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Nuclear Translocation Uncovers the Amyloid Peptide Aβ42 as a Regulator of Gene Transcription*◆

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Christian Barucker[‡§], Anja Harmeier[‡1], Joerg Weiske[¶2], Beatrix Fauler[‖], Kai Frederik Albring[¶], Stefan Prokop[‡‡], Peter Hildebrand[§§], Rudi Lurz[‖], Frank L. Heppner[‡‡], Otmar Huber[¶**], and Gerhard Multhaup[‡§3]**

*From the [‡]Institut fuer Chemie und Biochemie, Freie Universitaet Berlin, 14195 Berlin, Germany, the [§]Department of Pharmacology and Therapeutics, Faculty of Medicine, McGill University, Montreal, Quebec H3G 0B1, Canada, the [¶]Institute of Clinical Chemistry and Pathobiochemistry, Charite-Campus Benjamin Franklin, 12203 Berlin, Germany, the [‖]Max Planck Institute for Molecular Genetics, 14195 Berlin, Germany, the **Institute of Biochemistry II, Jena University Hospital, Friedrich Schiller University, 07743 Jena, Germany, and the [‡‡]Department of Neuropathology, [§§]Institute of Medical Physics and Biophysics, Charite-Universitätsmedizin Berlin, 10117 Berlin, Germany*

Background: Biological activities of nontoxic Aβ42 peptides remain unclear in Alzheimer disease.
Results: Aβ species are taken up in the nucleus of cells by a nonregulated mechanism, but only Aβ42 plays a role in gene transcription.
Conclusion: Aβ42 may act as a transcriptional regulator, similar to the cytoplasmic fragment AICD.
Significance: Genes regulated by nuclear Aβ42 could represent alternative targets for therapeutic approaches.

Although soluble species of the amyloid-β peptide Aβ42 correlate with disease symptoms in Alzheimer disease, little is known about the biological activities of amyloid-β (Aβ). Here, we show that Aβ peptides varying in lengths from 38 to 43 amino acids are internalized by cultured neuroblastoma cells and can be found in the nucleus. By three independent methods, we demonstrate direct detection of nuclear Aβ42 as follows: (i) biochemical analysis of nuclear fractions; (ii) detection of biotin-labeled Aβ in living cells by confocal laser scanning microscopy; and (iii) transmission electron microscopy of Aβ in cultured cells, as well as brain tissue of wild-type and transgenic APPPS1 mice (overexpression of amyloid precursor protein and presenilin 1 with Swedish and L166P mutations, respectively). Also, this study details a novel role for Aβ42 in nuclear signaling, distinct from the amyloid precursor protein intracellular domain. Chromatin immunoprecipitation showed that Aβ42 specifically interacts as a repressor of gene transcription with *LRP1* and *KAI1* promoters. By quantitative RT-PCR, we confirmed that mRNA levels of the examined candidate genes were exclusively decreased by the potentially neurotoxic Aβ42 wild-type peptide. Shorter peptides (Aβ38 or Aβ40) and other longer peptides (nontoxic Aβ42 G33A substitution or Aβ43) did not affect mRNA levels. Overall, our data indicate that the nuclear translocation of Aβ42 impacts gene regulation, and deleterious effects of Aβ42 in Alzheimer disease pathogenesis may be influenced by altering the expression profiles of disease-modifying genes.

The amyloid precursor protein (APP)[4] is first cleaved by the β-site APP-cleaving enzyme (BACE1) and sequentially processed by the γ-secretase complex to generate amyloid-β (Aβ) peptides of varying lengths encompassing 38, 40, 42, and 43 residues (1). Aβ generation, through amyloidogenic processing of APP, results in the simultaneous production of a C-terminal fragment corresponding to the APP intracellular domain (AICD). This has been reported to translocate into the nucleus and activate gene transcription (2, 3).

Aβ42 is hypothesized to be the main culprit in the pathogenesis of Alzheimer disease (AD) as it was postulated to impair synaptic function and initiate neuronal degeneration (4). In AD, soluble species of Aβ42 are more strongly correlated with disease symptoms than with amyloid plaques (5–9). Aβ42 was also reported to have an effect on differentiation and death of cultured neural stem or progenitor cells (10). Intraneuronal Aβ accumulation in brains of patients with AD, in animal models, and in cultured cells has suggested a pathophysiological role specific for Aβ40 and Aβ42 (11). Moreover, oxidative DNA damage in guinea pig primary neurons was shown to induce Aβ42 accumulation in the cytosol and to activate the p53 promoter (12). Similarly, under conditions of stress, Tau aggregates, which are the neuropathological hallmark of several neurodegenerative diseases, were found in the nucleus of neurons (13). In primary neurons, microinjection of Aβ42 rapidly

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[1] Present address: F. Hoffmann-La Roche AG, Pharma Research and Early Development, Discovery and Translational Area Neuroscience Basel, 4070 Basel, Switzerland.
[2] Present address: Bayer Pharma AG, 13353 Berlin, Germany.
[3] To whom correspondence should be addressed: Dept. of Pharmacology and Therapeutics, Faculty of Medicine, McGill University, 3655 Promenade Sir-William-Osler, Montreal, Quebec H3G 1Y6, Canada. Tel.: 514-398-3621; Fax: 514-398-2045; E-mail: gerhard.multhaup@mcgill.ca.

[4] The abbreviations used are: APP, amyloid precursor protein; Aβ, amyloid-β; AD, Alzheimer disease; AICD, APP intracellular domain; PS1, presenilin 1; TEM, transmission electron microscopy; tg, transgenic; qRT-PCR, quantitative real time PCR; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; MEF, mouse embryo fibroblast.

induced cell death, further underlining the neurotoxicity of intracellular amyloid (14). Accordingly, transgenic (tg) mice producing only intracellular Aβ developed neurodegeneration (15).

The presence of intraneuronal Aβ is explained by a dynamic relationship that exists between pools of intracellular and extracellular Aβ (16). Intraneuronal Aβ originates both from APP inside the neurons (17) and via uptake from the extracellular space. Aβ is internalized by neurons as well as by non-neuronal cells in culture, although the molecular events involved remain unclear (18–20). Aβ has been shown to accumulate within certain organelles, including the endosomes/lysosomes, and mitochondria (21), resulting in endosomal/lysosomal leakage, mitochondrial dysfunction, and apoptosis (22). Less well characterized is the peptide's ability to create channel-like pores in membranes (23–26).

Channel-like pores may allow direct passage of Aβ oligomers into the nucleus, although at present it is not known whether Aβ peptides have a biological activity in the nucleus. Here, we show that Aβ peptides of varying lengths such as Aβ38, Aβ40, Aβ42, and Aβ43 are internalized by neuroblastoma cells and are subsequently detected in the nucleus. The nuclear localization of internalized Aβ42 peptides was further confirmed by both confocal and transmission electron microscopy (TEM). We also demonstrated the presence of endogenous Aβ42 peptides in the nuclei of neurons of tg APP mice (APPPS1). Using the chromatin immunoprecipitation (ChIP) assay, Aβ42 was found to specifically interact with the *LRP1* and *KAI1* promoter. Thus, at higher concentrations the widely recognized neurotoxic form Aβ42 here acted at sublethal concentrations as a repressor of transcription of the genes *LRP1* and *KAI1*. This result was confirmed by quantification of the mRNA levels of the examined candidate genes by qRT-PCR. Furthermore, we found that Aβ42 increased the transcription of its own precursor gene *APP*. The mRNA levels were exclusively altered by the neurotoxic Aβ42 wild-type peptide, whereas neither Aβ38, Aβ40, nor Aβ43 had any effect on mRNA levels. Treatment with the nontoxic substitution peptide Aβ42 G33A, which was used as a control because it forms β-pleated sheet aggregates like the wild-type peptide (5), also did not have an effect. Although for all Aβ peptides tested a nuclear translocation was observed, albeit to a varying extent, only Aβ42 entailed gene regulation. Thus, the major deleterious effects in the pathogenesis could be mediated by Aβ42 gene control activity, because it specifically interacts as a repressor or activator, respectively, of gene transcription.

EXPERIMENTAL PROCEDURES

Cell Culture—SH-SY5Y and HEK-293 cells were purchased from DSMZ (ACC 209 and ACC 305) and were routinely cultured as described (5, 27). Briefly, SH-SY5Y cells were synchronized in the G_1 phase of the cell cycle by double-thymidine block (2 mM for 15 h; Sigma). After blocks, cells were grown in fresh medium without thymidine for 8 or 2 h (medium containing 20% (v/v) fetal calf serum) and released to progress through the cell cycle. As growth medium of MEF PS1/2 KO cells, we used DMEM high glucose (PAA), 10% fetal calf serum (PAA), 2 mM glutamine (PAA), and 0.1 mM nonessential amino acids (PAA).

Aβ Peptides and MTT Assay—Synthetic Aβ peptides (Peptide Specialty Laboratories, Germany) were monomerized and solubilized as described (5). Briefly, monomerized peptides were dissolved to 1 mg/ml in deionized water supplemented with ammonia to a final concentration of 0.13% (measured at pH 9.8). All peptides were used at a concentration of 1 μM. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed as described previously (5).

Nuclei Preparation and ELISA—Nuclei were isolated with the Nuclei EZ Prep nuclei isolation kit according to the manufacturer's instructions (Sigma) with minor modifications. Briefly, pellets containing nuclei were resuspended in PBS, sonicated, incubated at 95 °C, and centrifuged for 10 min at 20,000 × *g*. Aβ42 ELISAs using the C-terminal specific G2-13 antibody were performed as described (28). To quantify Aβ38, Aβ40, Aβ42, Aβ42 G33A, and Aβ43, the antibody 4G8 (HISS Diagnostics) recognizing Aβ residues 17–24 was used.

Immunodetection was performed with anti-histone H1 (MAB052), anti-actin (MAB1501), anti-calnexin (MAB3126), anti-GAPDH (MAB374) (all from Millipore), anti-lamin A/C (BD Biosciences), and flotillin1 (BD Biosciences) antibodies after electrophoresis (SDS or Tris-Tricine gels (Anamed)). Western blot analyses of aggregated peptides were performed as described previously (5). Briefly, 4× sample buffer was added to Aβ-containing fractions, and samples were electrophoresed on 10–20% Tris-Tricine gels (Anamed). Blots were incubated with 1.5 μg/ml W0-2 antibody (The Genetics Company (TGC), Switzerland) recognizing the Aβ residues 5–8 (29). Chemiluminescence was detected by ECL.

Chromatin Immunoprecipitation—ChIP was performed as described previously (30) with minor modifications. SH-S5Y5 cells were synchronized and grown on 100-mm dishes to a confluence of 80–90%. At the indicated times after the addition of 1 μM freshly dissolved Aβ peptide, cells were washed twice with PBS, fixed with 2 mM disuccinimidyl glutarate for 45 min at room temperature, and then cross-linked for 10 min at room temperature using 1% (v/v) formaldehyde. For immunoprecipitation, 2 μg of anti-NCoR (Santa Cruz Biotechnology), anti-Fe65 (Millipore), anti-Tip60 (Millipore), anti-Aβ (W0–2; TGC), or anti-NFκB p50 (Santa Cruz Biotechnology) antibodies were used. For PCR analysis, 2 μl of extracted DNA were used as template. Primers for the p50-binding motif in the *KAI1* proximal promoter were described previously (31) and for the *LRP1* promoter fragment were forward, 5′-GGG AGC CTG AAA TCC TAG AG-3′, and reverse, 5′-GGA AAG CGG TCC AAG AGT G-3′, and for the *HES1* promoter fragment were forward, 5-CTC CCA TTG GCT GAA AGT TAC-3′, and reverse, 5′-TGG CTA CTT GGT GAT CAG TAG-3′. PCR cycles were 2 min at 94 °C, 28–32 cycles at 94 °C for 15 s, annealing for *KAI1* promoter (30 s at 69 °C), for the *HES1* promoter (30 s at 57 °C) and *LRP1* promoter (30 s at 60 °C), elongation for 45 s at 72 °C, final extension for 3 min at 72 °C. PCR products were separated on 8% polyacrylamide gels.

Real Time PCR—Total RNA was isolated with the NucleoSpin RNA kit (Macherey & Nagel). RNA quality was determined by NanoDrop (PeqLab) and Bioanalyzer (Agilent

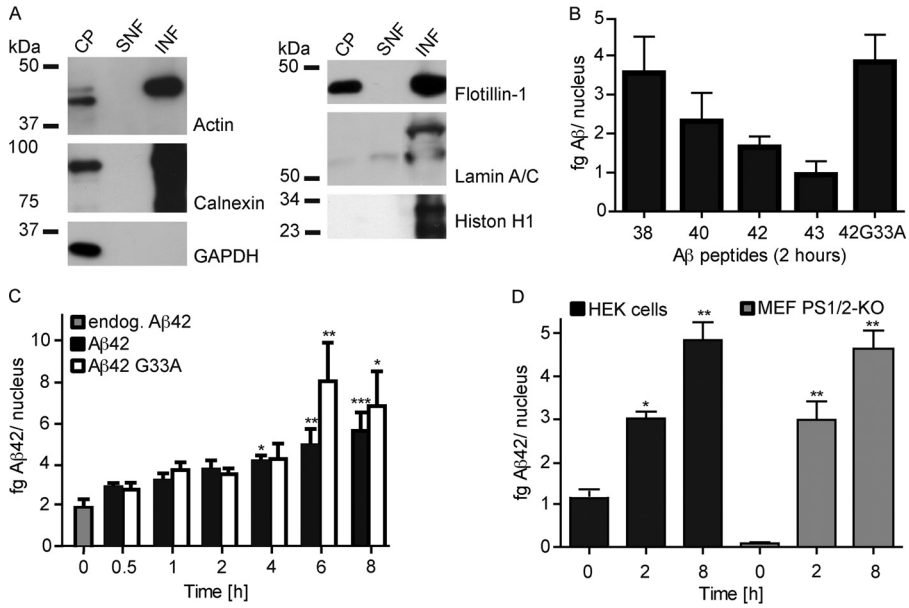

FIGURE 1. **Purity of cytoplasmic and nuclear fractions and nuclear Aβ uptake.** *A,* Western blot analysis of nuclear fractions for cytoplasmic or nuclear membrane marker proteins of SH-SY5Y cells. Cytoplasmic (*CP*) or nuclear membrane (insoluble nuclear fraction, *INF*) marker proteins are absent in the soluble nuclear fraction (*SNF*) that was analyzed for quantitative determination of Aβ by ELISA. Cytoplasmic or nuclear membrane marker proteins were found in the insoluble nuclear fraction (*INF*) that was not used for Aβ determination by ELISA. *B,* quantification of nuclear Aβ by ELISA after treatment of SH-SY5Y cells with freshly dissolved Aβ peptides. Aβ38, Aβ40, Aβ42, Aβ43, and Aβ42 G33A are displayed as detected in nuclear fractions of cells treated with individual peptides for 2 h using the 4G8 antibody to the epitope residues 17–24; number of experiments (n) = 4–8. *C,* nuclear fractions were analyzed for Aβ42 and Aβ42 G33A by ELISA using the Aβ42 C-terminal specific antibody G2-13 after treatment of SH-SY5Y cells for the indicated times. The *graph* shows an increase in Aβ compared with the endogenous level of untreated cells (*, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$); n = 4–7. *D,* HEK-293 and MEF PS1/2-KO cells were incubated for the indicated time points, and nuclear fractions were analyzed for Aβ42 by ELISA using the C-terminal specific antibody G2-13. Compared with the endogenous Aβ42 levels, the *graph* displays an increase over time (*, $p < 0.05$; **, $p < 0.005$), n = 3–5.

Technologies). For reverse transcription, the high capacity cDNA reverse transcription kit (Applied Biosystems) and for qRT-PCR the TaqMan gene expression assays HS01552282 (*app*), HS00172878_m1 (*he-1*), HS00174463_m1 (*KA11*), and HS01059295_m1 (*lrp1*) were used.

Immunofluorescence—SH-SY5Y cells were treated with biotinylated Aβ42 peptide 1 μM. Cells were fixed and permeabilized with 3.3% formaldehyde containing 0.5% Triton X-100 followed by 125 mM glycine in PBS containing magnesium and calcium. Cells were blocked with 5% fetal bovine calf serum followed by the primary antibody. Biotin-Aβ42 was detected with the monoclonal antibody AB (Sigma) or alternatively with Avidin Fluor488 (Sigma). Nuclei were stained with DAPI (Roche Diagnostics). Images were obtained using an LSM 510 meta (Carl Zeiss) confocal microscope.

Animal Studies and TEM—Transgene-negative littermate controls were obtained from the heterozygous breeding of *APPPS1*$^{+/-}$ mice. Genotyping was performed according to published protocols (32). All animal experiments were approved by the Berlin Office for Health and Social Services, Germany (O 0132/09).

SH-SY5Y cells were fixed with 2.5% glutaraldehyde in 50 mM sodium cacodylate buffer, pH 7.4, post-fixed in 0.5% osmium tetroxide, 0.1% tannic acid, and 2% uranyl acetate. Samples were embedded in Spurr's resin (Low Viscosity Spurr Kit, Ted Pella) and incubated at 60 °C. Grids were blocked in 20 mM Tris in 0.9% NaCl containing 0.4% BSA-c (Aurion), pH 8 buffer, and incubated with W0-2 or G2-13 primary antibody, followed by the secondary antibody labeled with 15 nm of gold (British BioCell).

Animals (12 months old) were perfused with PBS followed by fixative solution (4% formaldehyde, 0.2% glutaraldehyde in 50 mM sodium cacodylate buffer, pH 7.4). Hippocampi were dissected and post-fixed in the same solution at room temperature. Samples were embedded in LR-Gold resin (Science Services GmbH) and polymerized at 4 °C. Ultra-thin sections were incubated with the G2-13 primary antibody labeled with 10 nm colloidal gold (BB International). The sections were counterstained with uranyl acetate followed by lead citrate.

Labeling of mAb G2-13 was performed with colloidal gold (BB International). After centrifugation (10 min, 6700 × *g*), the supernatant of colloidal gold was counterstained with uranyl acetate and analyzed by TEM to ensure that the supernatant was free of gold aggregates. An amount of 500 μg of mAb G2-13 was dialyzed with 2 mM sodium tetraborate. Equal volumes of G2-13 and colloidal gold were incubated for 20 min at RT. The stability of the gold/protein ratio was assessed by titration with 10% NaCl. Colloidal gold was adjusted to pH 9 in 100 mM potassium carbonate. The protein/gold solution was incubated in 1% BSA for 20 min. After centrifugation, the pellet was resuspended in 20 mM TBS, 1% BSA, and 0.05% sodium azide, pH 8.2, and the solution was centrifuged for 5 min at 6700 × *g*. The supernatant contained G2-13 antibodies labeled with 10 nm of gold.

Modeling—The structure of the Aβ peptides was modeled using the NMR structure of Aβ42 fibrils (Protein Data Bank code 2beg) (33) as a template as described previously (5). All peptides were energetically minimized with help of the GROMOS 43B1 force field to avoid distorted geometries. Electrostatic surface potentials were calculated using the program

Adaptive Poisson-Boltzmann Solver with nonlinear Poisson-Boltzmann equation and contoured at ±5 *kT*/*e*.

Statistical Analysis—Statistical significance between treated cells and control groups was determined using the one-way analysis of variance Dunnett's multiple comparison test. The number of experiments and *p* values are given in the respective figure legends.

RESULTS

Aβ Peptides Can Be Detected in the Nucleus—We first examined uptake of Aβ into neuroblastoma SH-SY5Y cells by biochemical means. We exposed cells for 2 h to 1 μM concentrations of synthetic Aβ peptides with 38, 40, 42, and 43 residues. The purity of cytoplasmic and nuclear fractions was confirmed by Western blot analyses (Fig. 1*A*) before we analyzed them quantitatively for Aβ by ELISA. The nuclear uptake in SH-SY5Y cells was quantified using a monoclonal antibody recognizing the Aβ epitope formed by residues 17–24 (4G8). All peptides were detected in the nuclear fractions (Fig. 1*B*). Surprisingly, the nonaggregating Aβ38 peptide accumulated equally well as the 42-residue-long substitution peptide Aβ42 G33A (Fig. 1*B*). The latter is nontoxic and easily forms low *n* oligomers as we demonstrated previously by size exclusion chromatography (5) and presently by Western blot analysis (see below). To analyze the time course of Aβ uptake, SH-SY5Y cells were treated with Aβ42 and Aβ42 G33A peptides, respectively. The nuclear fractions were quantitatively analyzed by ELISA at different time points using the C-terminal specific Aβ42 monoclonal antibody G2-13 (Fig. 1*C*). In particular, Aβ42 and Aβ42 G33A began to accumulate in cell nuclei within the first 30 min of treatment and accrued thereafter over 8 h. We repeated the experiments using HEK-293 cells to examine whether the observed nuclear uptake in SH-SY5Y neuroblastoma nuclei was cell type-dependent. In addition, we analyzed nuclei of MEF PS1/2 knock-out cells that lack the catalytic subunit PS1 of the γ-secretase and treated the cells with Aβ42 (34). Nuclear translocation of Aβ in all these cells appeared similar, and results from MEF PS1/2 knock-out cells indicated that at least a portion of the intra-nuclear peptide was not a consequence of cellular γ-cleavage (Fig. 1*D*).

Notably, Aβ levels in Fig. 1, *B* and *C*, were quantified using monoclonal antibodies against two different epitopes. Values measured at the 2-h time point in Fig. 1, *B* and *C*, are similar for Aβ42 G33A and Aβ42, whereas the amount measured for Aβ42 in Fig. 1*B* is 50% lower compared with Fig. 1*C*. This reduction might be due to epitope masking of the monoclonal 4G8 through oligomer formation, as observed previously for other conformation-dependent Aβ antibodies (35). This rationale is consistent with our previous study (5) that showed AβG33A has a different aggregation profile compared with wild-type Aβ42.

Although cellular and nuclear uptake is readily facilitated for shorter Aβ segments, there is no obvious rule for longer forms that vary in hydrophobicity or aggregation behavior (5). Our modeling data indicate that longer Aβ peptides such as Aβ43 and the substitution peptide Aβ42 G33A possess an enlarged hydrophobic surface compared with Aβ42 (Fig. 2*A*). An enlarged hydrophobic surface patch is effectively shielded from the polar milieu upon oligomerization explaining why Aβ42 G33A tends to form higher oligomers compared with Aβ42. This rationale is consistent with Western blot data showing that similarly to Aβ43, freshly dissolved Aβ42 G33A migrates at molecular weights in the range of 12–20-mers and higher (Fig. 2*B*). Nevertheless, there is no apparent correlation between peptide hydrophobicity and nuclear uptake because the highly hydrophobic Aβ42 G33A behaves similarly to less hydrophobic peptides (Fig. 1*C*). These results imply that aggregation would not interfere with uptake.

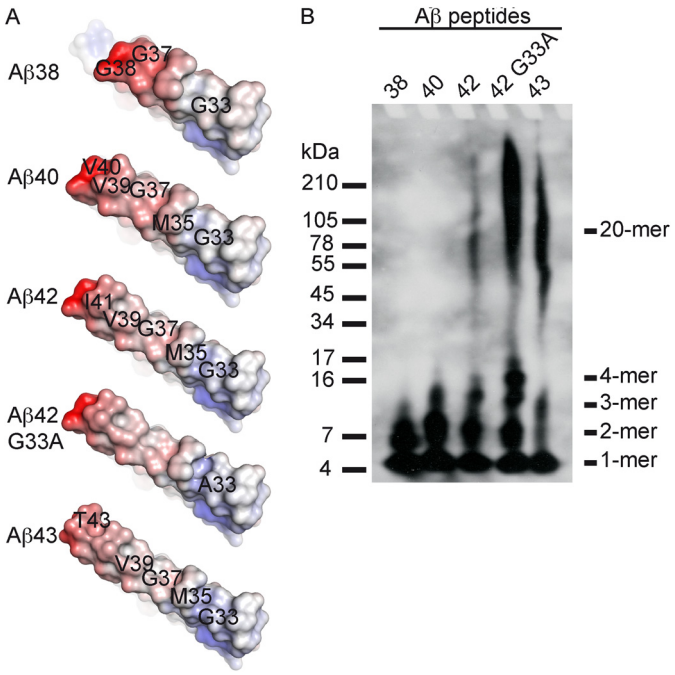

FIGURE 2. **Modeled surface representation of Aβ and Aβ aggregation analyzed by Western blot.** *A*, surface representation of Aβ38, Aβ40, Aβ42, Aβ42 G33A, and Aβ43 structures. The hydrophobic surface of longer Aβ peptides is enlarged. A continuous hydrophobic surface patch from Ile-31 to Met-35 is formed by Aβ42 G33A (computational model) compared with Aβ42 (Protein Data Bank code 2BEG) when the peptides adopt β-sheet conformation. This enlarged hydrophobic surface patch is effectively shielded from the polar milieu upon oligomerization explaining why Aβ42 G33A tends to form higher oligomers compared with Aβ42. Electrostatic surface potentials were calculated using the program Adaptive Poisson-Boltzmann Solver contoured from *red* (negative) over *white* (neutral) to *blue* (positive). *B*, freshly dissolved synthetic Aβ38 and Aβ40 mainly yielded monomers and dimers. Aβ42 and the substitution peptide Aβ42 G33A appear as monomers to tetramers. Aβ42 shows slight aggregation into high *n* oligomers, whereas a higher amount of high *n* oligomers is detected for the substitution peptide G33A. Aβ43 is present at monomers to trimers and high *n* oligomers.

To test whether concentrations of 1 μM Aβ42 were toxic, we monitored the viability of SH-SY5Y cells using the MTT assay, observing no substantial change when cells were exposed to 1 μM synthetic Aβ peptides for as long as 12 h (Fig. 3*A*). At 2.5 μM concentration, however, Aβ43 reduced viability to 75% and Aβ42 to 65% (Fig. 3*B*). Thus, all experiments executed with Aβ42 and other peptides at 1 μM concentrations were performed under sublethal conditions.

We next studied the presence of intra-nuclear Aβ peptides in cultured cells using confocal laser scanning microscopy. Fig. 4*A* shows Aβ42 localized in both the cytoplasm and nucleus of SH-SY5Y cells after 30 min of incubation and after 8 h. In the latter, large accumulations of Aβ42 were seen in the cytoplasm

and in the nucleus, as had been described previously for AICD complexes containing the adapter protein Fe65 and the histone acetyltransferase Tip60. Similar to AICD (36–38), intra-nuclear Aβ appeared as spherical spots (Fig. 4*A*). Finally, we confirmed the presence of intra-nuclear Aβ peptides by TEM not only in cultured SH-SY5Y cells (Fig. 4*B*) but also in brain tissue (Fig. 5). Synthetic Aβ was detected in the cytoplasm, mitochondria, and the nucleus in cultured SH-SY5Y cells by the monoclonal antibody W0-2 (Fig. 4*B*). In brain tissue from 19-month-old mice, the colloidal gold-labeled monoclonal G2-13 antibody directed against the C terminus of Aβ42 specifically recognized both endogenous mouse Aβ42 and Aβ42 derived from overexpressed human APP. Thus, Aβ42 was clearly evident in the nuclei of hippocampal neurons of wild-type mice and more strongly in neurons of tg APPPS1 mice, as expected due to the higher levels of expression (Fig. 5).

Taken together, the three independent methods consistently detected synthetic Aβ in the nucleus. Furthermore, the presence of human Aβ42 in the nucleus derived from transgenic mice overexpressing human APP was shown by TEM. These data indicate that exogenously added Aβ42 as well as endoge-

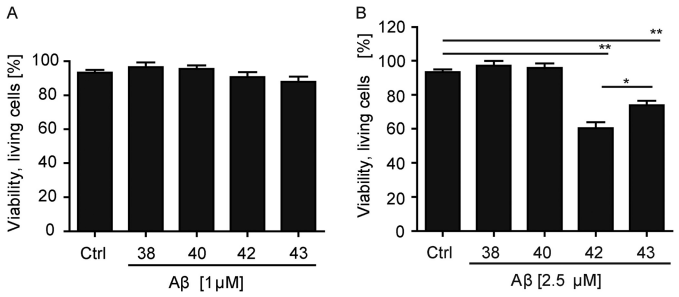

FIGURE 3. **Viability of SH-SY5H cells determined by the MTT assay after incubation with Aβ peptides.** *A,* SH-SY5Y cell viability was not significantly affected at a concentration of 1 μM peptides as indicated after 12 h of incubation, $n = 12–17$. *B,* both Aβ42 and Aβ43 exhibited significant toxicity, albeit to a different extent, at a concentration of 2.5 μM after 12 h of exposure compared with the vehicle control (*Ctrl*, cell culture medium supplemented with 0.13% ammonia) (*, $p < 0.05$; **, $p < 0.005$), $n = 12–17$.

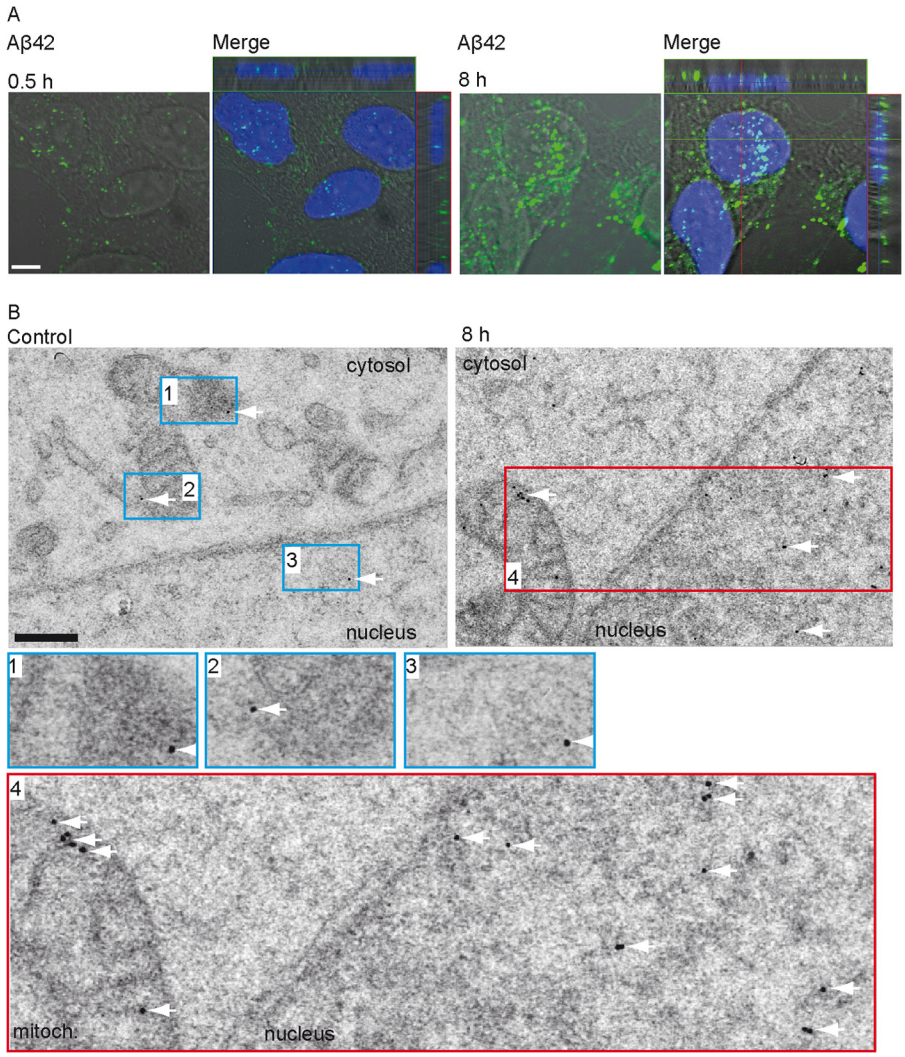

FIGURE 4. **Microscopic demonstration of intranuclear Aβ *in vitro*.** *A,* confocal laser scanning of SH-SY5Y cells treated with N-terminally biotinylated Aβ42. Z-scan images were taken from the top to the bottom of the cells to generate a pseudo three-dimensional image. Images taken in the center of a Z-scan, indicated by the cross in the orthogonal construction (*merge*), revealed Aβ42 peptides in the nucleus after 0.5 and 8 h of incubation time. Particularly after 8 h, the accumulation of Aβ is visible in the cytoplasm as well as in the nucleus. *Scale bar,* 5 μm. *B,* electron micrographs of SH-SY5Y cells that were either untreated (control) or treated with Aβ42 for 8 h. Staining shows endogenous Aβ in the cytoplasm (*1*), mitochondria (*2*), and the nucleus (*3*) of untreated SH-SY5Y cells; treatment of cells with Aβ42 for 8 h resulted in stronger signals of Aβ in the nucleus as well as in cytoplasm and mitochondria (*4*); see also enlarged images *1–4* for better visibility. *Scale bar,* 500 nm.

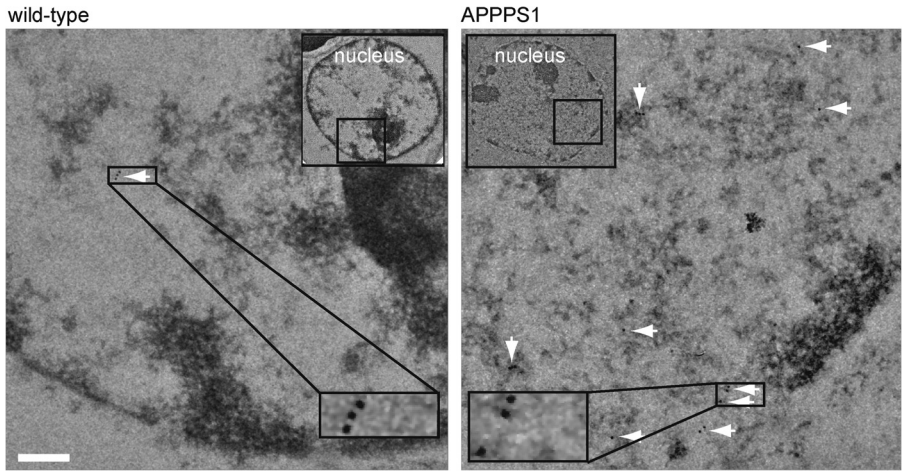

FIGURE 5. **Electron micrographs of hippocampal neuronal nuclei.** Wild-type and APPPS1 double tg mice were analyzed for Aβ42 with the colloidal gold-labeled antibody G2-13 against the C terminus of Aβ42 that recognizes both endogenous mouse Aβ42 and human Aβ42. APPPS1 mice express both mouse and human APP. *Upper insets* show entire nuclei at lower magnification of selected areas stained with immunogold-labeled Aβ42, and *lower insets* show higher magnifications of individual labeling (10 nm of gold, *arrow*). A considerably higher amount of Aβ42 was detected in the nuclei of APPPS1 mice, as expected due to the overexpression of human APP yielding a higher level of Aβ42. *Scale bar,* 210 nm.

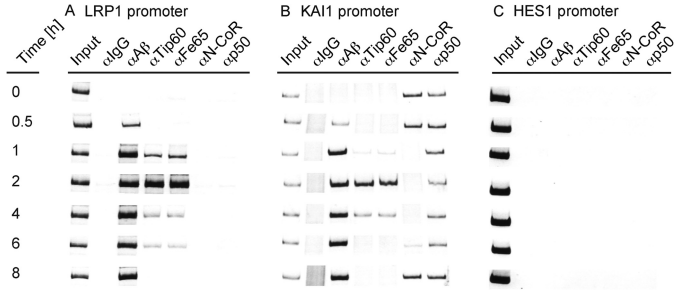

FIGURE 6. **Association of Aβ42 in SH-SY5Y cells with promoters analyzed by ChIP.** Shown are PCR amplificates of DNA precipitated with the respective antibodies. Upon Aβ42 treatment, nuclear Aβ42 associated with the promoters of *LRP1* (*A*) and *KAI1* (*B*), followed by the adapter protein Fe65 and the histone acetyltransferase Tip60, both of which dissociated after 4 or 6 h, whereas Aβ42 remained. Upon association of Aβ42 to the *KAI1* promoter, N-CoR dissociates within 1 h after treatment with Aβ42 and re-associates after 8 h. *C,* Aβ42 had no effect on the *HES1* promoter during time course analysis.

nous Aβ42 are taken up by cells, and low amounts are always present in the nucleus.

Aβ42 Associates with Gene Regulatory Elements—We next interrogated possible gene regulatory effects of nuclear Aβ42 on promoters known to be targets of the AICD (2, 3). To assess possible effects of nuclear Aβ42, we conducted ChIP assays to test whether Aβ42 peptides affected the same promoters as described previously for AICD. AICD is a component of the AFT complex containing AICD, Fe65, and Tip60, which has been shown to bind the *KAI1* (CD82) promoter and increases its transcription (36). Furthermore, the AFT complex was found to bind the *LRP1* promoter and suppress LRP1 mRNA synthesis (39). As a control, we used the *HES1* promoter that reportedly interacts with the Notch intracellular domain, which is generated by the γ-secretase complex and is known to modulate transcription of downstream genes, including *HES1* (39).

We found that treatment of SH-SY5Y cells with Aβ42 peptides resulted in the association of Fe65 and Tip60 with both *LRP1* and *KAI1* promoters after 1 h (Fig. 6, *A* and *B*). Association of Aβ42 with these promoters was already detectable after 30 min, indicating that Aβ peptides did not translocate into the nucleus with Fe65, unlike what has been reported for the AICD (2). The *KAI1* promoter is under the control of p50 and the co-repressor N-CoR (31). Interestingly, Aβ seemed to cause the dissociation of N-CoR. Similarly, a subset of NFκB-regulated genes was described to be activated upon export of the N-CoR corepressor complex as a consequence of high concentrations of AFT complexes (40). After 6 h of Aβ exposure, Fe65 and Tip60 were released from both promoters, whereas Aβ remained associated. Also, the *HES1* promoter, which is activated by the γ-secretase-derived Notch intracellular domain (39), was unaffected by Aβ42 treatment (Fig. 6*C*), further suggesting the specificity of Aβ42 activation indicating that the effects of Aβ42 on the tested promoters were specific. Thus, treatment of SH-SY5Y cells with Aβ42 resulted in the early association of the peptide with both *LRP1* and *KAI1* promoters, while the association of Fe65 and Tip60 with these promoters was delayed.

To investigate whether Aβ42 association to promoters impacts gene regulation, we quantified the corresponding mRNA levels of these genes by qRT-PCR. The early down-regulation of LRP1 and KAI1 mRNA by Aβ42 (Fig. 7, *A* and *B*) suggests a pathway different from AICD-regulated transcription (36, 39), perhaps because the AICD must first form a complex with Fe65 before entering the nucleus (41) where Tip60 is then recruited (40, 42). HES1 mRNA levels were not changed upon Aβ42 treatment (Fig. 7*C*), further confirming our ChIP results. No changes in mRNA expression were detected upon treatment with the reversed Aβ sequence 42-1 peptide.

Importantly, we detected increased APP mRNA levels upon Aβ42 treatment (Fig. 7*D*). Similarly to the detected effects on LRP1 and KAI1 mRNA levels, neither Aβ42 G33A nor Aβ38, Aβ40, and Aβ43 affected APP mRNA. Remarkably, the non-toxic Aβ42 G33A peptide (5), which is also highly present in the nucleus (Fig. 1), did not affect mRNA expression levels (Fig. 7, *A–D*). The latter can be regarded as the more rigid control, compared with the reverse sequence, because it forms β-pleated sheet aggregates like the wild-type peptide (5). Nota-

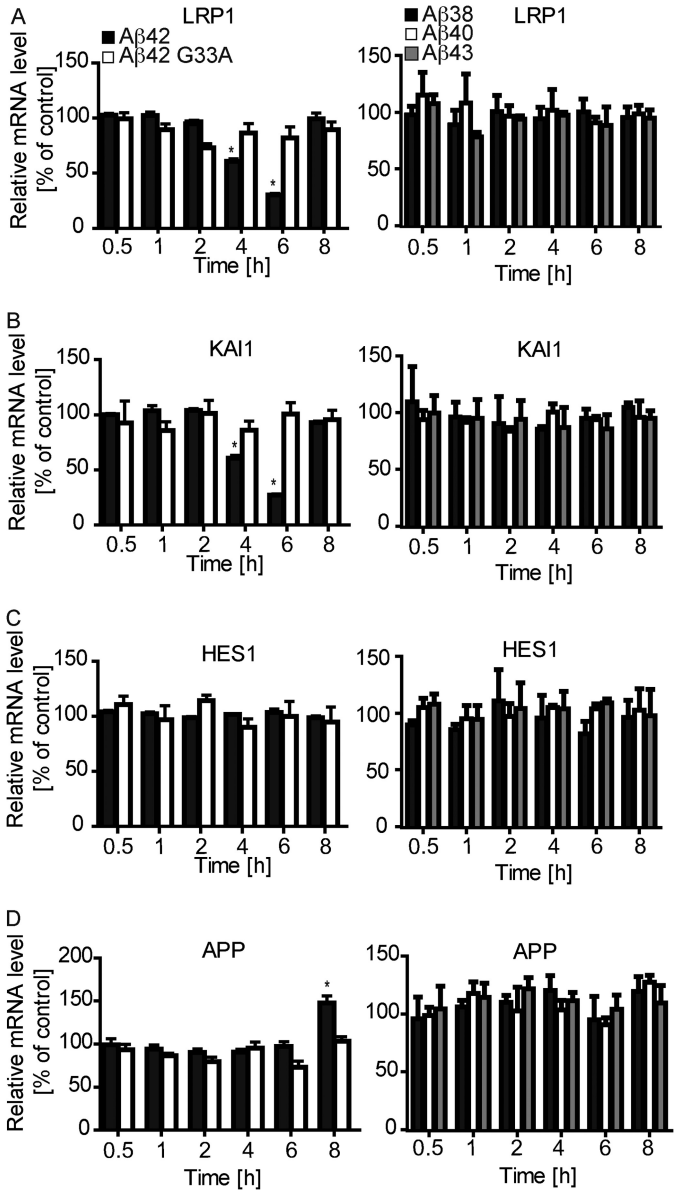

FIGURE 7. **Quantification of mRNA levels.** *A–D,* analysis of mRNA levels of LRP1, KAI1, HES1, and APP after treatment of SH-SY5Y cells with Aβ42 and Aβ42 G33A peptides. A significant decrease upon Aβ42 treatment after 4 and 6 h of incubation is detected for the mRNA levels of LRP1 (*A*) and KAI1 (*B*). Aβ42 did not change the mRNA levels of HES1 (*C*). Upon Aβ42 treatment, APP mRNA was up-regulated after 8 h (*D*). Note, none of the mRNA levels were altered by Aβ42 G33A (*A–D*) at the indicated time points (*, $p < 0.001$), $n = 4–7$. Treatment with Aβ38, Aβ40, and Aβ43 peptides did not affect the mRNA levels of LRP1, KAI1, HES1, or APP, $n = 3–6$.

bly, other tested Aβ species such as Aβ38, Aβ40, and Aβ43 also failed to alter mRNA levels (Fig. 7, *A–D*). Overall, the observed results indicate that the gene regulatory effects are specific to Aβ42.

DISCUSSION

Intraneuronal Aβ aggregates in the brains of AD patients and animal models have been detected with antibodies specific for Aβ40 and Aβ42. This suggests a pathophysiological role for the Aβ pool (11, 43, 44). Our present study demonstrates that in addition to intracellularly produced Aβ peptides, exogenous Aβ species of varying lengths can be taken up from the medium by the cells and translocated to the nucleus. Previously, intracellular soluble Aβ was shown not to colocalize with internalized transferrin, excluding clathrin-mediated endocytosis as the primary uptake mechanism (45). Nevertheless, clathrin-mediated Aβ endocytosis is possible and involves receptors that bind apolipoprotein E (apoE) (20). Aβ internalization by microglia was found nonsaturable, excluding receptor-mediated internalization (45). ApoE accelerates neuronal Aβ uptake, and the majority of endocytosed Aβ is suggested to traffic through early and late endosomes (46). Our data indicate that the uptake process may only be partially influenced by the overall peptide length and hydrophobicity and cannot be correlated with one of the reported mechanisms in the literature. Although the molecular mechanism of Aβ uptake may depend on the cell type and could be influenced by changes in Aβ composition and prevailing aggregation states, we successfully detected all major Aβ peptides (*i.e.* present in body fluids and cell culture supernatants (47)) in the nucleus of SH-SY5Y cells. Additionally, this study also detected Aβ42 peptides in the nuclei of HEK293 and MEF PS1/2 knock-out cells. Aβ entry into cells could potentially occur through ligand-receptor type interactions as follows: *e.g.* Aβ interacts directly or indirectly with integrins, receptor for advanced glycation end products (RAGE), and APP itself (48); interactions via TrkA, p75NTR, some G-proteins, NMDA, and AMPA receptors (49), and/or interactions via the prion protein (50, 51). Entry and transport could alternatively be limited to misfolded forms (*e.g.* aggregated Tau (52)), possibly involving a transcellular propagation (53) that could lead to wider cerebral Aβ distribution (54). Most likely, peptide conformation plays a role as revealed by the nontoxic control Aβ42 G33A, which easily forms low and high *n* oligomers (5). For example, in astrocytes, it was shown that oligomeric Aβ is more efficiently taken up than fibrillar Aβ (55). Thus, Aβ might be able to enter the nucleus as an oligomeric complex comprising as many as nine Aβ peptide molecules as subunits. It is known that protein complexes of such sizes can pass through nuclear membrane pores because only translocation into the nucleus of proteins larger than 40 kDa requires specific transport receptors (56). In any case, given that (i) we did not apply stress conditions such as oxidative (12) or heat stress (13) and (ii) Aβ does not possess a canonical nuclear localization signal or a nuclear export signal, these peptides are most likely diffusing freely into the nucleus. They are likely retained by binding to nondiffusible nuclear components and might accumulate there.

In accordance with our findings, we anticipate that low *n* oligomers of aggregation-prone Aβ species, as well as monomers (*e.g.* of Aβ34), are able to enter the nucleus. Also, it seems that peptide length, conformation, and oligomerization are important determinants of Aβ uptake into the nucleus, although their biophysical traits inconsistently influence the process.

Our ChIP results revealed that Aβ42 specifically associated with AICD-regulated promoters of *LRP1* and *KAI1*. Subsequent qRT-PCR analyses demonstrated that Aβ42 diminished mRNA levels of LRP1 and KAI1. Previously, Aβ was found to localize to the nucleus under conditions of oxidative stress (57). Moreover, Aβ42 was shown to bind to the *APP* promoter

sequence using ChIP analysis and electrophoretic mobility shift assays (57, 58). Here, we demonstrate an increase of APP mRNA levels upon Aβ42 treatment. Aβ likely contains a helix-loop-helix structure (59), which is common to certain transcription factors. Thus, a direct binding of metastable oligomeric structures of Aβ, described in preparations of amyloid-forming peptides such as α-synuclein Tau, prion, and Aβ42, could mediate an interaction with DNA. *In vitro*, DNA binds all soluble aggregated forms of Aβ42 indicating that DNA interaction is a general property of different soluble forms of Aβ42 unrelated to the extent of aggregation (60). The less- or non-neurotoxic Aβ species, including Aβ38, Aβ40, Aβ42 G33A, and Aβ43 (5, 61, 62), although possessing the ability to assemble into cross-β-fibrils (63), did not influence gene regulation. Thus, our novel findings suggest that there might be a functional activity of Aβ42 in the nucleus that is different from AICD (2, 3). Among the several Aβ species that can accumulate in the nucleus, only Aβ42 appears to impact the expression of LRP1, KAI1, and APP.

Most significantly, it is the overproduction of Aβ (*e.g.* mutations associated with early onset AD) that invariably leads to the modulation of Aβ load (6). This regulatory mechanism could induce APP transcription, thereby enhancing APP processing, Aβ production and uptake, and subsequent up-regulation of APP synthesis. Because the detection of intracellular Aβ is always accompanied by increased extracellular Aβ (64), such a proposed mechanism would account for the favored uptake of Aβ from the extracellular pool.

Notably, Aβ42, which is toxic *in vivo* at lower concentrations than applied here (49) for *in vitro* studies, specifically modulated gene regulation as reported herein. Accordingly, we hypothesize that the neurotoxic Aβ42 low *n* oligomers provoke changes in gene transcription *in vivo* at concentrations that are subtoxic *in vitro*. This view is supported by the consideration that soluble low *n* oligomers of Aβ42 peptides are neurotoxic, in particular Aβ42 dimers and tetra-/hexamers (5, 6, 9, 65).

In conclusion, our data indicate that Aβ42 has a specific transcriptional regulatory function. Our data imply that deregulation of Aβ target genes could be an alternative pathway for Aβ-induced neurotoxicity starting with processes such as Aβ accumulation.

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Nuclear Translocation Uncovers the Amyloid Peptide Aβ42 as a Regulator of Gene Transcription

Christian Barucker, Anja Harmeier, Joerg Weiske, Beatrix Fauler, Kai Frederik Albring, Stefan Prokop, Peter Hildebrand, Rudi Lurz, Frank L. Heppner, Otmar Huber and Gerhard Multhaup

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