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hnRNP C increases amyloid precursor protein (APP) production by stabilizing APP mRNA

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ABSTRACT

We have previously shown that heterogeneous nuclear ribonucleoprotein C (hnRNP C) and nucleolin bound specifically to a 29 nt sequence in the 3′-untranslated region of amyloid precursor protein (APP) mRNA. Upon activation of peripheral blood mononuclear cells, hnRNP C and nucleolin acquired APP mRNA binding activity, concurrent with APP mRNA stabilization. These data suggested that the regulated interaction of hnRNP C and nucleolin with APP mRNA controlled its stability. Here we have directly examined the role of the *cis* element and *trans* factors in the turnover and translation of APP mRNA *in vitro*. In a rabbit reticulocyte lysate (RRL) translation system, a mutant APP mRNA lacking the 29 nt element was 3–4-fold more stable and synthesized 2–4-fold more APP as wild-type APP mRNA. Therefore, the 29 nt element functioned as an APP mRNA destabilizer. RNA gel mobility shift assays with the RRL suggested the presence of endogenous nucleolin, but failed to show hnRNP C binding activity. However, wild-type APP mRNA was stabilized and coded for 6-fold more APP when translated in an RRL system supplemented with exogenous active hnRNP C. Control mRNAs lacking the 29 nt element were unaffected by hnRNP C supplementation. Therefore, occupancy of the 29 nt element by hnRNP C stabilized APP mRNA and enhanced its translation.

INTRODUCTION

A common pathological feature of Alzheimer's disease (AD) and Down's syndrome (DS) is the accumulation of extracellular insoluble amyloid deposits in neuritic plaques and around cerebral vessels. The primary component of these plaques is the β/A4 peptide derived from one of several β-amyloid precursor protein (βAPP) isoforms (1). Several lines of evidence suggest that overexpression (2,3) or improper processing (4,5) of βAPPs are associated with the development of AD and DS.

Increased APP mRNA levels have been observed in the central nervous system neurons or glia of some AD patients (6). Trisomy of the APP gene in DS results in a 4–5-fold overexpression of APP mRNA and protein (7,8), likely causing accelerated β/A4 production and deposition. The up-regulation of APP mRNA steady-state levels could be the result of either an increased rate of gene transcription, a decreased rate of APP mRNA turnover or both. In a growing number of examples, gene expression can be controlled by post-transcriptional events, such as regulated mRNA turnover and translation (9,10). Thus, dysregulated APP mRNA decay could contribute to elevated levels of APP mRNA and protein in AD and DS.

In mammalian cells, the susceptibility of mRNAs to degradation by cellular ribonucleases varies greatly. Half-lives ($t_{1/2}$) range from as little as 5–30 min for highly unstable cytokine and proto-oncogene mRNAs to >17 h for β-globin mRNA (11–15). Rates of degradation, however, are not static and can be rapidly modulated by extracellular stimuli or intracellular events. For example, treatment of resting cells with 12-*O*-tetradecanoylphorbol-13-acetate (TPA), calcium ionophore or mitogenic antibodies stabilizes several cytokine and lymphokine mRNAs (16–18). UV crosslinking of radiolabeled cytokine and proto-oncogene RNAs to cytoplasmic extracts of stimulated cells has identified multiple protein factors that may influence the decay of labile mRNAs (19–21). Indeed, there is substantial experimental support for activation of mRNA-binding proteins that specifically interact with RNase-sensitive sequences in the coding or 3′-untranslated regions (3′-UTRs) of labile cytokine or proto-oncogene mRNAs (16–21,22). The ensuing transient resistence to cytoplasmic decay contributes to a 50–100-fold increase in protein production upon cell activation (23,24). In hypoxic cells, overexpression of HuR, an RNA binding protein, resulted in stabilization of vascular endothelial growth factor mRNA (25). Thus, direct evidence linking RNA binding proteins to specific mRNA decay has been established.

Most forms of APP mRNA which are produced by alternative splicing and derived from a single gene share an identical 3′-UTR. We have previously shown that treatment of resting peripheral blood mononuclear cells (PBMC) with mitogens [TPA and phytohemagglutinin (PHA)] increased the half-life of APP mRNA from 4 to >12 h (26). During activation, we detected the appearance of several cytosolic, RNA-binding activities that specifically associated with a 29 nt region, 200 nt downstream of the stop codon of APP mRNA (27). These activities were

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purified, sequenced and found to be hnRNP C and nucleolin (28). Based on these data, we proposed that APP mRNA stability was mediated by the regulated interaction of hnRNP C and nucleolin with the 3′-UTR 29 nt element.

In this paper, we have employed a rabbit reticulocyte lysate (RRL) cell-free translation system to directly assess the roles of hnRNP C, nucleolin and the 29 nt element in APP mRNA turnover and translation. Our data show that the 29 nt element destabilizes APP mRNA. Mutation of this element increased the stability of APP mRNA and enhanced APP synthesis 2–4-fold. Western blotting and RNA gel mobility shift assays of the RRL demonstrated the presence of hnRNP C and nucleolin, although only the latter was able to bind APP RNA. Supplementation of the RRL with active hnRNP C stabilized wild-type APP mRNA, with a resultant 6-fold increase in APP synthesis. Therefore APP mRNA decay and translation are up-regulated by occupancy of the 29 nt instability element by hnRNP C.

MATERIALS AND METHODS

Plasmid construction

The construction of pCMV-APP695wt and mut cDNA vectors with a 5′ human cytomegalovirus early promoter and a 3′ SV40 polyadenylation signal has been described (26). The *Hin*dIII site in the 3′-UTR of APP695wt was deleted as follows. The plasmid pUC-18 was linearized with *Hin*dIII, the overhangs filled in with Klenow fragment and religated to produce pUCΔ*Hin*dIII. The APP695 cDNA insert was excised from pCMV-APP695wt with *Xba*I and cloned into pUCΔ*Hin*dIII at the *Xba*I site to produce pUCΔ*Hin*dIIIAPP695wt. The *Hin*dIII site in the APP695 3′-UTR was then deleted as for pUC-18 above, to produce pUCΔ*Hin*-dIIIAPP695wtΔ*Hin*dIII. APP695wtΔ*Hin*dIII cDNA was released with *Xba*I prior to overhang fill-in with Klenow fragment followed by blunt-end ligation into pT7T90 (29) at the *Sma*I site. This plasmid was designated pT7APP695wtΔ*Hin*dIIIT90.

pAPP751wt (from Dr D.Goldgaber, State University of New York) contained a full-length APP751wt cDNA insert in pGEM-9zf(–). pAPP751wt was digested with *Nde*I and *Bgl*II and the mutant *Nde*I–*Bgl*II fragment from pCMV-APP695mut was cloned in. pAPP751wt and mut were digested with *Xba*I and *Spe*I and the inserts were cloned into pT7T90 at the *Spe*I site. Constructs with the correct orientation were then digested with *Spe*I and *Eco*RV. The *Spe*I–*Eco*RV insert from pT7APP695wtΔ*Hin*dIIIT90 was ligated into each plasmid to produce pT7APP751wtΔ*Hin*dIIIT90 and pT7APP751mutΔ*Hin*dIIIT90. These plasmids contained full-length APP751Δ*Hin*dIIIwt or mut cDNAs downstream of a T7 RNA polymerase start site and upstream of a 90 nt poly(dT) tract immediately followed by a unique *Hin*dIII site (Fig. 1).

pHC12 (courtesy of Dr Gideon Dreyfuss, University of Pennsylvania) contained a full-length hnRNP C cDNA insert downstream of an SP6 RNA polymerase start site.

mRNA synthesis *in vitro*

pT7APP751wtΔ*Hin*dIIIT90, pT7APP751mutΔ*Hin*dIIIT90 and pSP6β-globinT90 (courtesy of Dr Richard Spritz, University of Wisconsin) were linearized by overnight digestion with *Hin*dIII. Linearized plasmids were deproteinated using an Ultrafree-Probind (0.45 μm) filter unit (Millipore Corporation, Bedford, MA) and transcribed with either the T7 or SP6 mMessage mMachine kit (Ambion, Austin, TX) following the manufacturer's protocol.

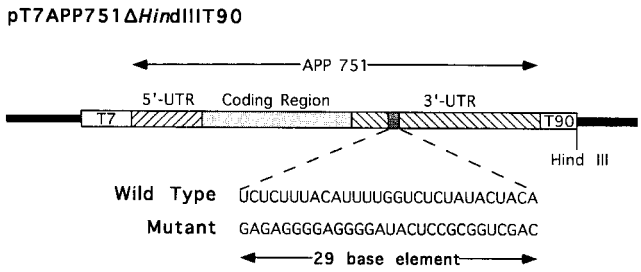

Figure 1. T7APP751T90 *in vitro* transcription vectors. Full-length APP751 cDNAs containing either a wild-type or mutant 29 nt element in the 3′-UTR were cloned into transcription vectors, downstream of a T7 RNA polymerase site and upstream of a 90 nt oligo(dT) tract. The *Hin*dIII site in the 3′-UTR of APP751 cDNAs was first deleted (see Materials and Methods) to enable linearization of the plasmids at the *Hin*dIII site that immediately followed the oligo(dT) tract.

Capped, polyadenylated (90 nt adenosine tail) mRNAs were extracted with phenol/chloroform, precipitated at –80°C and full-length transcripts selected with mini oligo(dT)–cellulose spin columns (5 Prime-3 Prime Inc., Boulder, CO). pHC12 was linearized with *Sty*I and transcribed using the SP6 mMessage mMachine kit. mRNAs were quantitated by absorbance at 260 nm and integrity verified by analysis on denaturing 1.2% agarose–formaldehyde gels.

In vitro mRNA translation/turnover reactions

An aliquot of 1 μg mRNA was translated at 30°C in 50 μl flexi rabbit RRL translation system (Promega, Madison, WI) containing 70 mM KCl, 1.8 mM $Mg(OAc)_2$ and 2–4 μl [^{35}S]methionine. At the indicated time points, 2 μl reaction mix were removed for analysis of radiolabeled proteins by 7.5% SDS–PAGE. Each sample was immediately snap-frozen at –80°C until all time points were completed. After electrophoresis, gels were dried and radioactive bands were quantitated with a phosphorimager (model 445SI; Molecular Dynamics). Experiments were repeated at least three times to ensure that the results were reproducible.

Synthesis of hnRNP C and preincubation with mRNAs

An aliquot of 1 μg hnRNP C or β-globin (control) mRNA was translated without [^{35}S]methionine in 50 μl flexi RRL for 2 h. The entire samples (hnRNP C RRL or β-globin RRL) were immediately frozen at –80°C in 5 μl aliquots. mRNAs were prebound to hnRNP C for 10 min at 30°C in 10 μl reactions containing 2 μl 1:5 diluted hnRNP C RRL, 2 μl 5× binding buffer (75 mM HEPES, pH 8, 50 mM KCl, 1 mM DTT, 50% glycerol) and 1 μg APP or β-globin mRNA. In control experiments, 1:5 diluted β-globin RRL was substituted for the hnRNP C RRL. Translation was initiated by addition of fresh flexi RRL, KCl, $Mg(OAc)_2$ and [^{35}S]methionine as described above. Experiments were repeated at least three times to ensure reproducibility.

Northern blotting

At the indicated times, 1 μl reaction mix was diluted with 10 μl depc-treated water and combined with 990 μl TRI reagent (Molecular Research Center Inc., Cincinnati, OH) and total RNA quantitatively isolated and analyzed by northern blotting as described previously (30). APP mRNA signals were quantitated

by phosphorimaging, normalized to those for 18S rRNA and plotted versus time.

RNA gel mobility shift assay

RNA band shift assays were performed as described previously (27) using ^{32}P-labeled APP RNA (APP-67, nt 2246–2313; 31) as the probe. Briefly, RRLs were incubated for 10 min at 30°C with radiolabeled APP-67 in 10 µl solution containing 15 mM HEPES (pH 8), 2 µg yeast tRNA, 10 mM KCl, 1 mM DTT and 10% glycerol. Following a 30 min digestion at 37°C with 20 U RNase T1 (Sigma), RNA–protein complexes were UV crosslinked for 5 min on ice in a Stratalinker (Stratagene) and resolved by 12% SDS–PAGE. After electrophoresis, gels were dried and analyzed by phosphorimaging. Protein markers were run alongside to identify the sizes of the crosslinked complexes. Unbound, RNase-digested probe was typically run off the bottom of the gel in order to better resolve the gel-shifted RNA–protein bands.

RESULTS

Translation and turnover of APP mRNAs in RRL cell-free translational system

In previous studies we implicated a 29 nt sequence 200 nt downstream of the stop codon as a putative APP mRNA destabilizing element (Fig. 1). We therefore examined the *in vitro* turnover and translation of capped, full-length, polyadenylated APP mRNAs with either an intact (wtAPP751A90) or mutated (mutAPP751A90) 29 nt element (see Materials and Methods). An aliquot of 1 µg each APP mRNA was added to a 50 µl translation reaction containing [^{35}S]methionine (see Materials and Methods) and incubated at 30°C. At various times, radiolabeled proteins from the translation reaction were examined by 7.5% SDS–PAGE. Although APP751A90 mRNAs code for polypeptides of ~83 kDa, we observed radiolabeled bands at 110 kDa (Fig. 2A), indicating that post-translational processing had occurred. Over the 90 min time course, mutAPP751A90 mRNA consistently coded for 2–4-fold as much APP as wtAPP751A90 (Fig. 2B).

To determine if differential APP production was due to differences in the rates of mRNA decay, we performed northern analyses of RNA samples isolated over the time course of translation. When normalized to signals for 18S rRNA, wtAPP751A90 mRNA decayed rapidly with a half-life of 45 min (Fig. 2C and D), while mutAPP751A90 mRNA was substantially more stable ($t_{1/2}$ > 120 min). Based on these data we conclude that the 29 nt sequence destabilizes APP mRNA. Enhanced APP production in the RRL programed with mutAPP751A90 mRNA likely reflects its greater stability.

Expression of active hnRNP C in the RRL

We have previously shown by RNA gel mobility shift assay that multiple cytoplasmic proteins specifically bound the APP mRNA 29 nt element (27,28). These binding activities were not detectable in resting PBMC but were rapidly up-regulated by TPA and PHA treatment. Concurrent with binding activity up-regulation, APP mRNA was stabilized (26). These data suggested that APP mRNA was resistant to RNase when the 29 nt element was occupied. Using conventional biochemical techniques, we purified and sequenced all of the protein constituents of the APP RNA–protein complexes (28). Surprisingly, only two

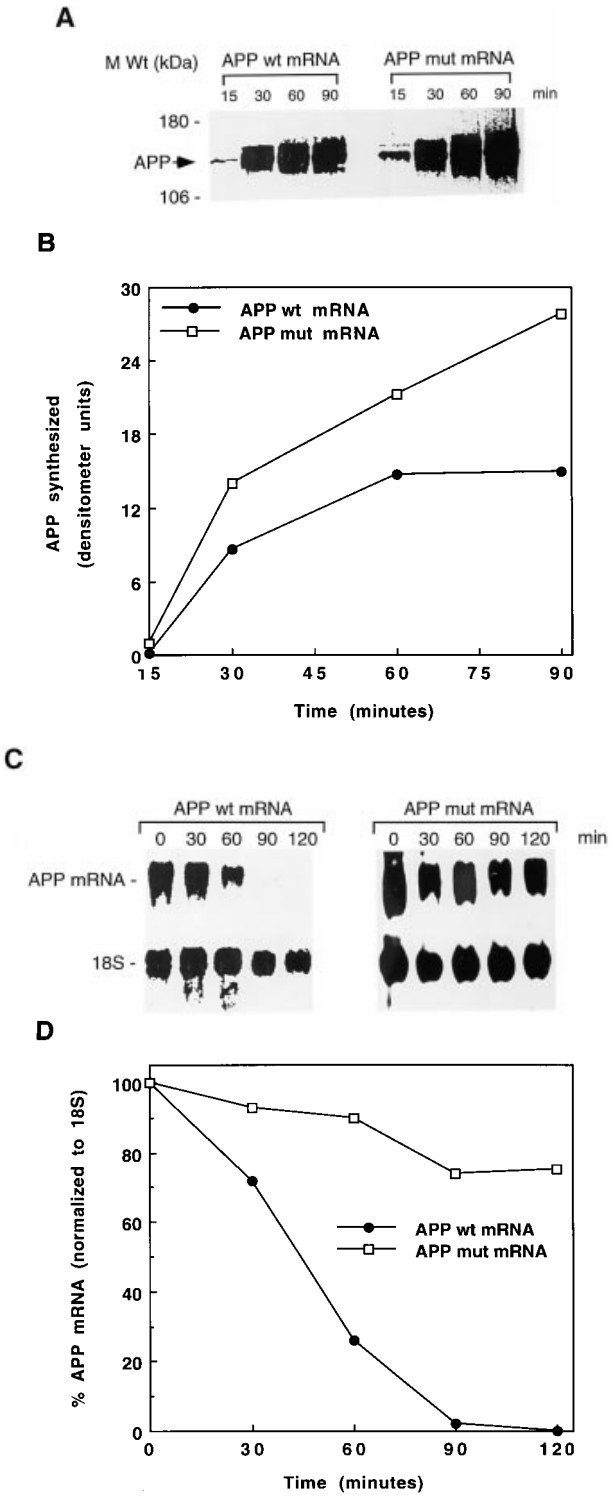

Figure 2. Translation and decay of wtAPP751A90 and mutAPP751A90 mRNAs in a RRL translational system. *In vitro* transcribed, capped and polyadenylated APP mRNAs (see Materials and Methods) were translated in a [^{35}S]methionine-supplemented RRL. (**A**) At the times indicated, aliquots were removed and separated by SDS–PAGE, followed by autoradiography. (**B**) The amounts of APP synthesized were quantitated using a phosphorimager and plotted versus time. (**C**) Aliquots of the translation reaction were removed at the indicated time points for quantitative isolation of total RNA followed by northern blotting and hybridization with cDNA probes for APP and 18S rRNA. (**D**)Autoradiograms were quantitated by phosphorimaging and APP-specific signals were normalized to those for 18S rRNA and plotted versus time.

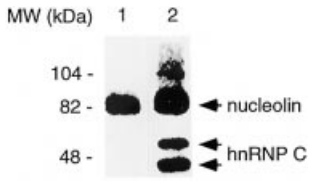

Figure 3. APP binding activities of nucleolin and hnRNP C in the RRL. *In vitro* transcribed, capped hnRNP C RNA or β-globin RNA were translated for 2 h in a RRL. A portion of each pre-programmed RRL was then subjected to RNA gel mobility shift assay with radiolabeled APP-67 (see Materials and Methods). Lane 1, pre-programmed with β-globin RNA; lane 2, pre-programmed with hnRNP C RNA. Gel-shifted nucleolin and hnRNP C bands are indicated with arrows and molecular size protein markers are on the left.

proteins, nucleolin and hnRNP C, were identified. Nucleolin–APP RNA complexes migrated at 104, 84, 73 and 65 kDa, while hnRNP C-APP complexes were at 47 and 42 kDa (27,28). No other complexes were observed.

To examine the role of these two protein factors on APP mRNA decay *in vitro*, we determined whether they were present (by western blotting) and active (by RNA gel mobility shift assay) in the RRL. Western blotting of the RRL using monoclonal anti-nucleolin and anti-hnRNP C antibodies showed the presence of full-length (110 kDa) nucleolin as well as the predicted nucleolin fragment of 70 kDa and full-length (42 kDa) hnRNP C (data not shown). RNA gel mobility shifts of the RRL were performed with radiolabeled wild-type APP RNA (nt 2246–2313, APP-67) as previously described (27). This APP RNA fragment included the entire 29 nt destabilizing element. A single shifted band of 84 kDa was observed (Fig. 3, lane 1) which, based on similarity to prior observations in mitogen-activated lymphocytes (27,28), likely represents the 70 kDa fragment of nucleolin. While full-length APP-67 has a molecular size of 24 kDa, only a portion of the RNA is protected by bound protein from the RNase T1 digestion employed in the gel shift assay. Thus the observed 84 kDa complex is consistent with the 70 kDa nucleolin fragment bound to an ~12 kDa APP RNA fragment. We did not detect any endogenous hnRNP C binding activity (Fig. 3, lane 1). The inability of endogenous hnRNP C to bind the APP 29 nt element may have been due to inactivation of the protein during RRL preparation.

hnRNP C was therefore synthesized by programming a 50 μl translation reaction (hnRNP C RRL) with 1 μg hnRNP C mRNA (see Materials and Methods). A control translation reaction was simultaneously performed with 1 μg β-globin mRNA (β-globin RRL). A portion of each lysate was then used in RNA gel mobility shift experiments with [^{32}P]APP-67. The β-globin RRL contained only endogenous nucleolin binding activity (Fig. 3, lane 1). The hnRNP C RRL showed two additional shifted bands with molecular masses of ~48 kDa (Fig. 3, lane 2). These likely represent synthesized hnRNP C binding activity (27,28). Thus, the RRL was able to produce an active form of hnRNP C that bound to the 29 nt element of APP mRNA.

hnRNP C specifically enhances translation of APP wild-type mRNA

WtAPP751A90, mutAPP751A90 or β-globin mRNAs were preincubated with either hnRNP C RRL or β-globin RRL for 10 min at 30°C in binding buffer (see Materials and Methods). At the end of 10 min, translation was initiated by addition of fresh complete

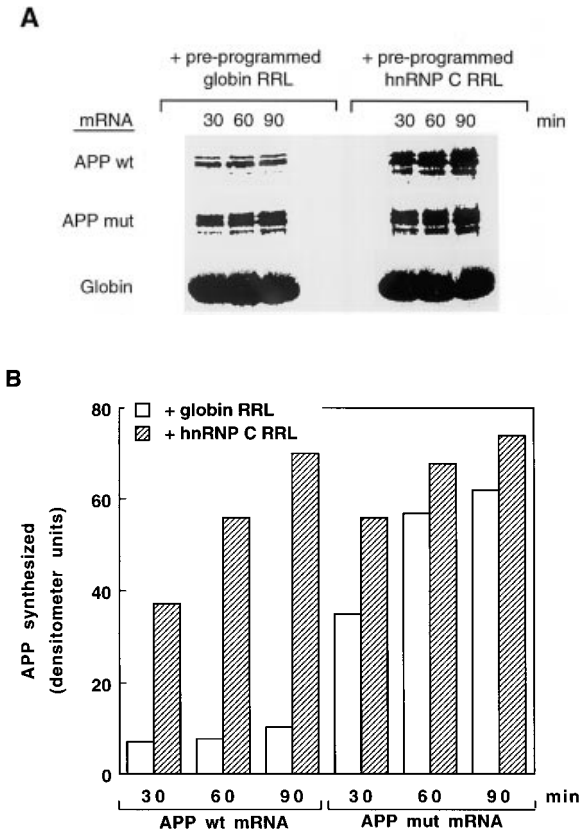

Figure 4. Influence of hnRNP C on *in vitro* translation of wtAPP751A90, mutAPP751A90 and β-globin mRNAs. Individual mRNAs were preincubated for 10 min at 30°C with either β-globin RRL (control) or hnRNP C RRL (see Materials and Methods). Translation was then initiated by addition of fresh RRL and other essential components (see Materials and Methods). At the times indicated, (**A**) the amounts of APP or β-globin synthesized were visualized by SDS–PAGE followed by autoradiography and (**B**) APP amounts were quantitated by phosphorimaging and plotted versus time.

RRL. After 90 min translation, aggregate APP synthesis was 6-fold higher when wtAPP751A90 mRNA was preincubated with hnRNP C RRL compared with β-globin RRL (Fig. 4A and B). hnRNP C had no effect on protein synthesis from mutAPP751A90 or β-globin mRNAs (Fig. 4A and B). Thus the effects of hnRNP C were specific and required the presence of the 29 nt element.

hnRNP C stabilizes APP wild-type mRNA

To determine whether hnRNP C stabilized wtAPP751A90 mRNA, we performed northern analyses. Samples were analyzed for APP mRNA during the preincubation and translation reactions, either in the presence of hnRNP C or β-globin. During preincubation, hnRNP C significantly inhibited decay of wtAPP751A90 mRNA. After 10 min, the reaction containing hnRNP C had 2- to 3-fold greater wtAPP751A90 mRNA than the control reaction (Fig. 5A and B). After translation began, wtAPP751A90 mRNA continued to decay rapidly in the control reaction and was undetectable after 30 min (Fig. 5A). However, in the presence of hnRNP C, wtAPP751A90 mRNA persisted for >60 min. The calculated half-lives based on the initial rates of decay were 3 min in the control reaction and 24 min in the presence of hnRNP C (Fig. 5B). The inhibition of APP mRNA decay was sufficient to account for the 6-fold increase in protein

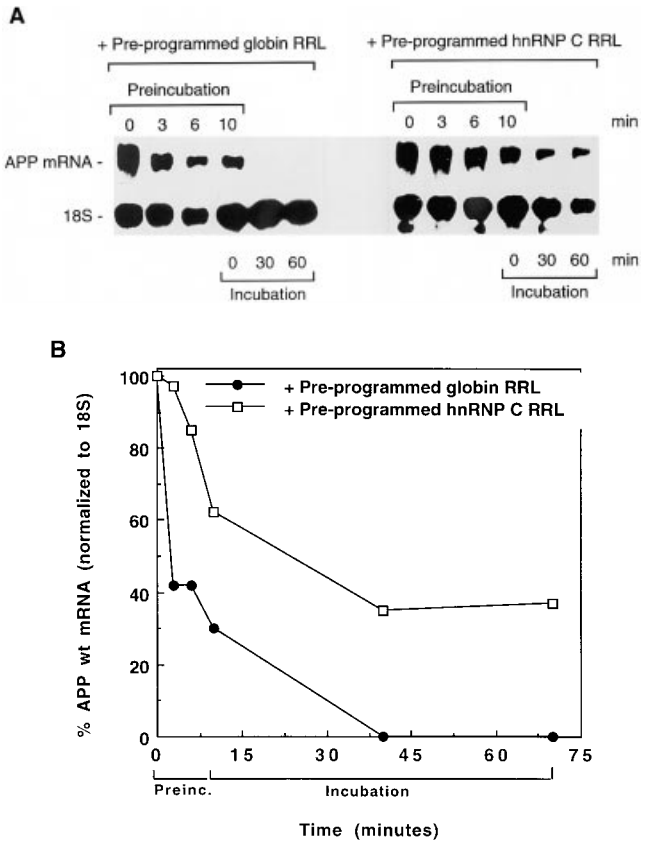

Figure 5. Effect of hnRNP C on the *in vitro* turnover of wtAPP751A90 mRNA. (**A**) Portions of the preincubation reaction and subsequent translation reaction were removed at the indicated times for quantitative isolation of total RNA and northern blotting with radiolabeled cDNA probes for APP and 18S rRNA. (**B**) Autoradiograms were quantitated by phosphorimaging and APP-specific signals were normalized to those of 18S rRNA and plotted versus time.

synthesis. Therefore, the dominant effect of hnRNP C was likely on APP mRNA stability rather than on translation. Addition of hnRNP C to the RRL system containing mutAPP751A90 mRNA did not alter its half-life.

DISCUSSION

Previous studies have suggested that APP expression may be modulated at the post-transcriptional level by variable mRNA decay (26). In quiescent PBMC, APP mRNA decayed with a half-life of 4 h (26). Activation of these cells with TPA and PHA stabilized APP mRNA ($t_{1/2}$ > 12 h). Cytosolic lysates prepared from activated cells contained two cellular proteins, hnRNP C and nucleolin, that bound specifically to a uridine-rich stretch of 29 nt in the 3′-UTR of APP mRNA (26–28). In mice, humans, guinea pigs and primates, this element is highly conserved in primary sequence (≥26 of 29 nt) and distance (~200 nt) from the stop codon. We therefore hypothesized that in quiescent PBMC, the 29 nt element targeted APP mRNA for rapid decay. However, upon cell stimulation, occupancy of this element by hnRNP C and nucleolin blocked APP mRNA decay.

Increased APP mRNA levels have been implicated as a likely cause of accelerated production and deposition of β/A4 peptide in the brains of patients with AD (6) and DS (7,8). Overexpression of APP in cell cultures generated abnormally processed neurotoxic derivatives leading to neurodegeneration (32). Transgenic mice overexpressing APP developed an AD-like histopathology, including brain deposition of β/A4 peptide and aberrant tau protein expression, accompanied by learning and memory impairment (33–35). Thus, APP mRNA stabilization leading to APP overproduction *in vivo* could have a major influence on the onset of AD and DS.

Further examination of these post-transcriptional events was therefore warranted. There are, however, several limitations to studying the mechanisms of mRNA turnover in intact cells. Calculation of mRNA half-lives requires that ongoing transcription be blocked with metabolic poisons like actinomycin D or the adenosine analog 5,6-dichloro-1-β-D-ribofuranosyl benzimidazole. These drugs can independently affect mRNA decay rates (36–38), suggesting that half-life assessments made in their presence may not be valid. A second limitation has been our inability to identify cell lines lacking constitutive hnRNP C and nucleolin binding activities.

A number of *in vitro* systems have been developed to overcome these obstacles and measure the decay of endogenous or exogenous mRNAs in the absence of transcriptional inhibitors. Such systems include post-mitochondrial supernatants (39), whole cell extracts (40), isolated polysomes (30,41) and RRLs (42–44). In this study, we employed an RRL cell-free translation system. We chose this system as exogenous mRNAs and *trans* factors can be easily added and mRNA decay assessed either in the absence of or during ongoing translation. For a number of rapidly decaying cytokine and proto-oncogene mRNAs, experimental evidence suggests that mRNA decay is tightly coupled to ongoing translation (45–47). The RRL system also faithfully mimics intracellular decay. mRNAs coding for IL-1α (42), insulin-like growth factor 1 (43) and several adenosine+uridine-rich mRNAs (44) that decay rapidly *in vivo* had half-lives of <10 min in the RRL system, while a stable mRNA such as β-globin was also stable in the RRL. Endogenous RRL proteins that specifically target IL-1α and other AUUUA-containing mRNAs for rapid degradation, have been identified (42). The RRL is therefore a valid and useful system for RNA stability studies.

Under optimal conditions for translation, wtAPP751A90 mRNA decayed with a half-life of 45 min (Fig. 2D). In previous studies, we observed that mutation of the uridine residues of the 29 nt element to guanidines and cytidines (see Fig. 1) entirely eliminated binding of hnRNP C and nucleolin (26,28). If hnRNP C and nucleolin competed with cellular ribonucleases for recognition of the 29 nt element, then mutation of the element must also protect APP mRNA from RNase-mediated decay. In fact, a mutant APP751A90 mRNA (see Fig. 1) was considerably more stable ($t_{1/2}$ > 120 min) in the RRL system and coded for twice as much APP as wild-type APP751A90 mRNA (Fig. 2). Thus the 29 nt element functioned as an APP mRNA destabilizer. The increase in APP mRNA stability upon mutation of this element can entirely account for the increase in APP production. Other 3′-UTR elements, such as the AUUUA repeats found in several cytokine mRNAs, can influence translational efficiency (48). However, our data do not support a role for the 29 nt element in translational control.

We next wished to determine whether addition of exogenous hnRNP C and nucleolin to the RRL could stabilize wild-type APP mRNA. Nucleolin is a major nucleolar phosphoprotein that autocatalyzes its own degradation. It also contains four consensus RNA-binding domains which are conserved among different species (48,49). Binding studies with pre-rRNA and selection–

amplification (SELEX) experiments revealed that nucleolin interacts specifically with short stem–loop structures that shared a common UCCCGA motif (48,49). This motif is not present in the APP 29 nt element. However, computer-aided folding of the APP mRNA 3′-UTR showed that the 5′-end of the 29 nt element also forms a short putative stem–loop. UV crosslinking of radiolabeled APP RNA to cell extracts from TPA/PHA-stimulated PBMC produced multiple RNA–nucleolin complexes of 104, 84, 73 and 65 kDa (26). The RRL used in these studies, however, contained a dominant RNA–nucleolin complex of 84 kDa (Fig. 3). This endogenous activity did not confer equivalent stability to wtAPP751A90 mRNA, as shown by the mutant version. This suggested that either additional nucleolin peptides and/or hnRNP C protein were required for maximal APP mRNA stability.

hnRNP C binding activity was not detected in the RRL. Upon supplementation of the RRL with exogenous hnRNP C, an 8-fold increase in wtAPP751A90 mRNA half-life was accompanied by a 7-fold increase in APP production. In this system, synthesis of APP is therefore largely controlled by the rate of decay of its mRNA. hnRNP C supplementation increased APP production from wtAPP751A90 mRNA to approximately that seen with mutAPP751A90 mRNA. It therefore seems likely that occupation of the 29 nt APP element by hnRNP C, possibly in cooperation with nucleolin, is a key factor in protecting APP mRNA from degradation by cellular ribonucleases.

In previous studies, we mapped the hnRNP C binding site to the sequence CUUUACAUUUG at the 5′-end of the 29 nt element (see Fig. 1). Others have shown that bacterially expressed recombinant hnRNP C, immobilized on protein A–Sepharose, tightly bound synthetic oligoribonucleotides at stretches of five uridines (50). A uridine-rich sequence therefore seems to be a requirement for hnRNP C binding. Recently, a highly conserved uridine-rich 26 nt regulatory element was identified in the 3′-UTR of GAP-43 mRNA (51). GAP-43 is a neuron-specific protein whose expression is significantly regulated at the post-transcriptional level. The GAP-43 3′-UTR element is very similar to the 29 nt APP element. It is therefore possible that hnRNP C may play a role in the stability of GAP-43 mRNA as well as other mRNAs containing U-rich sequences in their 3′-UTRs.

Based upon these data, we propose that APP expression is regulated post-transcriptionally by a 29 nt cytidine+uridine-rich destabilizing element in the 3′-UTR of its mRNA. The specific binding of hnRNP C and possibly nucleolin to this element protects APP mRNA from cellular RNase-mediated decay. The resultant mRNA stabilization has a dramatic effect on APP synthesis.

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