Expression of the human β-amloid peptide (Aβ) in a transgenic Caenorhabditis elegans Alzheimer disease model leads to the induction of HSP-16 proteins, a family of small heat shock-inducible proteins homologous to vertebrate αB crystallin. These proteins also co-localize and co-immunoprecipitate with Aβ in this model (Fonte, V., Kapulkin, V., Taft, A., Fluet, A., Friedman, D., and Link, C. D. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 9439-9444). To investigate the molecular basis and biological function of this interaction between HSP-16 and Aβ, we generated transgenic C. elegans animals with high level, constitutive expression of HSP-16.2. We find that constitutive expression of wild type, but not mutant, HSP-16.2 partially suppresses Aβ toxicity. Wild type Aβ-(1-42), but not Aβ single chain dimer, was observed to become sequestered in HSP-16.2-containing inclusions, indicating a conformation-dependent interaction between HSP-16.2 and Aβ in vivo. Constitutive expression of HSP-16.2 could reduce amyloid fibril formation, but it did not reduce the overall accumulation of Aβ peptide or alter the pattern of the predominant oligomeric species. Studies with recombinant HSP-16.2 demonstrated that HSP-16.2 can bind directly to Aβ in vitro, with a preferential affinity for oligomeric Aβ species. This interaction between Aβ and HSP-16.2 also influences the formation of Aβ oligomers in in vitro assays. These studies are consistent with a model in which small chaperone proteins reduce Aβ toxicity by interacting directly with the Aβ peptide and altering its oligomerization pathways, thereby reducing the formation of a minor toxic species.

Accumulation of the β-amyloid (Aβ)2 peptide in the brain has been proposed to be causally linked to Alzheimer disease (the “Amyloid Cascade” hypothesis (1)), even though the specific mechanisms by which the Aβ peptide induces AD pathology have not been resolved. Intracellular Aβ accumulation has also been proposed to underlie the muscle pathology observed in inclusion body myositis (2). To investigate Aβ toxicity in a genetically tractable model, we have engineered Caenorhabditis elegans nematodes to express the human Aβ-(1-42) peptide in either body wall muscle (3) or neurons (4).

In C. elegans transgenic models with muscle expression of Aβ, the peptide accumulates in intracellular cytoplasmic deposits (5) despite the inclusion of a signal peptide in the transgene construct. The appropriate removal of the signal peptide and the association of Abeta with hsp-3, an ER chaperone homologous to mammalian GRP78/Bif6 (6), have led us to propose that Abeta is routed to the secretory pathway in this model but is retrotranslocated out of the ER because it is recognized as an abnormal protein (4). We have also demonstrated a role for autophagosomes and lysosomes in the clearance of Abeta in this model, suggesting that Abeta may also exist in these subcellular compartments (8). Intracellular Abeta is observed in the muscles of IBM patients or in transgenic mouse models of IBM (9, 10), although the subcellular distribution of Abeta has not been determined. Intracellular Aβ has also been observed in human brain neurons (11), and the relevance of intracellular Aβ in Alzheimer disease has been supported by studies with the LaFerla 3× transgenic AD mouse model, where accumulation of intracellular Aβ precedes neurofibrillary tangle formation (12). A number of neurodegenerative diseases (Parkinson, Huntington, amyotrophic lateral sclerosis, etc.) are characterized by intracellular cytoplasmic accumulation of proteins causally associated with these diseases, and thus the C. elegans transgenic model described in this study may be generally relevant to the proteotoxicity underlying neurodegenerative diseases. In this context, a transgenic C. elegans strain expressing human Aβ has been used recently to investigate the roles of insulin-like signaling and heat shock factor in proteotoxicity (13).

A robust finding in these transgenic C. elegans models is the induction of the HSP-16 family of small chaperone proteins by Aβ expression (14, 15). HSP-16 proteins readily co-immunoprecipitate with Aβ in transgenic C. elegans worms and closely associate with intracellular Aβ deposits as observed by immunohistochemistry (16). The HSP-16 family proteins are homologous to αB crystallin and have been shown to have ATP-independent chaperone activity in vitro (17). These observations suggest that induction of HSP-16 expression by Aβ represents a protective response to the accumulation of an abnormal protein. This protective response could presumably alter Aβ toxicity by promoting Aβ sequestration, degradation, or refolding. Alternatively, the Aβ/HSP-16 interaction might alter Aβ multimerization, leading to a reduction in specific oligomeric or...
amyloidotic toxic species. Here we investigate the putative protective role of HSP-16 by examining the effect of constitutive overexpression of HSP-16 on Aβ toxicity and metabolism in vivo.

Our observations of the HSP-16 response to and interaction with Aβ in transgenic C. elegans models parallel observations made for the αB crystallin family proteins in Alzheimer brain. Ten genes have been identified in the human genome that encode small, αB crystallin-homologous proteins (18), five of which have been reported to have altered expression in AD brains. (To avoid nomenclature confusion, we will refer to these proteins using their unique HUGO identification.) Initial studies demonstrated increased immunoreactivity for CRYAB (αB crystallin) and HSPB1 (Hsp27/28) (19, 20) in AD brains. CRYAB has also been demonstrated to be increased in the temporal cortex of AD brain by mass spectrometry (21), and has been observed by immunoelectron microscopy to co-localize with Aβ in lens tissue from AD patients (22). Quantitative reverse transcription-PCR has also been used to demonstrate increased accumulation of CRYAB mRNA in select regions of postmortem AD brains (15). CRYAB immunoreactivity is also significantly increased in pathologic muscle tissue from patients with IBM, which is characterized by intracellular Aβ deposition (23) More recent immunohistochemical studies have found HSPB1, HSPB6 (Hsp20), HSPB2, and HSPB8 (Hsp22) associated with senile plaques (24, 25).

Although αB crystallin-homologous small heat shock proteins (sHSP) have been found reproducibly associated with deposits of Aβ (and other abnormal proteins), the biological relevance of this association is unclear. Studies examining the interaction of sHSPs with Aβ in vitro have produced inconsistent, and sometimes contradictory, results. Stege et al. (26) reported that co-incubation of Aβ-(1–40) with CRYAB resulted in a decrease in fibril formation and an increase in toxicity to hippocampal neurons. Liang (27), however, observed a dose-dependent increase in thioflavin T fluorescence (typically associated with increased amyloid fibril formation) when CRYAB was incubated with Aβ-(1–40). A more recent study found incubation of CRYAB with either Aβ-(1–40) or Aβ-(1–42) reduced fibril formation (28). Incubation of Aβ-(1–40) with either CRYAB (αA-crystallin) or a functional peptide derived from αA-crystallin (mini-αA-crystallin) inhibited both fibril formation and Aβ toxicity to PC12 cells (29). Similarly, co-incubation of an αB-crystallin-homologous protein from the protozoan parasite Babesia bovis with Aβ-(1–40) inhibited both fibril formation and toxicity to PC12 and SY5Y cells (30).

HSPB1 was reported to inhibit in vitro amyloidogenesis of Aβ-(1–42) (31), whereas a more recent study (25) failed to find an effect of HSPB8 on Aβ-(1–42) β-sheet formation or toxicity to human brain pericytes (although HSPB8 did inhibit β-sheet formation and toxicity of an Aβ-(1–40) peptide containing the Q22E substitution associated with hereditary cerebral hemorrhage with amyloidosis of the Dutch type).

The variance in the results described above may stem from the different sHSPs, Aβ peptides, and assays employed. However, none of these studies examined the effects of sHSP/Aβ interactions in vivo, which would address the biological relevance of this interaction more directly. We have therefore used transgenic co-expression of Aβ-(1–42) and HSP-16 in a well studied C. elegans model to investigate the biological effects of the interaction of these proteins in a living animal. We show for the first time that a small heat shock protein can suppress Aβ toxicity in vivo.

**EXPERIMENTAL PROCEDURES**

**Transgenic Constructions**—The HSP-16.2 coding region was recovered from genomic C. elegans DNA by PCR, using a forward primer tagged with a 5’ KpnI site, and a reverse primer tagged with a 5’ EcoRI site. The PCR fragment was cleaved with KpnI and EcoRI and then inserted between the KpnI and EcoRI sites of myo-3/GFP expression vector pPD118.20, replacing the GFP coding sequence with HSP-16.2. The resulting plasmid, pCL137, was subjected to in vitro mutagenesis (Stratagene QuickChange kit) to generate a myo-3/HSP-16.2 R94G plasmid (pCL187). A parallel in vitro mutagenesis was also performed to introduce the R94G mutation into the HSP-16.2 Escherichia coli expression construct described previously by Leroux et al. (17). The pCL137 and pCL187 plasmids were independently co-injected along with marker plasmid pCL26 (mtl-2/GFP), which produces strong intestinal GFP expression. Heritable extrachromosomal lines were obtained by screening for GFP fluorescence. A completely stable chromosomally integrated strain, CL392, was derived from extrachromosomal myo-3/HSP-16.2 strain CL1392 by γ-irradiation as described previously (3).

**Paralysis Scoring**—Synchronous populations of transgenic animals were generated by limited egg lay at 16 °C, and worms were allowed to develop for 48 h, thus reaching the third larval stage (L3). The worms were then upshifted to 25 °C and then scored for paralysis, typically starting 24 h after upshift. Animals were scored as paralyzed if they failed to propagate a full sinusoidal contraction after prodding, or if they were associated with a “halo” of ingested bacterial lawn, indicative of an inability to move to access food. In experiments measuring paralysis in extrachromosomal transgenic strains, sibling worms containing the transgene were identified by GFP fluorescence of the marker transgene included in the transgenic array. Identification of transgenic and nontransgenic worms was performed after paralysis scoring to prevent observer bias.

**Immunoblotting and Immunohistochemistry**—For immunoblot analysis, fourth larval stage nematode populations were harvested, washed free of E. coli by low speed centrifugation, and immediately flash-frozen in liquid nitrogen in the presence of protease inhibitors (Sigma protease inhibitor mixture, P2714). Frozen samples were solubilized by boiling 5 min in Laemmli sample buffer, and protein concentrations were determined by a modified Bradford assay using Coomassie Plus–200 reagent (Pierce) per the manufacturer’s description. Protein samples were fractionated by SDS-PAGE using 4–12% acrylamide NuPAGE BisTris gels (Invitrogen) and transferred to Nybond ECL nitrocellulose membranes (Amersham Biosciences). Blots were probed with anti-HSP-16.2 antisera (gift of Peter Candido) at 1:10,000 dilution, anti-Aβ monoclonal 6E10 (Abcam, ab10146) at 0.7 μg/ml, or anti-actin monoclonal JLA20 (Developmental Studies Hybridoma Bank, University of Iowa) at 1:100 dilution, and horseradish peroxidase-conjugated
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secondary antibodies. ECL (Amersham Biosciences) was used for signal detection.

For immunofluorescence, nematode populations were fixed in 4% paraformaldehyde and permeabilized using collagenase/β-mercaptoethanol treatment as described previously (3). Anti-HSP-16.2 antisera was used at 1:1000 dilution, and 6E10 was used at 10 μg/ml. Secondary antibodies fluorescently labeled with Alexa dyes (Molecular Probes) were used at 20 μg/ml. Images were acquired using a Zeiss Axioptot epifluorescence microscope equipped with a digital deconvolution retrofit and Slidebook analysis software (Intelligent Imaging Innovations).

For quantification of β-amyloid, live adult transgenic worms were stained with the fluorescent amyloid dye X-34 as described previously (5), and amyloid deposits were imaged under shortwave illumination after destaining. Projection images of optical sections of each stained worm were generated to capture whole body amyloid signal. To exclude the effects of background staining (see Fig. 5B), deposits were first identified by inspection, and then the masking and measurement functions of Slidebook were used to generate measurements of total amyloid signal per anterior region of each worm.

Electron Microscopy—Nematodes were prepared for observation in the electron microscope as published previously (32). Briefly, fourth larval stage animals were flash-frozen in a high pressure freezer (Balzers HPM 010) according to the technique of Dahl and Staehein (33), in which the samples are freeze-substituted with 2% osmium tetroxide and 0.05% uranyl acetate in acetone at −80 °C for 5 days, gradually warmed to room temperature, infiltrated with Araldite/Embed-812 (Electron Microscopy Sciences), and polymerized. Thin sections were stained with 2% uranyl acetate and Reynold’s lead citrate. The body wall muscles of at least eight animals in each category were imaged at 80 kV on a Phillips CM10 electron microscope.

Biochemical Procedures—Recombinant His6-HSP-16.2 fusion protein was purified from a previously described BL21(DE3) E. coli strain containing a pRSET A expression vector in which the HSP-16.2 coding sequence was cloned downstream of a 4-kDa polyhistidine tag, resulting in an easily purified protein active in vitro chaperone assays (17). Induced cell lysates were run through Polyprep columns (Bio-Rad) packed with nickel-Sepharose 6 Fast Flow resin (Amersham Biosciences), and captured His6-HSP-16.2 was eluted with phosphate-buffered saline (PBS) containing 500 mM imidazole and 0.05% Triton X-100. Appropriate fractions were dialyzed against PBS containing 0.2% Triton X-100 and 20% glycerol. His6-HSP-16.2 protein preparations were subsequently biotinylated using an EZ Link Sulfo-NHS-biotinylation kit (Pierce) per the manufacturer’s instruction.

Oligomerized Aβ-(1–42) was prepared as described by Barghorn et al. (34). Lyophilized 50-μg aliquots of Aβ-(1–42) (Sigma) were solubilized in 10 μl of hexafluoro-2-propanol and incubated at 37 °C for 90 min to remove secondary structure. After removal of hexafluoro-2-propanol by evaporation, Aβ precipitates were first resuspended in 2.2 μl of dimethyl sulfoxide (Me2SO) leading to an initial concentration of 5 mM peptide and then diluted to 400 μM peptide with PBS containing 0.2% SDS. The resuspended peptide was incubated for 6 h at 37 °C, diluted 3-fold with distilled water, then incubated an additional 18 h at 37 °C. This incubation resulted in the formation of soluble Aβ oligomers in the 38–48-kDa range.

For HSP-16.2 pulldown reactions, 5 μg of biotinylated HSP-16.2 was rocked with Aβ oligomer preparations in 1 ml of PBS + 0.02% Triton X-100 at 4 °C for 30 min. To capture HSP-16.2-Aβ complexes, 10 μl of streptavidin-agarose beads (pre-washed in PBS/Triton X-100 buffer) were added to the binding reaction, and the reaction was rocked for another 30 min at 4 °C. The streptavidin-agarose beads were subsequently recovered by low speed centrifugation and washed extensively with cold PBS containing 0.02% Triton X-100. For immunoblot analysis, bead pellets were solubilized in sample buffer, and bound proteins were fractionated as described above.

RESULTS

The HSP-16 family in C. elegans consists of four closely related proteins (HSP-16.1, 16.2, 16.41, and 16.48) encoded by six genes. (There are 14 other more divergent αB-crystallin homologs in the C. elegans genome.) As it is not known if any of the HSP-16 proteins have specialized functions, we chose to engineer overexpression of HSP-16.2, which has been the most studied member of the HSP-16 family. To engineer high level, constitutive HSP-16.2 expression, a genomic fragment containing the hsp-16.2 coding region (including its single intron) was fused to the promoter of the myo-3 gene, which encodes a myosin protein expressed specifically in body wall muscle cells. The chimeric myo-3/HSP-16.2 construct, along with a marker transgene, was introduced into wild type C. elegans strains by microinjection. To control for nonspecific transgene effects, we sought to generate a control transgene in parallel containing a single missense mutation predicted to interfere with HSP-16.2 chaperone function. Given that there are no known natural mutations in HSP-16.2, we engineered an R94G substitution, which is equivalent to the R120G mutation in αB-crystallin that is associated with desmin myopathy (see Fig. 1A). The R120G mutation has been shown to reduce in vitro αB-crystallin chaperone activity (35, 36).

The wild type and mutant myo-3/HSP-16.2 transgenes were introduced by genetic mating into strain CL4176, which has been engineered to have temperature-inducible expression of myo-3-driven human Aβ-(1–42) (15). Temperature upshift of CL4176 third larval stage animals results in induction of Aβ expression and an irreversible paralysis that begins ~24 h after upshift. As shown in Fig. 1B, CL4176 worms containing the myo-3/HSP-16.2 transgene show significantly delayed paralysis, whereas the myo-3/HSP-16.2 R94G transgene has minimal effects on the time of paralysis (Fig. 1C). This experiment takes advantage of the meiotic instability of the extrachromosomal myo-3/HSP-16.2 transgenes, which results in populations of sibling transgenic and nontransgenic animals, allowing phenotypic comparisons of these genotypes under identical conditions. These results demonstrate that HSP-16.2 can partially counter Aβ toxicity and that this effect requires the wild type chaperone activity of HSP-16.2.

To investigate the biochemical effects of HSP-16.2 overexpression, the myo-3/HSP-16.2 transgene was chromosomally integrated by γ-irradiation, yielding a completely stable trans-
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CL2392 (integrated myo-3/HSP-16.2) was crossed to three independent inducible myo-3/Aβ-(1–42) lines, and the resulting dual transgenic lines were assayed for paralysis. For all dual transgenic lines, the presence of the myo-3/HSP-16.2 transgenes significantly reduced paralysis onset, producing a stronger effect than that observed for the extrachromosomal myo-3/HSP-16.2 transgene (likely because of nonmosaic expression of the integrated transgene; see representative plot in Fig. 3A). To determine whether this suppression of paralysis rates was associated with a decrease in accumulation of Aβ in the dual transgenic strains, Aβ levels in the single and dual transgenic lines were assayed by immunoblot (Fig. 3B). Worms were harvested at 24 h after Aβ transgene induction, a time point when there was significantly less paralysis in the dual transgenic strains. Nevertheless, no differences were observed in either monomeric or oligomeric species detected with the anti-Aβ monoclonal antibody 6E10 on immunoblots. This result indicates that HSP-16.2 does not promote degradation of Aβ and rules out nonspecific effects on transcription of the Aβ transgenes by the myo-3/HSP-16.2 transgene (e.g. because of competition for myo-3 transcription factors).

If HSP-16.2 does not affect Aβ levels, might it alter the cellular distribution of Aβ? To investigate this possibility, dual transgenic lines were examined by immunohistochemistry, probing fixed worms with primary antibodies specific for Aβ and HSP-16. Intramuscular Aβ deposits were dramatically redistributed into the HSP-16.2 inclusions (Fig. 4A). To examine the specificity of this apparent sequestration of Aβ into the HSP-16.2 inclusions, we generated another dual transgenic strain, which contained the myo-3/HSP-16.2 transgene and an inducible myo-3/Aβ single chain dimer construct. Unlike Aβ-(1–42), the Aβ single chain dimer does not form amyloid...
fibrils when constitutively expressed (37) and does not strongly associate with endogenous HSP-16 (16). As shown in Fig. 4B, induced Aβ single chain dimer does not redistribute into the HSP-16.2 inclusions, arguing that the redistribution of Aβ into the inclusions is because of a specific interaction between HSP-16.2 and Aβ, rather than a nonspecific co-aggregation. However, the sequestration of Aβ into the HSP-16.2 inclusions is also observed in animals expressing HSP-16.2 R94G (Fig. 4C), suggesting that sequestration per se may not be the mechanism of HSP-16.2 suppression of Aβ toxicity.

Although HSP-16.2 did not appear to alter overall levels of Aβ, it might alter folding or multimerization pathways, altering the accumulation of conformational variants of Aβ or specific oligomeric species. To determine whether HSP-16.2 altered the Aβ multimerization pathway leading to amyloid fibril formation, the myo-3/HSP-16.2 transgene was introduced into strain CL2006. This previously characterized strain constitutively expresses Aβ-(1–42) in muscle cells, resulting in the formation of intracellular amyloid deposits (3, 5). (Amyloid deposits are not detected in transgenic strains with acute, induced Aβ expression such as CL4176.) Immunoblot of CL2006 and its myo-3/HSP-16.2 dual transgenic derivative revealed that the myo-3/HSP-16.2 transgene did not appreciably change the levels of Aβ monomer or oligomer species (Fig. 5A), as described above for the inducible Aβ dual transgenic strains. However, quantitation of the in vivo amyloid load, using the fluorescent amyloid-specific dye X-34, indicated that co-expression of the myo-3/HSP-16 did significantly reduce amyloid formation (Fig. 5, B, C, and D).

To investigate directly whether HSP-16.2 can bind to Aβ and modulate Aβ oligomerization, in vitro experiments were performed with recombinant HSP-16.2 and synthetic Aβ-(1–42). Samples containing Aβ-(1–42) oligomers (“globulomers”) were prepared by incubation of Aβ in Me₂SO, as described previously (34), to mimic the collection of high molecular weight oligomeric Aβ species detected in the worm transgenic models. The mixed Aβ species were incubated with biotinylated HSP-16.2, and HSP-16.2-Aβ complexes were recovered by avidin affinity pulldown. As shown in Fig. 6A, Aβ was recovered with HSP-16.2 in this pulldown experiment, with oligomeric species predominating. This preferential binding of HSP-16.2 to Aβ oligomers in vitro recapitulates the preferential co-immunoprecipitation of Aβ oligomers with HSP-16 in lysates from transgenic worms expressing Aβ (Fig. 2A) (16). To investigate if this HSP-16.2/Aβ interaction can alter Aβ oligomerization, HSP-16.2 was co-incubated with Aβ-(1–42)
DISCUSSION

We have demonstrated that overexpression of HSP-16.2, a C. elegans chaperone protein homologous to αB crystallin, can suppress toxicity associated with human Aβ-(1–42) in a C. elegans AD model. Chaperone expression has not been demonstrated previously to suppress Aβ toxicity in an in vivo model, although the toxicity observed when primary rat hippocampal neurons are transfected with an adenovirus vector driving Aβ expression is reduced if the neurons are co-transfected with an HSP70-expressing vector (38). HSP70 overexpression has been observed to suppress toxicity in fly (39) and mouse models (40) of polyglutamine repeat diseases. In both these models, HSP70 overexpression suppressed toxicity without altering the nuclear inclusions associated with toxic polyglutamine protein expression. HSP40 (DnaJ) family chaperones have also been found to suppress toxicity in fly polyglutamine repeat disease models (39, 41). HSP70 overexpression has also been observed to suppress α-synuclein toxicity in a fly model of Parkinson disease, again without altering the formation of the Lewy body-like deposits observed in this model (42). These observations are consistent with our results demonstrating that chaperone suppression of Aβ toxicity occurs without reduction of toxic protein accumulation per se.

The formation of HSP-16.2-containing inclusions in transgenic worms overexpressing this protein was unexpected, but perhaps not surprising, given that sHSPs typically function as large oligomers, and avian HSP25 expressed in HeLa cells has also been observed to form inclusions (43). The R120G mutant form of αB-crystallin, associated with desmin-related myopa-

spontaneous oligomers generated in aqueous Aβ solution) or high molecular weight oligomers (lane 2, oligomers generated by MeSO incubation) were mixed with biotinylated recombinant HSP-16.2, and bound complexes were recovered using streptavidin-agarose beads (lane 3, low molecular weight Aβ input; lane 4, high molecular weight Aβ input). Note poor recovery of monomeric Aβ (arrow, lane 3). The bound portion of this immunoblot (lanes 3 and 4) was exposed for a longer time period than the input half to highlight the preferential recovery of Aβ oligomers (arrow). Aβ detection was by monoclonal antibody 6E10. B, inhibition of in vitro oligomerization by HSP-16.2. Monomeric Aβ was incubated in MeSO as described under “Experimental Procedures” along with control protein (bovine serum albumin, BSA) and/or HSP-16.2. Aliquots of the oligomerization reactions were fractionated after a 6-h incubation. Note that inclusion of HSP-16.2 in the oligomerization reaction strongly reduces the accumulation of higher molecular weight oligomers detected by immunoblot after completion of the oligomerization reaction. C, wild type HSP-16.2 is more efficient at inhibiting Aβ oligomerization than HSP-16.2 R94G. Monomeric Aβ was incubated in MeSO with 4 μg of BSA, with or without 0.5 μg of HSP-16.2 (WT or R94G) for 6 h, diluted 3-fold, and then incubated another 12 h before fractionation. (These conditions were chosen to highlight the differences in the oligomerization inhibition activities of WT and R94G HSP-16.2.) Lane 1, Aβ + BSA only; lane 2, Aβ + BSA + WT HSP-16.2; lane 3, Aβ + BSA + HSP-16.2 R94G. Note that the prominent reduction of an ~70-kDa Aβ species by WT HSP-16.2 (arrow) is not seen in the reaction containing HSP-16.2 R94G.
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HSP-16, has also been reported to form toxic amyloidal oligomers in vitro (44). We have not observed HSP-16 inclusions in wild type worms induced to express HSP-16 by heat shock, which may be due to the lower levels of naturally induced HSP-16 and/or the co-induction of other stress proteins that interact with HSP-16. The observation that Aβ-(1–42), but not the Aβ single chain dimer, is sequestered into the HSP-16.2 inclusions argues that HSP-16.2 recognition of Aβ is conformation-dependent and occurs despite the formation of HSP-16.2 inclusions. Similarly, we have also observed that an aggregating form of GFP, but not soluble GFP, is sequestered into HSP-16 inclusions (32). Taken together, these data imply that in vivo HSP-16 binding involves the recognition of a conformational motif generally present on some species of aggregation-prone proteins.

We have attempted to complement these overexpression studies by examining the effect of reducing HSP-16.2 expression by RNA interference, which we would predict to enhance the toxicity associated with Aβ expression. Our results from these experiments have been inconsistent, which we attribute to the multiple hsp-16 genes present in C. elegans and the existence of compensatory cross-regulation between members of this gene family. We have demonstrated previously that RNA interference knockdown of the ER chaperone HSP-3 leads to a compensatory up-regulation of related ER chaperone HSP-4, which effectively mitigates the biological effects of HSP-3 knockdown (45).

How does HSP-16.2 suppress Aβ toxicity? Given the direct interaction between this chaperone and Aβ, the simplest models posit that this interaction alters Aβ metabolism, thereby reducing the amount of toxic Aβ species. Our results indicate that the overall amount of Aβ is not reduced by HSP-16.2 overexpression, arguing against the idea that HSP-16.2 promotes degradation of Aβ. Likewise, the predominant forms of SDS-stable Aβ oligomers are similarly not detectably changed by HSP-16.2 overexpression. In transgenic worms constitutively expressing Aβ, HSP-16.2 overexpression does significantly reduce amyloid load. Amyloid deposition does not correlate with toxicity in this C. elegans model (46), so this result does not directly explain the reduction in paralysis rate resulting from HSP-16.2 overexpression. We interpret the reduction in amyloid formation as indicative of HSP-16.2 modulating multimerization pathways of Aβ, possibly leading to changes in the accumulation of a (nonamyloidic) toxic species. (This hypothetical toxic Aβ species may constitute a minor fraction of all Aβ, and thus be difficult to detect by immunoblot-based methods.) To investigate directly whether HSP-16.2 can modulate Aβ oligomerization, we examined the effect of co-incubation of recombinant HSP-16.2 with synthetic Aβ-(1–42), using an established oligomerization protocol (34). Co-incubation with HSP-16.2 was found to decrease the formation of higher molecular weight Aβ species, consistent with the proposal that the chaperone activity of HSP-16.2 acts to direct Aβ along pathways leading to less toxic oligomeric species. This interpretation was further supported by our demonstration that the R94G mutant of HSP-16.2, which shows significantly less protection against Aβ toxicity in vivo, also showed a reduced capacity to inhibit the formation of Aβ oligomers in vitro. Given our current data, we cannot exclude the possibility that HSP-16.2 could also act downstream of the formation of toxic Aβ species to intervene in the toxic process itself, perhaps by counteracting perturbations of cellular protein folding caused by Aβ accumulation (47).

The simple C. elegans model described in this study does not replicate many aspects of human AD (e.g. formation of extracellular senile plaques or neuronal dysfunction). The rationale for using this model is that it can capture some of the disease-relevant biology that underlies AD and IBM, such as the mechanism(s) by which Aβ perturbs cell function, and the protective responses of cells to toxic Aβ accumulation. C. elegans does not naturally produce the Aβ peptide, so the interaction of HSP-16.2 and Aβ is likely to result from a general function of HSP-16.2 that can influence multimerization/aggregation pathways of potentially toxic proteins. Our results suggest that the HSP-16.2 interaction with Aβ involves recognition of a conformational epitope associated with Aβ oligomerization. In vitro studies have demonstrated that many proteins can potentially form toxic oligomers (48), suggesting that cells may have a continual need to counteract toxic protein oligomerization. Interestingly, transgenic overexpression of HSP-16.2 has also been observed to increase the life span in C. elegans (7). We speculate that an evolved function of small heat shock proteins may be to bind intermediate multimers that occur during the formation of higher molecular weight oligomers, thus reducing the formation of toxic oligomer species. Small heat shock proteins may therefore play a role in a range of neurodegenerative diseases associated with toxic protein aggregates. If this hypothesis is true, we would predict that risk alleles for some neurodegenerative diseases may be associated with genes encoding small heat shock proteins, and furthermore, diminished small heat shock protein function with age may play a role in the age-dependent onset of these diseases.

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