp105α, Hsp70, Hsp40, and Hsp27, seem to suppress cell toxicity caused by an expansion of the polyglutamine tract. These findings suggest that increasing the expression levels or enhancing the function of chaperones in neurons may open up a promising approach to the treatment of polyglutamine diseases, although more studies are required to determine the precise mechanism of neurodegeneration of CAG repeat diseases.

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