Clorocinol promotes the degradation of metal-dependent amyloid-β (Aβ) oligomers to restore endocytosis and ameliorate Aβ toxicity

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Alzheimer’s disease (AD) is a common, progressive neurodegenerative disorder without effective disease-modifying therapies. The accumulation of amyloid-β peptide (Aβ) is associated with AD. However, identifying new compounds that antagonize the underlying cellular pathologies caused by Aβ has been hindered by a lack of cellular models amenable to high-throughput chemical screening. To address this gap, we use a robust and scalable yeast model of Aβ toxicity where the Aβ peptide transits through the secretory and endocytic compartments as it does in neurons. The pathogenic Aβ 1–42 peptide forms more oligomers and is more toxic than Aβ 1–40 and genome-wide genetic screens identified genes that are known risk factors for AD. Here, we report an unbiased screen of ~140,000 compounds for rescue of Aβ toxicity. Of ~30 hits, several were 8-hydroxyquinolines (8-OHQs). Clioquinol (CQ), an 8-OHQ previously reported to reduce Aβ burden, restore metal homeostasis, and improve cognition in mouse AD models, was also effective and rescued the toxicity of Aβ secreted from glutamatergic neurons in Caenorhabditis elegans. In yeast, CQ dramatically reduced Aβ peptide levels in a copper-dependent manner by increasing degradation, ultimately restoring endocytic function. This mirrored its effects on copper-dependent oligomer formation in vitro, which was also reversed by CQ. This unbiased screen indicates that copper-dependent Aβ oligomer formation contributes to Aβ toxicity within the secretory/endoosomal pathways where it can be targeted with selective metal binding compounds. Establishing the ability of the Aβ yeast model to identify disease-relevant compounds supports its further exploitation as a validated early discovery platform.


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Alzheimer’s disease (AD) is a common and devastating neurodegenerative disorder that is projected to increase in frequency as our population ages. The lack of disease-modifying therapies requires new approaches to address the underlying mechanisms of cellular dysfunction and identify potential therapeutic agents. The amyloid-β peptide (Aβ) plays a major role in AD and ultimately leads to neuronal death and cognitive impairment (1). Aβ peptides of ~40 aa are generated by the successive cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. Mutations in APP or γ-secretase cause familial AD and bias APP cleavage toward a 42-aa Aβ peptide that predominates in Aβ plaques, is more aggregation prone, and is toxic to neurons (2, 3). Although Aβ plaques are a common, conspicuous feature of pathology in diseased brains, increasing evidence suggests that small oligomers of Aβ are the most toxic species (4, 5).

The conservation of protein homeostasis mechanisms—such as protein trafficking and chaperone networks—among all eukaryotes makes yeast a powerful discovery platform for modeling the cellular toxicities caused by neurodegenerative disease proteins (6). In neurons, the cleavage of APP to generate the Aβ peptide occurs within the secretory and endosomal pathways (7). APP is trafficked through the secretory pathway to the plasma membrane and subsequently internalized and recycled through endosomal vesicles and the trans-Golgi network back to the plasma membrane (7). During this recycling, the Aβ peptide is liberated from APP by β/γ-secretases, thus enabling the peptide to interact with multiple membranous compartments within the cell.

We have taken advantage of the great conservation of the secretory and endocytic pathways between yeast and neurons to study Aβ in a simple, highly tractable model organism—budding yeast. By expressing Aβ as a fusion to an endoplasmic reticulum (ER) targeting signal, we have mimicked in yeast the multicompartmental distribution of Aβ (8). This approach bypasses the need to recapitulate the entire APP processing pathway and generates an Aβ peptide with exactly the same sequence as is found in the human brain. The ER targeting signal directs cotranslational transport of the primary translation product into the ER, where the signal sequence is removed by signal peptidase. The peptide then transits through the secretory pathway and is secreted from the cell. In yeast, the cell wall prevents secreted Aβ from diffusing away, allowing it to interact with the plasma membrane and undergo endocytosis. As in the human nervous system (2), the aggregation-prone 42-aa peptide is more prone to forming oligomeric species than the 40-aa peptide (9) and is more toxic (8).

This model allowed us to take advantage of yeast genetics to perform a completely unbiased screen of the yeast genome for suppressors or enhancers of Aβ toxicity. Of the ~6000 genes we tested, we recovered only a handful of modifiers. There are

Significance

Identifying disease-modifying therapies for Alzheimer’s disease (AD) has been an insurmountable challenge. To provide a new discovery tool for high-throughput compound screening, we used a simple yeast model that makes toxic amounts of β-amyloid (Aβ), a peptide central to AD pathology. Previous genetic analysis established that Aβ compromises yeast biology in a manner relevant to human AD. We screened 140,000 compounds for reversal of toxicity and identified a class of protective metal-binding compounds related to clioquinol (CQ), a compound that alleviates Aβ toxicity in mouse AD models. Treatment with CQ promoted rapid degradation of Aβ oligomers, rescuing cellular processes perturbed by this insidious peptide and restoring viability. Our approach provides a method for identifying compounds that may eventually help treat AD.
~25,000 genes in the human genome and less than 20 (10) have been shown to confer risk for AD. However, several of the yeast genes that alter Aβ toxicity are either direct homologs or interacting partners of human risk factors (8, 10). For example, YAP1β02, the yeast homolog of PICALM, one of the most highly validated risk factors for AD (10, 11); INP52, is homologous to SYNJ1, which interacts with the risk factor BIN1 (12, 13); and SLA1 is homologous to SH3KBP1, which interacts with the risk factor CD2AP (14–16). All of these proteins are involved in clathrin-mediated endocytosis in yeast and humans. In addition to ameliorating the toxicity of Aβ in yeast, these proteins reduced Aβ toxicity in both Caenorhabditis elegans and rat cortical neuronal models (8). The recovery of genes that promote clathrin-mediated endocytosis in unbiased genome-wide screens suggested that Aβ poisons this process (8). Indeed, this peptide provided sufficient endocytosis of a transmembrane receptor (Ste3), an activity crucial for neuronal function. Importantly, the mechanism of action of these risk factors had not previously been linked to Aβ. Thus, the yeast model has already provided key insights on the nature of Aβ’s cellular toxicity in the human brain.

In this study, we used our yeast Aβ model to identify small molecules that ameliorate toxicity. In an extremely stringent and unbiased screen of 140,000 compounds, we identified a small number of cytotoxic protective compounds, including 8-hydroxyquinolines (8-OHQs). Members of this family bind metals and are among the few compounds that have been shown to alleviate Aβ toxicity in mouse models of AD (17, 18), and to show early potential as an AD therapeutic (19). Here, we investigate the mechanism of action for the most efficacious member of this family, cloroquinol (CQ).

Results

Screen for Compounds That Rescue Aβ Toxicity in Yeast. To screen for compounds that ameliorate Aβ toxicity in yeast, we used a strain with a compromised drug efflux system that expresses Aβ under the control of a galactose-inducible promoter (8). This strain allows for direct measurement of Aβ toxicity that provided sufficient dynamic range and high signal-to-background signal to confidently identify rescuing compounds. Approximately 140,000 compounds were tested in duplicate in 384-well plates using increased growth (optical density) as an end point. The high reproducibility enabled robust detection of “hits” (Fig. L4). Only ~30 compounds passed our rigorous scoring criteria (Z score > 3), establishing the high stringency of the screen. Strikingly, one-half of all recovered hits were 8-OHQs (Fig. 1B and S1).

Notably, the effectiveness of this class of compounds against Aβ toxicity has been established in neurons and mouse models. Two closely related 8-OHQs—CQ and PBT2—decrease Aβ accumulation and alleviates cognitive and behavioral symptoms in mouse models of AD (17, 18). 8-OHQs are moderate-strength metal chelators that can extract metals weakly bound to proteins and both redistribute metals within the cell and act as ionophores (17, 18). PBT2 is one of only a few compounds effective in phase II clinical trials with human AD patients (19). CQ rescued our yeast Aβ model in a highly dose-dependent manner (Fig. 1 C and D), but at higher concentrations it inhibited the growth of both Aβ-expressing cells and control strains (Fig. 1C). The increase in optical density of Aβ-expressing cells with CQ was a direct consequence of increased viable colony-forming units and not an artifact of increased cell size (Fig. S2).

Due to our previous findings that different 8-OHQs have distinct activities in different models of proteotoxicity (20), we compared the efficacy of CQ in all of our yeast proteotoxicity models. The Aβ, α-synuclein (i.e., Parkinson disease), TDP-43 (i.e., frontotemporal dementia and ALS), and htt2Q (i.e., Huntington disease) yeast models have comparable levels of toxicity, but in each case the nature of the cellular toxicity is distinct as are the genetic hits obtained from unbiased genome-wide modifier screens (8, 21–23). CQ had significant, yet modest efficacy against α-syn and htt2Q and no effect on TDP-43 (Fig. 1E). However, it rescued the Aβ model more potently than it did any other model, indicating a considerable degree of selectivity.

Although CQ showed some protection in other models, we previously showed that CQ does not rescue the toxicity caused by diverse yeast genes when overexpressed from the same galactose-regulated promoter (20). In addition, CQ was unable to rescue growth defects caused by five different gene deletion mutations and seven toxic compounds with diverse modes of action (Table S1). Given the selectivity of CQ, we focused on investigating its activity in the yeast Aβ toxicity model.

CQ Restores Endosomal Trafficking. Our recent unbiased screen for suppressors of Aβ toxicity uncovered several proteins involved in clathrin-mediated endocytosis whose homologs are AD risk factors in humans (8). We directly tested the effect of Aβ on clathrin-mediated endocytosis using Ste3, a G-protein–coupled receptor for the mating pheromone. Indeed, steady-state Ste3 endocytosis was severely perturbed by Aβ and rescued by the genetic suppressors involved in this process (8). Here, we extended the analysis with another widely used reporter of endocytic function, a putative 13-pass transmembrane transporter for methionine, Mup1. This reporter provides a facile means of investigating stimulus-dependent trafficking: the endocytosis of Mup1 is rapidly induced by the addition of methionine.

As expected, Mup1-GFP localized primarily to the plasma membrane of WT cells in the absence of methionine (Fig. 2, Upper Left). After 30 min of methionine treatment, Mup1-GFP was endocytosed and exclusively detected in endosomes and the vacuole (Fig. 2, Upper Right). In the absence of methionine, Aβ expression perturbed steady-state trafficking of Mup1, detected by its localization to both the plasma membrane and to intracellular foci (Fig. 2, Lower Left). The addition of methionine promoted
CQ restores endosomal trafficking of Mup1-GFP. Mup1-GFP endocytosis was monitored in WT and Aβ strains (CQ, 0.8 μM). Cultures without methionine (left) or with 30-min methionine treatment (right) enabled visualization of stimulated endocytosis of Mup1-GFP from the plasma membrane to the vacuole.

Fig. 2. CQ restores endosomal trafficking of Mup1-GFP.

CQ Rescues a C. elegans Model of Aβ Toxicity. To establish that the ability of CQ to rescue the toxicity of Aβ in the secretory compartment was conserved in neurons, we tested a nematode model of Aβ toxicity. In this model, Aβ is expressed in the secretory pathway of glutamatergic neurons (a neuronal subtype particularly relevant to AD) using the eat-4 promoter and a C. elegans ER signal sequence (8). An advantage of C. elegans is that the transparency of the cuticle allows surviving neurons to be visualized in living animals expressing GFP in the same neurons. Aβ expression produced an age-dependent loss of glutamatergic neurons. Importantly, the endocytosis-related genes we originally identified in our yeast screen, which are also known AD risk factors in humans, suppress Aβ toxicity in this nematode model (8). Expressing Aβ in glutamatergic neurons enables a quantitative measure of neurodegeneration and differs from previous approaches in the nematode where Aβ expressed within the body wall muscle cells caused a motor phenotype (24, 25).

As previously described, the percentage of worms with the WT number of neurons decreased from 3 to 7 d post larval hatching [Fig. 3A] (8)]. Because the cuticle of the worm is relatively impenetrable, we used an established protocol to administer CQ acutely at the earliest larval stage (26). After 24 h, the worms were transferred to normal growth media and allowed to develop and age in the absence of CQ. Even with this single early dosing, CQ increased the percentage of worms with the WT number of neurons. Rescue was apparent at both 3 and 7 d post CQ treatment (Fig. 3A and B). Thus, as in yeast, the toxicity of secreted forms of Aβ is rescued by CQ in glutamatergic neurons. This encouraged us to take advantage of the yeast model to further investigate CQ’s mechanism of action.

CQ Rescue of Aβ Toxicity Requires Metal Binding. Because 8-OHQs are known metal binding compounds, we first asked whether this functionality was required for the rescue of Aβ toxicity. We tested CQ analogs lacking either the hydroxyl group or the aromatic nitrogen. Together, these groups coordinate the bidentate binding of transition metals ions. Because direct analogs of CQ lacking these groups were not available, we tested analogs of chloroxine (COCl) (Fig. 4). This 8-OHQ is identical to CQ except that it has a chlorine atom in place of the iodine atom. It was as effective at alleviating Aβ toxicity in yeast as was CQ (Fig. 4B, “Max”). We then tested COCl analogs lacking either the hydroxyl group (COCl-OH) or the nitrogen atom (COCl-N). Both were inactive against Aβ, indicating CQ efficacy requires bidentate metal binding (Fig. 4B).

Next, we tested the ability of exogenous metals to alter CQ activity. We used copper, iron, and zinc, all of which are known to bind CQ (27). On their own, none of these metals affected Aβ toxicity (Fig. 4C). When coadministered at a concentration that was equimolar with CQ, each metal had completely different effects (Fig. 4C). Copper abolished rescue of Aβ by low concentrations of CQ and also abolished the growth inhibition caused by higher CQ concentrations. Iron shifted the dose–response, increasing the required dose for rescue and reducing the growth inhibitory effects of CQ. Zinc had no effect on CQ efficacy or growth inhibition at higher concentrations. These data suggest that CQ binds copper, and to a lesser extent iron, to alleviate Aβ toxicity.

Effects of Metals and CQ on In Vitro Aβ Assembly. Metal ions, including copper, iron, and zinc, bind to Aβ both in vitro and within Aβ plaques from the brains of AD patients (28, 29). In vitro assembly reactions with Aβ, the addition of copper favors the formation of nonfibrillar, amorphous aggregates (29) and oligomers (30, 31) that are more toxic to cells than amyloid fibers of Aβ (18, 30–32). Metal binding compounds, such as CQ and PBT2, can remove metals from Aβ in vitro (17, 18, 33, 34). These compounds also increase the amount of soluble Aβ in the brains of AD mouse models in vivo and stimulate the extraction of Aβ from plaques in brain samples from AD patients (17). Because the predominant form of Aβ 1–42 in yeast is small oligomers (8) and copper appears central to rescue of Aβ toxicity (Fig. 4C), we...
tested the effects of copper on Aβ oligomerization in vitro, and asked how CQ affects this process.

We first tested the effects of copper on Aβ 1–42 fiber formation using Thioflavin T (ThT), which binds β-sheet–rich proteins (i.e., amyloid fibrils). Copper strongly inhibited amyloid fiber formation when present in Aβ assembly reactions (Fig. 5A) (29). To characterize the species of Aβ that accumulate in the presence of copper, we used an antibody known as A11. This antibody, which was raised against a form of Aβ that mimics toxic oligomers, recognizes specific prefibrillar conformers of Aβ that are highly toxic to neurons (35). Aβ assembly reactions were carried out with both equimolar (1:1) and excess (5:1) copper relative to Aβ for 30 min and then subject to dot blot analysis with A11 and another antibody that detects total Aβ (6E10). Copper substantially increased the amount of A11-reactive Aβ (Fig. 5B). CQ had no effect on the formation of A11-reactive Aβ species on its own, but it strongly antagonized the oligomer-potentiating effects of copper (Fig. 5C).

**CQ Dramatically Reduced Aβ Peptide Levels by Increasing Degradation.**

To determine whether CQ has a similar ability to modulate Aβ within the context of the secretory compartment of a living cell, we turned to yeast. First, we examined the effects of CQ on the accumulation of Aβ using denaturing SDS gels. CQ dramatically reduced the accumulation of Aβ, with a sharp dose dependence that mirrored the sharp dose dependence of CQ's ability to rescue Aβ toxicity (Fig. 6A, Upper). CQ had no effect on total protein levels (Fig. 6A, Lower).

We next tested whether exogenous metals antagonized the ability of CQ to reduce Aβ levels. Indeed, they did so in a manner similar to their effects on the rescue of Aβ toxicity (Fig. 4C). Copper completely eliminated CQ's effects on Aβ accumulation; iron affected it modestly; and zinc had no effect (Fig. 6B). We next asked what species of Aβ were most affected by CQ. Nondenaturing gels, which preserved oligomer structure, demonstrated a decrease in most forms of Aβ when cultures were treated with CQ (Fig. 6C, Right). The levels of a control protein (YFP) expressed from the same promoter did not change (Fig. 6C, Left). Thus, the ability of CQ to rescue Aβ toxicity directly correlated with its ability to prevent the accumulation of oligomeric Aβ in a manner dependent on metal binding.

The decrease in oligomeric Aβ could be due to either its reduced accumulation or accelerated degradation. To distinguish between these two possibilities, we used two methods to assess degradation rates. First, we treated cells with the protein synthesis inhibitor, cycloheximide (CHX), and monitored Aβ levels by immunoblotting. After expressing Aβ for 24 h with either DMSO or CQ, cells were treated with CHX for 15 or 45 min. In CQ-treated cells, Aβ was rapidly degraded within 15 min of inhibiting translation (Fig. 6D). This increased degradation was not observed with a control protein (Pgk1). In contrast, the Aβ peptide that accumulated in cells treated with DMSO did not significantly degrade during this same time frame (Fig. 6D).

Inhibiting translation in this global manner might affect all cellular degradation pathways and confound interpretations. We therefore used [35S]methionine pulse labeling and immunoprecipitation. Cells expressing Aβ were transiently labeled with [35S]methionine to allow incorporation of radiolabeled methionine into newly synthesized proteins. After chasing cultures with nonlabeled methionine, we monitored the remaining Aβ by immunoprecipitating the peptide from cell lysates. In agreement with the CHX experiment, CQ specifically increased degradation of Aβ (Fig. 6E and Fig. S3). Together, the two experiments indicate that the stability of Aβ oligomers formed within the secretory pathway is dependent on metals and that, by affecting these oligomers, CQ can directly antagonize the cellular pathologies caused by Aβ.

**Discussion**

We have used an unbiased phenotypic small-molecule screen of ~140,000 compounds to identify a clinically relevant class of compounds that prevent the toxic effects of Aβ expressed within the secretory pathway. Although multiple metal-related Aβ pathologies and modes of action for CQ—and PBT2—have been described and postulated (17, 18, 28), our data suggest a mechanism of action in the yeast model that is not ionophore based (18). Rather, CQ promoted the degradation of metal-dependent Aβ oligomers within the secretory and endosomal compartments.

![Diagram](Image)

**Fig. 5.** Effects of copper and CQ on Aβ oligomers. (A) ThT fluorescence of Aβ assembly reactions in the presence of copper (micromolar) at indicate molar ratios compared with Aβ. EM shows representative fields of Aβ with no or 50 µM Cu²⁺. (B) Dot blot analysis of Aβ assembly (10 µM) in the presence or absence of both copper and CQ at 1:1 and 5:1 molar ratios. A11 monitors oligomeric Aβ conformations and 6E10 monitors total Aβ. (C) Dot blot (Left) and quantitation (Right) of Aβ assembly reactions in the presence of indicated concentrations of copper and CQ.
Empowered by significant improvements in DNA sequencing, a considerable effort is now underway to catalog polymorphisms in the human genome and identify those that contribute to pathologies related to protein homeostasis. It is now clear that the total number of human polymorphisms is much greater than previously realized (40), creating an intense need for rapid and tractable model systems to explore genotype–phenotype causality. Our yeast Aβ system meets this need. A previous unbiased genetic screen for modifiers of Aβ toxicity allowed us to link toxicity to recently discovered human AD risk alleles (8) with no previously known relationship to Aβ. Here, the facile yeast system enabled high-throughput compound screening that allowed us to rapidly identify relevant compounds and interrogate their mechanism of action. This ultimately validates our approach as we pursue new compounds with unknown protective mechanisms. Although this initial screen primarily revealed the central importance of metals in cellular Aβ pathology, we anticipate that other compounds with distinct modes of action will be identified.

In general, for AD and other protein-misfolding diseases, yeast chemical genetic approaches will help elucidate new targets and “druggable” pathways and aid discovery and validation (38). It is imperative to integrate several orthogonal model systems and many other recent advances (10, 22, 38, 41, 42) to further our understanding of, and eventually our ability to treat, devastating diseases such as AD.

Methods

Constructs and Yeast Strains. The signal sequence:Aβ (ssAβ) construct has been described (8). The chemical screening strain used here was generated by integrating either GAL1-regulated Aβ or YFP constructs into the trp1 and ura3 loci of a WT strain and deleting the drug efflux pump (pdr5::KanMX) using standard methods (Table S1). The MUP1-GFP Aβ strain was generated by recombining a PCR product of GFP and a downstream (Table S2).

Small-Molecule Screen and Compounds. Small-molecule screening was carried out as described (20). Briefly, late log phase Aβ cultures grown in 2% (wt/vol) raffinose-containing media were diluted (OD600 = 0.03) into galactose-containing media. Compounds (100 nL) were pinned to 384-well plates containing diluted culture and incubated for 40 h at 30 °C. OD600 values were then used to calculate Z scores (OD600 well – OD600 plate average)/OD600 plate average. Compounds were purchased as follows: CQ (Sigma-Aldrich); CuCl2 (Sigma-Aldrich); CuCl2·OH (Santa Cruz Biotechnology); CuCl2·N (Frontier Scientific Services).

Growth Assays. Growth assays with compounds were performed by either Bioscreen C or 384-well plates analysis. In both cases, log phase Aβ cultures grown in 2% (wt/vol) raffinose-containing media were diluted (OD600 = 0.02) into galactose-containing media, compounds or DMSO added, and cultures grown at 30 °C with either intermittent shaking (Bioscreen C) or endpoint (384-well) OD600 readings. Data were typically expressed as the difference in OD600 between compound- and DMSO-treated cultures. For all metal experiments, metals were added at the same time as CQ. Specificity experiments were performed in conditions with at least 50% reduction in growth to gene deletion or toxic compounds (Table S2). Toxic compound concentrations were selected to inhibit growth of WT yeast by at least 50%.

MUP1-GFP Assay. The effects of Aβ expression on Mup1-GFP endocytosis were performed as described (38). Cultures induced for ~16 h with SGalMet in a Bioscreen C with or without CQ (0.8 μM), methionine (50 μg/mL) added for 30 min, and GFP localization then imaged by fluorescence microscopy.

C. elegans EQT Treatment. The C. elegans strain (UA166) expressing an ssAβ construct in glutamatergic neurons has been described (8). Compound treatment (20) and analysis of glutamatergic neurons (8) have been described.

In Vitro Aβ Oligomer Formation. Lyophilized, chemically synthesized Aβ(1–42) (American Peptide Company) was dissolved at ~1 mg/mL in pH 11.5 NaOH and flash frozen. Exact concentration was measured using amino acid analysis (Dana Farber Molecular Biology Core). For THT assays, Aβ was dissolved in PBS (pH 7.2) to a final concentration of 10 μM and THT was added to a final concentration of 20 μM. The solution was allowed to aggregate at 30 °C under quiescent conditions and fluorescence at 480 nm was monitored for 12 h using a Tecan Safire plate reader. For dot blots, 300 μL of solutions
containing 10 μM Aβj with varying concentrations of Cu and CQ were loaded onto a 0.1-μm nitrocellulose membrane using the Bio-Rad vacuum filter trap device. The membrane was then blocked in 5% (v/v) nonfat dry milk in PBS for 1–2 h followed by an overnight incubation in A11 antibody (Millipore; 1:250 in 5% (v/v) milk/PBS) and a horseradish peroxidase-conjugated anti-rabbit secondary antibody (Sigma). Quantification of dot blots was performed using ImageJ (NIH). Transmission electron microscopy was performed by incubating Aβj solutions aggregated for ~12–15 h on a Nickel Formvar grid followed by negative staining by 2% (wt/vol) uranyl acetate.

Effect of CQ on Aβ Levels. The ssAβj and YFP strains were grown to log phase at 30 °C and diluted to an O.D. of 0.01 (YFP) or 0.03 (Aβ) in media containing galactose with either 0.6 μM Cu or DMSO and grown in a Bioscreen C plate at 30 °C to an O.D. of ~0.6 (~16 h). Lyastes were generated and blots probed as previously described (8).

Analysis of Aβ Degradation. For CHX experiments, Aβ yeast strains were grown to log phase with end-specific A β monoclonals: Evidence that an initially deposited species is Aβ 42/40. Neuroreport 13(2):215-221.


