Expression of the Anti-amyloidogenic Secretase ADAM10 Is Suppressed by Its 5′-Untranslated Region

Received for publication, February 10, 2010, and in revised form, March 25, 2010. Published, JBC Papers in Press, March 26, 2010. DOI 10.1074/jbc.M110.110742

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Proteolytic processing of the amyloid precursor protein by α-secretase prevents formation of the amyloid β-peptide (Aβ), which is the main constituent of amyloid plaques in brains of Alzheimer disease (AD) patients. α-Secretase activity is decreased in AD, and overexpression of the α-secretase ADAM10 (a disintegrin and metalloprotease 10) in an AD animal model prevents amyloid pathology. ADAM10 has a 444-nucleotide-long, very GC-rich 5′-untranslated region (5′-UTR) with two upstream open reading frames. Because similar properties of 5′-UTRs are found in transcripts of many genes, which are regulated by translational control mechanisms, we asked whether ADAM10 expression is translationally controlled by its 5′-UTR. We demonstrate that the 5′-UTR of ADAM10 represses the rate of ADAM10 translation. In the absence of the 5′-UTR, we observed a significant increase of ADAM10 protein levels in HEK293 cells, whereas mRNA levels were not changed. Moreover, the 5′-UTR of ADAM10 inhibits translation of a luciferase reporter in an in vitro transcription/translation assay. Successive deletion of the first half of the ADAM10 5′-UTR revealed a striking increase in ADAM10 protein expression in HEK293 cells, suggesting that this part of the 5′-UTR contains inhibitory elements for translation. Moreover, we detect an enhanced α-secretase activity and consequently reduced Aβ levels in the conditioned medium of HEK293 cells expressing both amyloid precursor protein and a 5′-UTR-ADAM10 deletion construct lacking the first half of the 5′-UTR. Thus, we provide evidence that the 5′-UTR of ADAM10 may have an important role for post-transcriptional regulation of ADAM10 expression and consequently Aβ production.

Alzheimer disease (AD)4 is the most common form of dementia worldwide. The major pathological hallmarks of AD are neurofibrillary tangles and amyloid plaques (1). Amyloid plaques are composed of the amyloid-β peptide (Aβ), which is derived by proteolysis from the β-amyloid precursor protein (APP) (2). Two proteases, termed β- and γ-secretase, generate Aβ. The aspartyl protease β-site APP cleaving enzyme 1 (BACE1) was identified as β-secretase and cleaves APP into two fragments (summarized in Ref. 3). The extracellular N-terminal domain of APP, APPsβ, is released upon shedding by BACE1. Recently, it was reported that a N-terminal proteolytic derivative of APPsβ could bind to the death receptor DR6, thereby triggering axon pruning and neuronal death under conditions where trophic factors are reduced (4). The remaining membrane-bound C-terminal stub of APP is the immediate precursor for Aβ generation by γ-secretase (5). Alternatively, APP is also cleaved in a nonamyloidogenic pathway by α-secretase within the Aβ domain, thereby preventing the formation of Aβ (6, 7). Processing of APP by α-secretase generates the soluble APPsα ectodomain, which may have neuroprotective and neurotrophic properties (8). The resulting membrane-bound C-terminal fragment is further cleaved by γ-secretase to produce p3, a N-terminally truncated Aβ derivative (9).

Three members of the ADAM (a disintegrin and metalloprotease) family of metalloproteases are described to have α-secretase activity, namely ADAM9, ADAM10, and ADAM17 (10–12). These ADAMs are cell surface proteases, which cleave a variety of different substrates including adhesion molecules, cytokines, growth factors, and growth factor receptors (13). For ADAM10, in vivo evidence exists suggesting that it acts as α-secretase. Neuronal overexpression of ADAM10 in APP[V171I] transgenic mice results in increased shedding of APPsα, a decrease in the formation of Aβ, and a significant reduction of the amyloid plaque load. In contrast, overexpression of a dominant negative variant of ADAM10 leads to an enhanced formation of amyloid plaques (14). In addition, in situ hybridization analysis in human cortical neurons as well as in the brains of mice from different stages of development revealed coexpression of ADAM10, APP, and BACE1 mRNA

‡‡ This work was supported by the Deutsche Forschungsgemeinschaft (Collaborative Research Center SFB596 “Molecular Mechanisms of Neurodegeneration,” to S. L., S. F. L., and C. H.), a fellowship of the Hans und Ilse Breuer Foundation (S. Z.), a predoctoral fellowship (Molekulare Medizin–Förderung der Forschung und Lehre, FoFoLe) by the Ludwig-Maximilians-University, Munich (G. B.), the Bundesministerium für Bildung und Forschung (“Degenerative Dementias: Target Identification, Validation, and Translation into Treatment Strategies” (S. F. L. and C. H.)), and by the Center of Integrated Protein Science Munich (CIPSM).

1 The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. S1.

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3 Supported by a Forschungsprofessur of the Ludwig-Maximilians-University.
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(15), suggesting that ADAM10 and BACE1 are the primary enzymes involved in APP shedding. Interestingly, it was reported that ADAM10 protein levels are decreased in platelets of AD patients, and hence the release of APPs from platelets is significantly reduced (16). Additionally, APPs was found to be decreased in the cerebrospinal fluid of sporadic AD patients (16–18) and AD patients carrying the Swedish APP mutation (19), indicating that decreased α-secretase activity and/or enhanced BACE1 activity might contribute to the pathogenesis of AD (20).

Besides APP, ADAM10 cleaves other neuronal substrates such as the L1 adhesion molecule, N-cadherin, and ephrin, suggesting that ADAM10 plays an important role for neurite outgrowth and migration (13, 21). Indeed, ADAM10-deficient mice die early in development showing multiple defects in the developing central nervous system as well as defective somite segmentation and defects in the cardiovascular system (22). In addition, ADAM10 plays a role in inflammation and cancer development (13).

Little is known about the regulation of ADAM10 expression and activity. Recently, it was shown by immunocytochemical analysis that ADAM10 protein levels seem to be decreased in the neurons of AD patients in comparison with controls (23). In contrast to these observations, it was shown that in hippocampal samples of severe AD cases, ADAM10 mRNA levels were up-regulated by a factor of two, most likely because of a defensive response; however, an increase in ADAM10 activity was not analyzed (24). Moreover, it was reported that ADAM10 protein and the mRNA level were elevated in various forms of cancer (25–27). In addition, it was recently demonstrated that ADAM10 transcription could be stimulated by all-trans-retinoic acid in neural and monocytic blood cells (28, 29). Besides the regulation on the transcriptional level, ADAM10 is regulated post-translationally. The prodomain of ADAM10 is a potent inhibitor of ADAM10 activity (30) and is released upon maturation in the Golgi apparatus by proprotein convertases like furin and PC7 (31).

BACE1 expression is regulated by a number of independent post-transcriptional control mechanisms (summarized in Ref. 3). Among these, BACE1 protein expression was shown to be significantly repressed by its 5′-UTR (32–36). It was demonstrated that the GC-rich region within the BACE1 5′-UTR forms a translational barrier that may impede scanning of the ribosome along the 5′-UTR or initiate translation (34). Moreover, translation of an upstream open reading frame (uORF) in the 5′-UTR was shown to be involved in translational repression of BACE1 (35, 36). Recently, it was demonstrated that under stress conditions the translation preinitiation complex bypasses this uORF and structured regions in the 5′-UTR of BACE1, which leads to increased BACE1 translation (37).

By sequence comparison, we found that the 5′-UTR of ADAM10 has similar properties compared with the 5′-UTR of BACE1. Here, we demonstrate that the 5′-UTR of ADAM10 suppresses translation of ADAM10 in vitro and in vivo. Repression of ADAM10 expression is mediated by a very strong inhibitory element within the first 259 nucleotides of the ADAM10 5′-UTR.

EXPERIMENTAL PROCEDURES

Antibodies—The following monoclonal antibodies were used: anti-green fluorescent protein (GFP) antibody (Clontech), anti-V5 antibody (Invitrogen), anti-β-actin antibody (Sigma), and 6E10 antibody against Aβ1–16 (Signet Laboratories). The polyclonal antibody 192wt against APPsβ, a kind gift from Drs. Dale Schenk and Peter Seubert (Elan Pharmaceuticals), and the polyclonal antibody 6687 against the C terminus of APP were described before (38, 39). Horseradish peroxidase-conjugated anti-mouse and anti-rabbit IgG antibodies were obtained from Promega.

cDNA Constructs—Human ADAM10 without 5′-UTR was amplified by PCR from peak12-ADAM10 and subcloned into pcDNA6/V5-His A (Invitrogen) using HindIII and XhoI, thereby introducing the last 12 nucleotides of the ADAM10 5′-UTR (numbering 433–444 in supplemental Fig. S1) directly in front of the ADAM10 initiation codon. The 5′-UTR (numbering 1–432 in supplemental Fig. S1) was amplified from pCP53AB.1 (28) and introduced after PCR using the Nhel and HindIII sites. All of the deletion constructs and constructs with mutated upstream ATGs (uATG1 to ATA and uATG2 to GTG) were generated by PCR. Similarly, firefly luciferase was cloned into pcDNA6/V5-His A using HindIII and XhoI, thereby introducing a stop codon in front of the XhoI restriction site. The 5′-UTR of ADAM10 and variants thereof were introduced by PCR using Nhel and HindIII restriction sites. All of the cDNAs were verified by sequencing.

Cell Culture and cDNA Transfections—HEK293 cells, African green monkey COS7 cells, and human neuroblastoma SH-SY5Y cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 2 mM glutamine. HEK293 cells stably overexpressing APP695 were cultured in the same medium supplemented with 100 μg/ml hygromycin. Transfections were carried out using Lipofectamine 2000 (Invitrogen) according to the supplier’s instructions.

Protein Analysis—1.8 × 10⁶ HEK293 or HEK293-APP695 overexpressing cells were plated in 6-cm dishes and transiently transfected with 8 μg of cDNA encoding ADAM10 variants and 0.1 μg of pEGFP-N1 (Clontech) where indicated for a transfection control. 24 h after transfection, the cell lysates were prepared with lysis buffer containing 50 mM Tris/HCl, pH 7.6, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, and protease inhibitors (Sigma) and analyzed for ADAM10, β-actin, and GFP protein levels by immunoblotting. For analysis of APP processing, the cell culture medium was replaced 24 h after transfection, and the cells were incubated overnight in fresh medium. The cell lysates were prepared and analyzed for ADAM10 and APP expression. Equal amounts of the conditioned media were analyzed for APPs0, APPsβ, and Aβ. Detection was performed using ECL and ECL Plus (Amersham Biosciences) or CDP-Star (Applied Biosystems) for Aβ detection. Quantification was performed using the FluorChem 8900 (Alpha Innotech) and AlphaEase FC software. ADAM10 levels were normalized to β-actin and GFP protein levels where indicated.

Quantification of Aβ—Secreted Aβ40 peptides in conditioned medium were quantified by a sandwich immunoassay
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Expression of ADAM10 Is Repressed by Its 5′-UTR—We next investigated whether the 5′-UTR may affect expression of ADAM10. We transiently transfected HEK293 cells with C-terminally V5-tagged ADAM10 cDNA constructs with or without the 5′-UTR. 24 h after transfection, we observed a significantly lower ADAM10 expression in the presence of the 5′-UTR (Fig. 1A). Consistent with previous findings for ADAM10 and ADAM17, we predominantly detected the immature form of ADAM10 upon overexpression (12, 44–46). Quantification of ADAM10 levels revealed a 3-fold increase of ADAM10 protein in cells transfected with the cDNA construct lacking the 5′-UTR (Fig. 1B). This probe detects ADAM10 transcripts of 4.4 kb in heart, 3.3 kb in brain, 2.2 kb in liver, 1.5 kb in skeletal muscle, 2.0 kb in pancreas, and 1.8 kb in lung as reported previously for human tissue and cell lines (data not shown) (41–43). In agreement with these data, expressed sequence tag analysis revealed that the ADAM10 5′-UTR may affect expression of ADAM10 RNA expression was normalized to endogenous glyceraldehyde-3-phosphate dehydrogenase according to the ΔΔCt method.

In Vitro Translation—In vitro translation of in vitro transcribed mRNA (mMESSAGE mMACHINE kit; Ambion) was carried out using nuclelease-treated rabbit reticulocyte lysate (Promega) as described (34).

RESULTS

ADAM10 Transcripts Contain a GC-rich 5′-UTR—Sequence analysis revealed that the ADAM10 mRNA contains a long 5′-UTR with similar properties as the 5′-UTR of BACE1, which was shown to be involved in translational repression of BACE1 (32–36). The 5′-UTR of ADAM10 was cloned from a human macrophage cDNA library (41) and contains 444 nucleotides, has a high GC content (69%), and has two uORFs. It is highly similar to a predicted ADAM10 5′-UTR of chimpanzee and of rhesus monkey (supplemental Fig. S1), which may indicate that the 5′-UTR of ADAM10 might have a physiological function. To confirm that native ADAM10 transcripts contain the 5′-UTR, we performed multiple tissue Northern blot analysis using the first 432 nucleotides of the 5′-UTR as a probe. This probe detects ADAM10 transcripts of ~4.4 kb in heart, brain, liver, skeletal muscle, pancreas, and lung as reported previously for human tissue and cell lines (data not shown) (41–43). In agreement with these data, expressed sequence tag analysis revealed a clone from human spleen (NCBI DA941750) that lacks only the first 13 nucleotides of the ADAM10 5′-UTR (supplemental Fig. S1).

Quantitative Real Time PCR—Total RNA was isolated from HEK293 cells 24 h after transfection with ADAM10 variants using the RNeasy mini kit (Qiagen). Subsequently, the RNA was treated twice with DNase I (DNA-free; Ambion). cDNA was synthesized from 0.5 μg of total RNA using SuperScript II reverse transcriptase (Invitrogen) with oligo(dT) primers. Quantitative real time PCR was performed with 2× Power SYBR Green PCR Master Mix (Applied Biosystems) and 1.0 μM of each primer pair (ADAM10–2228 forward primer, 5′-CATTCAGCAACCCGAGGTCAG-3′; and ADAM10-V5 reverse primer, 5′-ATCGAGACCGAGGAGGGTGGT-3′ (note that the amplified product of this primer pair results only from reverse transcribed V5-tagged ADAM10 cDNA) and glyceraldehyde-3-phosphate dehydrogenase forward primer, 5′-TCAGTGCCACCCAGGAC-3′; and glyceraldehyde-3-phosphate dehydrogenase reverse primer, 5′-CAGTGGAGCTTCCCGTGTCAG-3′). Quantification was performed with the 7500 Fast Real Time PCR System (Applied Biosystems). For each RNA sample, triplicates were analyzed with each primer set, and relative ADAM10 RNA expression was normalized to endogenous glyceraldehyde-3-phosphate dehydrogenase according to the ΔΔCt method.

Luciferase-Reporter Assay—1.8 × 105 HEK293 cells were seeded in 24-well plates and transfected with 0.4 μg of the indicated firefly luciferase constructs and 10 ng of Renilla luciferase-vector as transfection control. 24 h after transfection, the cell lysates were prepared, and luciferase activity was measured with the dual luciferase reporter assay system (Promega) according to the supplier’s protocol. Quantification was performed using an LB96V luminometer (Berthold Technologies) and analyzed with WinGlow software (Berthold Technologies). Firefly luciferase activity was normalized to Renilla luciferase activity.

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Translational Repression of ADAM10

Expression of ADAM10 Is Repressed by Its 5′-UTR—We next investigated whether the 5′-UTR may affect expression of ADAM10. We transiently transfected HEK293 cells with C-terminally V5-tagged ADAM10 cDNA constructs with or without the 5′-UTR. 24 h after transfection, we observed a significantly lower ADAM10 expression in the presence of the 5′-UTR (Fig. 1A). Consistent with previous findings for ADAM10 and ADAM17, we predominantly detected the immature form of ADAM10 upon overexpression (12, 44–46). Quantification of ADAM10 levels revealed a 3-fold increase of ADAM10 protein in cells transfected with the cDNA construct lacking the 5′-UTR.
5'UTR compared with the construct with the 5'UTR of ADAM10 (Fig. 1B). Significantly, the 5'UTR had no effect on the respective mRNA levels, demonstrating that the 5'UTR did not alter ADAM10 transcription (Fig. 1C). A similar repression of ADAM10 expression caused by the presence of the 5'UTR was detected in African green monkey COS7 cells and in the human neuroblastoma cell line SH-SY5Y (Fig. 1D), demonstrating that the translational repression is not cell type-specific.

To further provide evidence that the 5'UTR of ADAM10 is involved in lowering ADAM10 protein expression by reducing ADAM10 mRNA translation, we investigated whether the 5'UTR of ADAM10 could inhibit the expression of a heterologous ORF. We cloned the ADAM10 5'UTR in front of a firefly luciferase reporter construct and transfected HEK293 cells with this construct or with luciferase lacking the 5'UTR. Firefly luciferase activity was measured in cell lysates and normalized to Renilla luciferase, which was used as a transfection control. We could detect approximately four times more luciferase activity in cells transfected with luciferase lacking the 5'UTR compared with the construct with the 5'UTR of ADAM10 (Fig. 2A), indicating that the 5'UTR represses translation. To provide further evidence, we performed an in vitro translation assay with equal amounts of in vitro transcribed luciferase mRNA with and without the 5'UTR of ADAM10 (Fig. 2B, upper panel). We detected robust levels of luciferase protein only in the reaction mixture containing luciferase mRNA without the 5'UTR of ADAM10 (Fig. 2B, lower panel). Taken together, these results demonstrate that the 5'UTR of ADAM10 efficiently represses translation of ADAM10.

The uORFs of the ADAM10 5'UTR Are Not Involved in Translational Control of ADAM10—Translational control of eukaryotic protein synthesis could be mediated by uORFs within the 5'UTR of mRNAs (47). After translation of such uORFs, the ribosome could (a) terminate and reinitiate translation, (b) synthesize an N-terminal extended protein, or (c) terminate and dissociate from the mRNA, resulting in a translational repression of the main ORF (47). Additionally, mutations that introduce or disrupt a uORF are responsible for some human diseases, demonstrating that uORFs could be critically involved in protein expression (48–50). Therefore, we investigated whether translational repression might be mediated by the two short uORFs, coding for putative peptides of 11 (uORF1) or 4 amino acids (uORF2) (supplemental Fig. S1). Both upstream codons reside in a suboptimal context with a pyrimidine base each at positions −3 and +4 for both uORFs, whereas the initiation start codon is flanked by an adenine at position −3 and a guanine at position +4 and is thus in a good context for translation initiation (51). We mutated the corresponding uATG of the first uORF to ATA and from the second uORF to GTG and transiently transfected these constructs in HEK293 cells. ADAM10 protein levels were determined in the cell lysates by immunoblotting (Fig. 3A). Mutation of uORF1 (ATG1KO) as well as mutation of the second uORF (ATG2KO) showed no significant change in ADAM10 expression compared with cells transfected with the wild-type 5'UTR ADAM10 construct (Fig. 3B). These results suggest that the two uORFs do not contribute significantly to the translational repression mediated by the 5'UTR of ADAM10. Instead, other features of the 5'UTR such as the high GC content, which favors the formation of complex secondary structures, might be responsible for translational repression of ADAM10.

The First Half of the ADAM10 5'UTR Contains an Inhibitory Element for Translation—The mRNA structure prediction program Mfold suggests a stable secondary structure with several stem loops for the 5'UTR of ADAM10 (data not shown) (52), suggesting that ADAM10 translation might be inhibited by
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complex secondary structures within the 5′-UTR. To identify regions within the 5′-UTR, which affect translation efficiency of ADAM10, we analyzed a series of deletion mutants of the 5′-UTR (Fig. 4). Successive deletion of the first 259 nucleotides of the ADAM10 5′-UTR resulted in a strong increase in ADAM10 protein expression (Fig. 4, A and B). Interestingly, deletions from the 5′ end of the 5′-UTR result in 40- and 130-fold increases of ADAM10 expression for the constructs missing the first 215 and 259 nucleotides of the ADAM10 5′-UTR, respectively (Fig. 4B). Because mRNA levels only increased 2.6- and 5.4-fold, respectively, for these samples (data not shown), the very robust effects on protein synthesis are unlikely to be solely attributed to mRNA stability. Instead, these findings suggest that there is a very strong translational inhibitory RNA element within the first 259 nucleotides of the ADAM10 5′-UTR. In contrast to these findings, deletion mutants from the 3′ end of the ADAM10 5′-UTR resulted in decreased ADAM10 protein levels (Fig. 4, C and D). Quantification of ADAM10 protein levels revealed a decrease in ADAM10 expression between 80% for the Δ201–432 ADAM10 construct and 90% for both Δ251–432 ADAM10 and Δ341–432 ADAM10 constructs compared with the wild-type 5′-UTR (Fig. 4D). However, deletion of nucleotides 151–432 of the ADAM10 5′-UTR resulted in a similar ADAM10 expression compared with the control (Fig. 4D), probably because inhibitory elements for translation are missing or destroyed. For all four analyzed constructs, the decrease in ADAM10 protein expression was accompanied by a similar reduction of ADAM10 mRNA levels (data not shown), indicating that the second half of the 5′-UTR of ADAM10 is important for mRNA stability and efficient translation of ADAM10 under these conditions.

To further confirm these findings, we investigated whether the above described mutants of the 5′-UTR of ADAM10 exert a similar effect on the translation of firefly luciferase. We transfected HEK293 cells with luciferase encoding vectors containing the wild-type 5′-UTR of ADAM10, the 5′-UTR containing uORF mutants, and 5′-UTR deletion mutants in front of luciferase. Firefly luciferase activity was measured in cell lysates and normalized to Renilla luciferase (Fig. 5). Importantly, all of the investigated ADAM10 5′-UTR mutations affected luciferase expression in a similar way as in the natural ADAM10 context. Consistent with the data shown above, mutations of the two uORFs within the 5′-UTR do not show a significant change in luciferase activity, confirming that these uORFs contribute very little if at all to the inhibitory effect of the ADAM10 5′-UTR. Similarly, we observed a decrease in luciferase activity when we transfected HEK293 cells with a 5′-UTR-luciferase construct lacking nucleotides 251–432 of the ADAM10 5′-UTR. Moreover, when we expressed constructs lacking the first 215 or 259 nucleotides of the ADAM10 5′-UTR in front of luciferase, we observed a 5–10-fold increase in luciferase activity, respectively. Probably due to a shorter half-life time of luciferase compared with ADAM10 (53, 54), the observed effects were not as pronounced as those reported above for the native ADAM10.

**FIGURE 4. Effects of 5′-UTR deletions on ADAM10 expression.** HEK293 cells were transfected with the indicated 5′-UTR ADAM10 cDNA constructs. Representative Western blots for V5-tagged ADAM10 and quantification of ADAM10 signals from cells transfected with ADAM10 cDNA constructs lacking either parts of the 5′-end (A and B) or the 3′-end (C and D) of the 5′-UTR are shown. The values were normalized to β-actin and GFP levels. In both cases the ADAM10 signal for cells transfected with 5′-UTR ADAM10 was set to 1. The results are expressed as the means ± S.D. of at least three (C) or six (D) independent experiments.

**FIGURE 5. Effects of uORF mutations and deletions of the ADAM10 5′-UTR on the expression of a Firefly luciferase reporter.** HEK293 cells were transfected with firefly luciferase (Luc) cDNA constructs containing the indicated variants of the ADAM10 5′-UTR and Renilla luciferase as a transfection control. Firefly luciferase activity was normalized to Renilla luciferase activity, and the signal for firefly luciferase containing the entire 5′-UTR of ADAM10 was set to 1. The results are expressed as the means ± S.D. of three independent experiments made in triplicate.
observed a 2-fold increase of APPsβing the first 259 nucleotides (Fig. 6) after 48 h we also detected small amounts of mature ADAM10 after transfection (compare Fig. 4). A tremendous increase in ADAM10 expression, similar to that observed 24 h after transfection (Fig. 6). Again we detected a tremendous increase in APPsβ secretion in these cells compared with mock transfected cells (Fig. 6). These data indicate that the 5'-UTR could have an important physiological role for post-transcriptional regulation of ADAM10 expression and consequently Aβ production.

**DISCUSSION**

Approximately 10% of vertebrate mRNAs contain atypically long 5'-UTRs with a high GC content and uORFs. Among these transcripts, many mRNAs encode proto-oncogenes and regulatory protein products that are implicated in cell growth and proliferation (55, 56). BACE1 translation is also repressed by its long GC-rich 5'-UTR (32–34). The ADAM10 5'-UTR has similar properties as the 5'-UTR of BACE1. Our findings demonstrate that the 444-nucleotide-long, very GC-rich 5'-UTR of ADAM10 is sufficient to repress translation of ADAM10.

Several cis-acting elements within 5'-UTRs, like uORFs, secondary structures or binding motifs bound by specific RNA binding proteins could be involved in translational regulation of certain transcripts (55–57). Recently, it was demonstrated that uORFs within 5'-UTRs typically reduce protein expression by 30–80%, with a modest effect on mRNA levels (58). For example, uORFs in the 5'-UTR of huntingtin, embryonic proinsulin, BACE1, and GADD34-mRNA are involved in translational repression of the downstream cistrons (37, 59–61). The translational repression by uORFs seems to be dependent on the following properties: uAUG context (Kozak sequence), increased distance from the cap, evolutionary conservation, and number of uORFs (58). The 5'-UTR of ADAM10 contains two uORFs; however, the uAUGs of both uORFs are in an unfavorerd context for initiation because they have pyrimidines at positions −3 and +4 (51). Both uORFs of the human ADAM10 5'-UTR are nearly identical with the predicted sequences from chimpanzee and rhesus monkey, but the initiation codon of uORF1 at positions 43–45 of the 5'-UTR is located close to the cap and thus is not expected to be involved in translational repression of ADAM10 (58). In agreement with this prediction, we found that mutation of uATG1 to ATA (ATG1KO) does not influence significantly ADAM10 translation in HEK293 cells. Similarly, mutation of uATG2 to GTG (ATG2KO) did not change ADAM10 protein levels as well, arguing that both uORFs do not have significant effects on the translational repression of the 5'-UTR of ADAM10. Consistent with these findings, it was reported that the translational suppression of the platelet-derived growth factor B chain and the NR2A subunit of the N-methyl-d-aspartate receptor was not primarily mediated by uORFs within the 5'-UTRs of their mRNAs. Instead, because of the high GC content of these 5'-UTRs, it was assumed that translation was inhibited by several stable secondary structures (62, 63).

Interestingly, it was reported that stable stem loops with a free energy of −30 to −61 kcal/mol are sufficient to impose a strong block on ribosomal scanning along the 5'-UTR and
Importantly, we observed a significant increase in APP structures, as was described for the 5'UTR of luciferase. These data suggest that the first 259 nucleotides of the 5'-UTR predominantly inhibit translation of ADAM10, most likely because of the formation of complex secondary structures, as it was described for the 5'-UTRs of platelet-derived growth factor B chain mRNA and shank1 mRNA (62, 68). Importantly, we observed a significant increase in APPso secretion and at the same time a decrease in αβ generation in HEK293-APP695 cells transiently transfected with Δ1–259 5'-UTR ADAM10 cDNA.

It was described that RNA binding proteins are involved in translational control of certain genes by binding to secondary structure elements like stem loops within 5'-UTRs. The best known examples are iron-regulatory proteins, which control iron homeostasis by regulating the translation of ferritin heavy and light chain mRNAs by binding to the 5'-UTR near the cap and thereby preventing translation initiation (57, 69). Expression of ADAM10 may thus be regulated by similar mechanisms. Therefore, our findings open future research toward translational regulation of ADAM10 and may provide the basis for the identification of novel therapeutic targets.

Acknowledgments—We thank Dr. Manfred Brockhaus for the Aβ40 specific antibody BA24 and Drs. Dale Schenk and Peter Seubert for the polyclonal antibody 192wt. We also thank Dr. Michael Willem and Dr. Harald Steiner for critical reading of the manuscript.

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