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# Two-hybrid system as a model to study the interaction of β-amyloid peptide monomers

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**ABSTRACT** **The kinetics of amyloid fibril formation by β-amyloid peptide (Aβ) are typical of a nucleation-dependent polymerization mechanism. This type of mechanism suggests that the study of the interaction of Aβ with itself can provide some valuable insights into Alzheimer disease amyloidosis. Interaction of Aβ with itself was explored with the yeast two-hybrid system. Fusion proteins were created by linking the Aβ fragment to a LexA DNA-binding domain (bait) and also to a B42 transactivation domain (prey). Protein–protein interactions were measured by expression of these fusion proteins in *Saccharomyces cerevisiae* harboring *lacZ* (β-galactosidase) and *LEU2* (leucine utilization) genes under the control of LexA-dependent operators. This approach suggests that the Aβ molecule is capable of interacting with itself *in vivo* in the yeast cell nucleus. LexA protein fused to the *Drosophila* protein bicoid (LexA-bicoid) failed to interact with the B42 fragment fused to Aβ, indicating that the observed Aβ–Aβ interaction was specific. Specificity was further shown by the finding that no significant interaction was observed in yeast expressing LexA-Aβ bait when the B42 transactivation domain was fused to an Aβ fragment with Phe-Phe at residues 19 and 20 replaced by Thr-Thr (AβTT), a finding that is consistent with *in vitro* observations made by others. Moreover, when a peptide fragment bearing this substitution was mixed with native Aβ-(1–40), it inhibited formation of fibrils *in vitro* as examined by electron microscopy. The findings presented in this paper suggest that the two-hybrid system can be used to study the interaction of Aβ monomers and to define the peptide sequences that may be important in nucleation-dependent aggregation.**

An aggregated form of β-amyloid peptide (Aβ), a 39- to 42-aa peptide, is the principal component of amyloid in the core of plaques, which are characteristic of the Alzheimer disease (AD) brain (1, 2). Aβ is posttranslationally derived from a much larger amyloid precursor protein (APP) encoded by a gene on chromosome 21 in band q21 (3–6). The strongest evidence for involvement of APP in AD comes from familial mutations discovered close to or within the Aβ domain (7–12). At least one of these mutations (codons 670 and 671 of APP-770) has been shown to increase secreted Aβ *in vitro* (13, 14). It is therefore likely that dysfunction in APP or Aβ metabolism may play a role in AD.

Since Aβ was detected in senile plaques, it was assumed that this peptide was a result of abnormal cleavage of APP. It is now accepted that Aβ is secreted by cells in culture and is found as a soluble peptide in the cerebrospinal fluid (CSF) of AD patients and in comparable concentrations in age-matched control patients (15–17). Soluble Aβ has also been detected in the plasma of healthy individuals (15). The measurement of soluble Aβ in CSF of patients free of neurodegenerative disease indicated an increase in peptide levels with age (18). Therefore, physiological factors that can induce Aβ aggregation may be more important in the development of AD pathology than the concentration of Aβ *per se*.

Extracellular and cerebrovascular amyloid deposits are composed of Aβ variants which differ at their carboxyl termini (1, 2, 19, 20). Neuritic plaques have been found to contain high levels of Aβ-(1–42) (1, 19, 21). Kinetic studies on Aβ aggregation have demonstrated that amyloid formation is a nucleation-dependent phenomenon (22), and that a lag time precedes aggregation, the length of which may depend on protein concentration. The nucleation event may therefore be the rate-determining step of *in vivo* amyloidosis.

Nucleation-dependent polymerization is observed in protein crystallization, microtubule assembly, flagellum assembly, phage capsid assembly, actin polymerization (23), and a small subset of human proteins that characterize amyloid diseases (24–26). Interaction between two monomers, a thermodynamically unfavorable intermolecular interaction, may be a critical step in nucleation. The experimental yeast system presented herein provides an opportunity to study the interaction of Aβ monomers *in vivo*.

## EXPERIMENTAL PROCEDURES

**Bacterial and Yeast Strains.** Manipulations of bacterial strains and of DNAs were by standard methods (27, 28) unless otherwise noted. *Escherichia coli* maximum-efficiency DH5α competent cells (GIBCO/BRL) were used as hosts throughout. Yeast strain EGY48 was obtained from the laboratory of Roger Brent (Massachusetts General Hospital, Boston).

**Construction of Bait and Prey Plasmids.** To construct the bait plasmid (LexA-Aβ fusion), primers to the cDNA for the APP-770 isoform cloned into the *Hin*dIII and *Xba* I sites of pRcCMV were used. For amplification, the primers used were 5′-AAGGCCTGGATCCTGGATGCAGAATTCCGACATGAC-3′ at the 5′ end and 5′-AAGGCCTCTCGAGGTCGACCTACGCTATCAGCAACCACCGCACC-3′ at the 3′ end. This primer set amplified a 163-bp fragment that was digested with *Bam*HI and *Xho* I to obtain a 143-bp *Bam*HI–*Xho* I fragment, which was then ligated into pEG202 (29) at those sites. This places the open reading frame for Aβ into translational phase with the LexA sequence of pEG202. The downstream primer was constructed to contain a synthetic stop codon after Ala-42 of Aβ. The bait fusion protein is produced constitutively from pEG202, a 2-μm $HIS3^+$ plasmid under the control of the *ADH1* promoter and encoding the LexA carboxyl-terminal oligomerization region, which contributes to operator occupancy by LexA derivatives (29).

The prey plasmid (B42-Aβ fusion) was constructed by digesting the 163-bp PCR-amplified fragment designed for bait

Abbreviations: Aβ, β-amyloid peptide; AβTT, Aβ peptide with $Phe^{19}$-$Phe^{20}$ replaced with Thr-Thr; APP, amyloid precursor protein; AD, Alzheimer disease; HA, hemagglutinin; ONPG, *o*-nitrophenyl β-D-galactopyranoside; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside.

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(see above) with *Eco*RI and *Xho* I. This *Eco*RI–*Xho* I fragment was placed in pJG4-5, a 2-μm *TRP1* plasmid (30, 31), in translational frame with the codons for the simian virus 40 large T nuclear localization signal, the B42 transactivation domain, and the hemagglutinin (HA) epitope tag. The prey fusion protein (16 kDa) will be inducible in yeast grown on minimal medium (MM) containing 2% galactose and 1% raffinose (Gal/Raf) but not in yeast grown on 2% glucose (Glc). Amino acids 3 and 4 (glutamate, phenylalanine) of Aβ (at the point where it is fused to the HA tag) are generated by codons in the *Eco*RI site.

To construct the mutant prey plasmid that contained the AβTT-encoding sequence, a fragment was constructed from a 147-bp oligonucleotide representing the mutation of $Phe^{19}$-$Phe^{20}$ to $Thr^{19}$-$Thr^{20}$ within Aβ synthesized on a Millipore model 8909 Expedite nucleic acid synthesis system as follows. The oligonucleotide 5′-AGGCCTGAATTCCGACATGACTCAGGATATGAAGTTCATCATCAAAAATTGGTGACTACTGCAGAAGATGTGGGTTCAAACAAAGGTGCAATCATTGGACTCATGGTGGGCGGTGTTGTCATAGCGTAGGTCGACCTCGAGAGGCCT-3′ was annealed with a complementary short oligonucleotide, 5′-AGGCCTCTCGAGGTCGACC-3′, and filled in by Klenow DNA polymerase (BRL). The fragment was extracted with phenol/chloroform and purified with the qiaquick-spin PCR purification kit (Qiagen). The sample was then digested with *Eco*RI and *Xho* I and placed into a ligation reaction mixture in a 7:1 ratio with *Eco*RI/*Xho* I-digested pJG4-5 prey plasmid. The plasmids were propagated and grown in DH5α subcloning-efficiency competent cells from BRL.

The accuracy of the reading frames in the bait and prey plasmids was verified with an automated Applied Biosystems sequencer employing 373 software. Sequences were confirmed to be correct by the analysis features of SEQUENCE EDITOR and MACVECTOR software (data not presented).

Western blot analyses were performed (32) to show that the bait and prey plasmids expressed the expected fusion proteins (data not presented).

**Transformation of Strain with Reporter, Bait, and Prey Plasmids.** The selection strain was made by transforming the EGY48 yeast strain with a *URA3 lacZ* (β-galactosidase) reporter plasmid and the *HIS3* bait plasmid by the lithium acetate method (27). The yeast selection strain harboring the bait and reporter plasmids was transformed with the prey plasmid DNA (27), and tryptophan utilization phenotype was used (in addition to Ura and His markers for bait and *lacZ* reporter plasmids, respectively) for selection of transformants with prey plasmids.

**Determination of Bait–Prey Interaction.** Yeast strains containing the appropriate bait and prey plasmids were grown to an $OD_{600}$ of 0.5, diluted 1000-fold, and spotted on plates containing Glc $Ura^-$ $His^-$ $Trp^-$ 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) medium or Gal/Raf $Ura^-$ $His^-$ $Trp^-$ X-Gal medium to assess the transcriptional activation of the *lacZ* reporter gene. Suitably diluted cell suspensions were also spotted on Gal/Raf $Ura^-$ $His^-$ $Trp^-$ $Leu^-$ medium and Glc $Ura^-$ $His^-$ $Trp^-$ $Leu^-$ medium to assess the transcriptional activation of the *LEU2* gene.

***β*-Galactosidase Activity in Liquid Cultures of Yeast.** Cells were assayed for β-galactosidase activity by the *o*-nitrophenyl β-D-galactopyranoside (ONPG) method (27). The experiment was repeated in triplicate and the plotted data represent an average value of the values for the three samples. The statistical significance was computed with Student's *t* test in a two-tailed analysis.

**Immunoprecipitation and Western Blot.** Extracts were made from EGY48 cells that contained a prey plasmid encoding B42-Aβ and a bait plasmid encoding LexA-Aβ. Cells were grown in 100 ml of Glc or Gal/Raf medium (in which B42-Aβ expression was induced) to an $OD_{600}$ of 0.6–0.8, pelleted by centrifugation, resuspended in 500 μl of RIPA buffer (25), lysed by beating with glass beads five times for 2 min each, and spun twice for 5 min in a microcentrifuge (10,000 × *g*) at 4°C to remove the beads and cell debris. Five microliters of the supernatant was taken as a control, and 15 μl of rabbit anti-LexA antiserum [kindly donated by Roger Brent (33)] was added to the remainder, which was incubated at 4°C for 4 hr on a rotating platform. LexA-containing proteins were precipitated from this remainder with 50 μl of protein A-Sepharose CL-4B (Sigma). The entire pellet was dissolved in Laemmli sample buffer, subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE; Integrated Separation System, Hyde Park, MA), and blotted onto nitrocellulose. Tagged Aβ fusion proteins were identified by Western analysis of the blotted proteins with the 12CA5 monoclonal anti-HA antibody (34). Cell extracts and immunoprecipitates were also subjected to immunoblotting with monoclonal anti-Aβ antibodies 4G8 and 6E10. Western blot analysis was performed with ECL chemiluminescence reagents using the protocol supplied by the vendor (Amersham).

**Electron Microscopy.** Dilutions of Aβ for incubation with the octapeptide QKLVTTAE were performed as in ref. 41 (ratio of Aβ to octapeptide was 1:10). The photomicrographs were obtained with a JEOL JEM-100S electron microscope at 80 kV (×155,000 magnification).

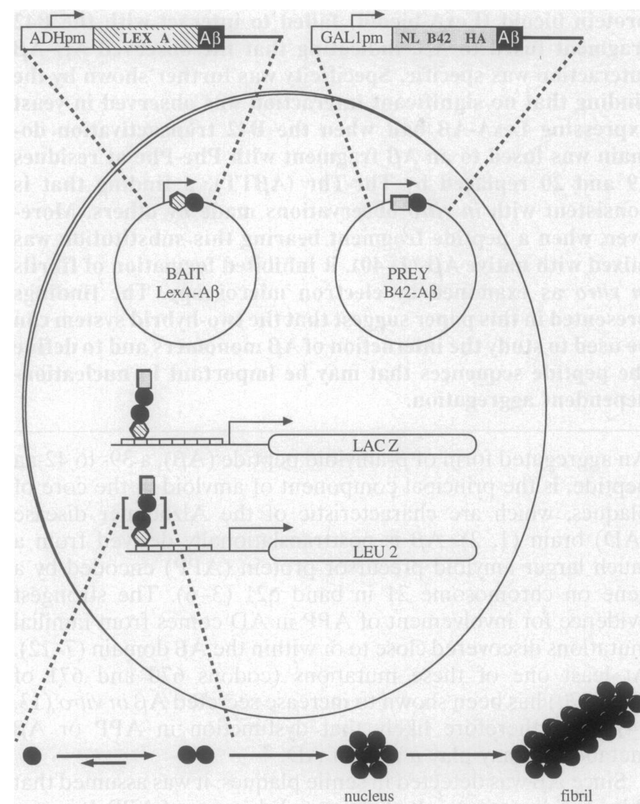


FIG. 1. Schematic representation of yeast strain EGY48 transformed with bait (LexA-Aβ fusion), prey (B42-Aβ fusion), and *lacZ* reporter plasmids. Bait fusion protein (LexA-Aβ) is produced constitutively under the control of the *ADH* promoter and binds to the upstream region of reporter genes at LexA operator sites; prey fusion peptide expression is driven by the *GAL1* promoter and is inducible by galactose. Prey fusion protein is expressed in the presence of galactose. If the Aβ portion of the prey protein binds the Aβ moiety of the bait fusion peptide, transcription from the reporter genes is triggered. The system therefore provides an opportunity to examine interaction between two monomeric Aβ molecules, an essential first step in the nucleation event leading to fibril formation.

## RESULTS

The experimental system established by Brent *et al.* (described in ref. 29) is depicted in Fig. 1. The selection strain contains either Aβ or bicoid as a bait fused in-frame to the bacterial LexA protein, which by itself has no transcriptional activation function in yeast (33). The host strain contains *LEU2* and *lacZ* reporters carrying LexA operators instead of native upstream activating sequences. A strain containing the bait (LexA-Aβ) and reporters (*LEU2* and *lacZ*) remains inert for the expression of leucine utilization or β-galactosidase activity unless it also contains a vector (prey) that expresses an interacting protein as a fusion molecule consisting of nuclear localization sequences from simian virus 40, the B42 acid blob transactivation domain, and an epitope tag from influenza virus HA protein (35). In this system, conditional expression of library-encoded proteins is directed by the *GAL1* promoter (achieved by growing yeast cells in Gal/Raf minimal medium).

We first determined whether EGY48 strains containing the LexA protein alone, LexA-Aβ fusion protein, or LexA-bicoid permitted the expression of *lacZ* or leucine genes. When EGY48 strains containing the individual LexA fusion baits were spotted at equal density on minimal medium plates containing Gal/Raf Ura$^-$ His$^-$ medium, similar growth rates were observed, indicating that none of the baits was toxic to yeast. These strains failed to grow on Gal/Raf Ura$^-$ His$^-$ Leu$^-$ medium and did not form blue colonies on Gal/Raf Ura$^-$ His$^-$ X-Gal medium (data not presented), indicating that none of the bait proteins by themselves could permit the expression of leucine or β-galactosidase phenotypes.

B42-Aβ prey plasmid was introduced into the yeast strain containing LexA-Aβ bait protein. Equal dilutions of this yeast strain were spotted on Gal/Raf Ura$^-$ His$^-$ Trp$^-$ X-Gal and Glc Ura$^-$ His$^-$ Trp$^-$ X-Gal media to measure expression of β-galactosidase, and on Gal/Raf Ura$^-$ His$^-$ Trp$^-$ Leu$^-$ and Glc Ura$^-$ His$^-$ Trp$^-$ Leu$^-$ media to check the expression of the leucine utilization phenotype. The B42-Aβ prey plasmid, when introduced into the yeast strain with LexA-Aβ bait, showed growth on minimal medium plates devoid of leucine (Fig. 2*A*) and showed blue colonies on X-Gal medium in the presence of Gal/Raf as the carbon source (Fig. 2*C*) but showed no growth (Fig. 2*B*) and no blue colonies (Fig. 2*D*) in the presence of glucose. These results indicate that the interaction between LexA-Aβ and B42-Aβ is triggered by expression of the B42 fusion protein under the influence of the *GAL1* promoter. When LexA-bicoid (Fig. 2) or LexA protein alone (data not presented) was used as bait, introduction of B42-Aβ plasmid did not result in growth on leucine plates or blue colonies on X-Gal medium in the presence of Gal/Raf, indicating that the

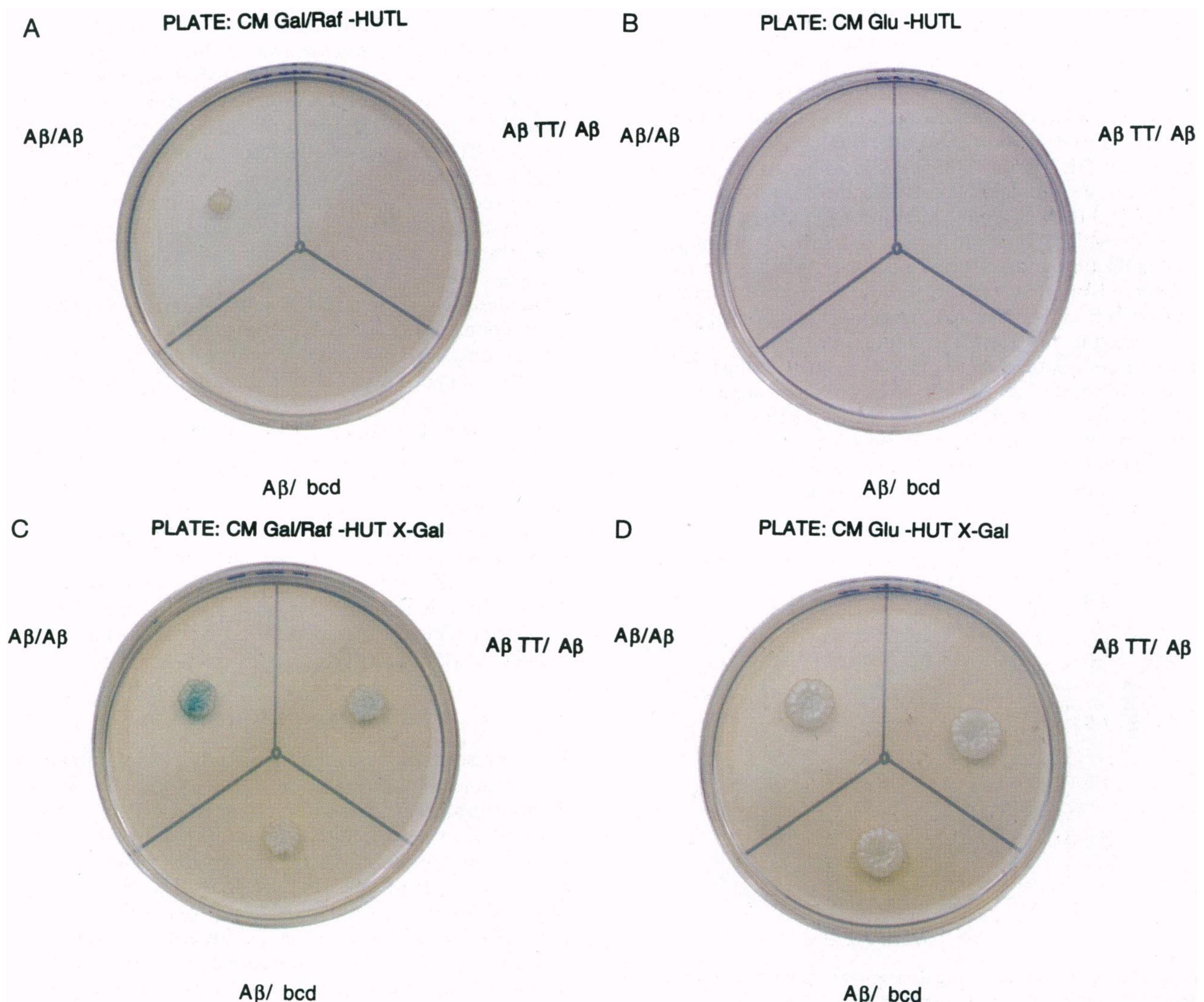


FIG. 2. Four-plate screen to examine interaction. B42-Aβ/LexA-Aβ strain, B42-AβTT/LexA-Aβ strain, and B42-Aβ/LexA-bcd strain were grown on plates containing Gal/Raf Ura$^-$ His$^-$ Trp$^-$ Leu$^-$ minimal medium (*A*), Glc Ura$^-$ His$^-$ Trp$^-$ Leu$^-$ minimal medium (*B*), Gal/Raf Ura$^-$ His$^-$ Trp$^-$ X-Gal minimal medium (*C*), or Glc Ura$^-$ His$^-$ Trp$^-$ X-Gal minimal medium (*D*).

interaction between LexA-Aβ and B42-Aβ is specific and most likely due to the intermolecular interaction between the Aβ molecules derived from the bait and the prey. When B42-AβTT prey plasmid was introduced into the yeast strain bearing LexA-Aβ bait, minimal growth was observed in plates devoid of leucine and no blue (or only very weakly blue) colonies were observed on X-Gal medium in the presence of glucose or Gal/Raf as carbon source (Fig. 2). This indicates that the Aβ molecule substituted at positions 19 and 20 with threonine residues interacts poorly with the wild-type Aβ peptide.

We next attempted to quantitate the observed Aβ–Aβ interaction by the ONPG colorimetric assay. Fig. 3 clearly indicates that there was significantly higher β-galactosidase activity in the yeast strain expressing B42-Aβ prey and LexA-Aβ bait compared with the yeast cells with B42-Aβ prey/LexA-bicoid bait (≈2.5-fold, $P = 0.01$, Student's *t* test) or B42-AβTT prey/LexA-Aβ bait (≈2-fold, $P = 0.02$). These results indicate that the Aβ–Aβ interaction inferred from Fig. 2 was significantly greater than the interaction between AβTT and Aβ or Aβ and bicoid monomers.

In an attempt to obtain direct *in vivo* evidence for the interaction between B42-Aβ prey and LexA-Aβ bait proteins, immunoprecipitates obtained by using antibodies against bait protein were subjected to immunoblotting with antibodies against the prey protein. Yeast cells expressing LexA-Aβ bait and B42-Aβ or B42-AβTT prey proteins were grown in glucose-containing medium and switched to glucose or Gal/Raf liquid minimal medium. The cells were harvested and cell extracts were prepared from equal numbers of cells. One aliquot of cell extract was subjected to immunoprecipitation with an anti-LexA antibody and the immunoprecipitates were subjected to Tris/tricine SDS/PAGE followed by immunoblotting with the monoclonal anti-HA antibody. If the two Aβ molecules or Aβ–AβTT molecules interact *in vivo*, one should be able to isolate the bait–prey complexes with antibody specific to the bait. Indeed, prey-specific HA immunoreactivity was observed (at 16 kDa; Fig. 4, lane 1) from the immunoprecipitates obtained from Aβ/Aβ cells grown in the presence of galactose, but not from the immunoprecipitates obtained from these cells subjected to glucose in the medium (Fig. 4, lane 2), indicating that the two Aβ fusion proteins interact inside the yeast cell nucleus. When Aβ/Aβ cell extracts were directly subjected to immunoblotting with anti-HA antibody, the 16-kDa band was observed in extracts derived from these cells grown in the presence of galactose (Fig. 4, lane 3), but no

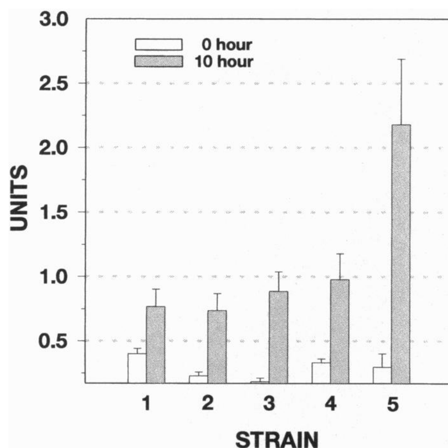


FIG. 3. Assay to determine β-galactosidase activity present in each of the strains tested after 0 hr (open bars) and 10 hr (filled bars) of incubation in Gal/Raf complete minimal medium. Bars: 1, B42-Aβ/LexA-bicoid; 2, B42 alone/LexA alone; 3, B42 alone/LexA-Aβ; 4, B42-AβTT/LexA-Aβ; 5, B42-Aβ/LexA-Aβ. These data are typical of three replicated experiments.

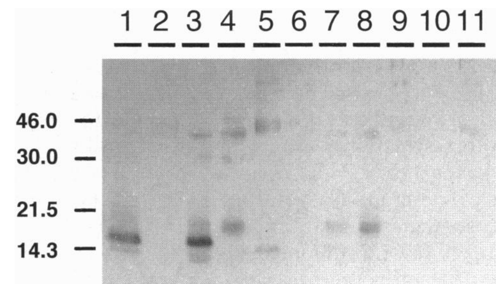


FIG. 4. Immunoprecipitates obtained by using LexA antiserum with extracts of B42-Aβ/LexA-Aβ strain grown in Gal/Raf medium (lane 1) or Glc medium (lane 2), cell extracts of B42-Aβ/LexA-Aβ cells grown in the presence of Gal/Raf (lane 3) or Glc (lane 4). Lanes 5–8 are similar to lanes 1–4 except that B42-AβTT/LexA-Aβ yeast strain was used. Lanes 9 and 10 represent immunoprecipitates and cell extracts obtained from LexA-Aβ strain containing no prey plasmid and from B42 alone/LexA alone yeast strain, respectively. Samples were electrophoresed in a Tris/tricine SDS/15% polyacrylamide gel and immunoblotted with anti-HA antibody.

immunoreactive band was observed for cells grown in the presence of glucose (Fig. 4, lane 4). The LexA immunoprecipitates obtained from AβTT/Aβ yeast grown on galactose resulted in very low levels of the anti-HA-immunoreactive 16-kDa band, seen only upon prolonged exposure (Fig. 4, lane 5; band not seen at this exposure). No 16-kDa band resulted from immunoprecipitates grown on glucose even on prolonged exposure (Fig. 4, lane 6). The anti-HA-immunoreactive 16-kDa band was, however, observed in cell extracts obtained from AβTT/Aβ yeast grown on galactose (Fig. 4, lane 7) but not in AβTT/Aβ yeast grown on glucose (Fig. 4, lane 8; the AβTT prey protein seems to run slightly lower than the Aβ prey protein). The observed molecular mass of 16 kDa is consistent with that predicted for a prey fusion protein and was also observed when anti-Aβ antibodies were used with cell extracts from Aβ/Aβ cells grown on galactose (data not presented). No immunoreactive bands were obtained in LexA immunoprecipitates derived from the EGY48 strain expressing the LexA-Aβ bait but containing no prey plasmid (Fig. 4, lane 9) and in cell extracts or immunoprecipitates from E9Y48 strain.

The observed weak interaction of AβTT with the native Aβ molecule was also examined by electron microscopy. An octapeptide fragment, QKLVTTAE, representing the FF-to-TT substitution at positions 19 and 20 in Aβ-(17–24) is capable of inhibiting fibril formation of the native Aβ-(1–40) (Fig. 5). Aβ-(1–40) at 250 μM, incubated for 4 days in water, showed significantly greater fibril formation (Fig. 5*A*) compared with the amount seen when Aβ-(1–40) at 250 μM was incubated with the octapeptide at 2.5 mM under the same conditions (Fig. 5*B*).

## DISCUSSION

In the present study we demonstrate that two monomers of Aβ are capable of interacting in a eukaryotic cell. We further demonstrate that this interaction is specific by using the *Drosophila* protein bicoid as a bait protein in this system. This interaction was found to be positive by using the *lacZ* and *LEU2* reporter systems (Fig. 2). Quantitation of β-galactosidase activity by the ONPG assay supported these conclusions (Fig. 3). Furthermore, direct evidence of interaction was obtained by subjecting immunoprecipitates obtained by using antibodies against bait protein to immunoblotting with antibodies raised against the HA epitope present on the prey protein (Fig. 4).

Hilbich *et al.* (36) have previously reported that a well-preserved hydrophobic core around aa 17–24 is important for

the formation of β-sheet structure and amyloid properties. When stained with Congo red, peptide Aβ-(10–42) or Aβ-(10–43) containing the FF-to-TT substitution (equivalent to the substitution in AβTT) did not reveal birefringence and showed decreased β-sheet content when compared with the native peptide by circular dichroism spectroscopy and by infrared spectroscopy. Moreover, when these substituted peptides were mixed with the native Aβ-(10–43) fragment at equimolar concentration, they inhibited the formation of filaments *in vitro* (below 15%), as detected by electron microscopic analysis (36). Our data suggest that the octapeptide fragment QKLVTTAE, representing the FF-to-TT substitution in Aβ-(17–24), is also capable of inhibiting the aggregation of native Aβ-(1–40) (Fig. 5; unpublished results). The results presented in this paper clearly demonstrate that AβTT fusion protein interacts poorly with the native Aβ (Figs. 2–4). Hilbich *et al.* (36) suggest that the Aβ and AβTT monomers may form oligomers that do not fit into the structure of a filament. Our data suggest that inhibition of filament formation by peptides representing the TT substitution (at 10-fold molar abundance compared with the native peptide) may be explained by a weak interaction between the Aβ and AβTT monomers. Alternatively, it is also possible that AβTT peptide may directly interfere with the fiber formation process.

Recent evidence has indicated that the cellular forms of prion protein ($PrP^c$) can form protease-resistant prion protein ($PrP^{sc}$) in a cell-free system in which $PrP^{sc}$ is used as a seed (37). This conversion did not require biosynthesis of new $PrP^c$, asparagine-linked glycosylation, or the presence of its normal glycosylphosphatidylinositol anchor, suggesting that oligomer

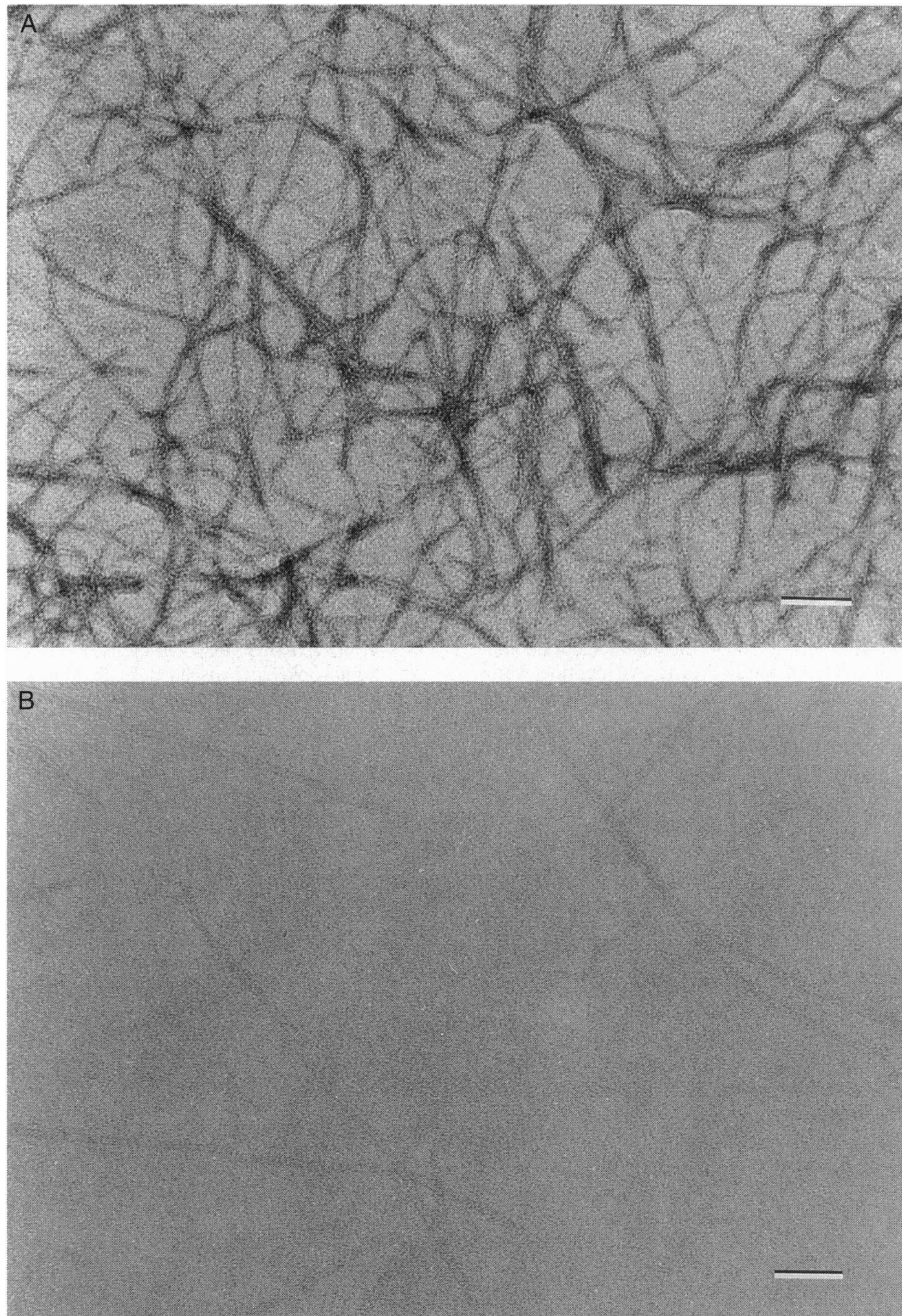


FIG. 5. Transmission electron micrographs of Aβ-(1–40) peptide incubated for 4 days in water (*A*) and Aβ-(1–40) peptide incubated in a 1:10 ratio with the octapeptide QKLVTTAE for 4 days in water (*B*). (Bar = 0.1 μm.)

formation between $PrP^{sc}$ and $PrP^{c}$ is sufficient for the conversion reaction to occur. Amyloid fibrils characterize several human diseases in which the presence of amyloid deposits is coincident with organ dysfunction. There is often a positive correlation between severity of the disease and the extent of fibril formation (38). Amyloid formation exhibits nucleation-dependent kinetics (22, 39–41). The slow and thermodynamically unfavorable interactions between individual monomers may be the rate-limiting step in aggregation. The yeast system described in this paper offers an opportunity to study the interaction of monomeric Aβ peptides. Although the peptides were expressed as fusion proteins, the results presented in this paper suggest that the observed interaction is due to the Aβ peptide domain. Results presented in Fig. 4 also suggest that no covalent higher-order bait–prey aggregates can be observed on the gel. This system may therefore provide an opportunity to freeze-frame the monomer–monomer interaction. Experimental system(s) such as the one presented in this paper may thus be used to study monomer–monomer interactions in other proteins that participate in nucleation-dependent amyloid formation.

There are some caveats in the present study. Although our results indicate that the interaction of LexA-Aβ and B42-Aβ fusion proteins occurs mainly because of amino acids within the two Aβ domains, it is possible that the conformation of the fusion proteins may influence this effect. These interactions take place in the yeast cell nucleus under conditions where the LexA-Aβ bait complex is bound to the LexA operator site. Whereas the yeast system described here is useful in studying intermolecular interactions, the intramolecular interactions may not be fully captured in a fusion-protein context. Furthermore, it is possible that interaction between bait-Aβ and prey-Aβ may not be relevant to fibril formation. This system will therefore have to be carefully validated by using molecules that are known to accelerate or inhibit the monomer–monomer interaction in Aβ fibrillogenesis.

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