The 3'-Untranslated Region of the $\alpha_{2C}$-Adrenergic Receptor mRNA Impedes Translation of the Receptor Message*

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We report that two subtypes of $\alpha_2$-adrenergic receptors ($\alpha_{2A/D}$ and $\alpha_{2C}$-AR) are ectopically expressed with dramatically different efficiencies and that this difference is due to a 288-nucleotide (nt) segment in the 3'-untranslated region (3'-UTR) of the $\alpha_{2C}$-AR mRNA that impairs translational processing. NIH-3T3 fibroblasts were transfected with receptor constructs (coding region plus 552 nt, $\alpha_{2C}$-AR; coding region plus 1140 nt, $\alpha_{2A/D}$-AR) and a vector conferring G418 resistance. Transcription was driven by the murine sarcoma virus promoter element, and the receptor gene segment was upstream of an SV40 polyadenylation cassette. Drug-resistant transfectants were evaluated for expression of receptor mRNA and protein. 90% of the NIH-3T3 $\alpha_{2C}$-AR transfectants expressed receptor mRNA, but only 14% of the clonal cell lines expressed receptor protein. In contrast, 90% of the NIH-3T3 $\alpha_{2A/D}$-AR transfectants expressed receptor protein (200–5000 fmol/mg). Similar results were obtained following transfection of DTMF-2 cells with the two receptor constructs. The role of the 3'-UTR of the $\alpha_{2C}$-AR in mRNA processing was determined by generating new constructs in which the 3'-UTR was progressively truncated from 552 to 470, 182, or 74 nt to the stop codon. Truncation of the 3'-UTR resulted in the expression of receptor protein in the G418-resistant transfectants (nt 74, 100%; nt 143, 80%; nt 182, 50%). The level of mRNA in the transfectants expressing the receptor protein was not greater than that in nonexpressing clones, and the differences in protein expression did not reflect altered mRNA stability in the truncated constructs. The $\alpha_{2C}$-AR mRNA with the longer 3'-UTR underwent translational initiation as it was found in the polysome fraction, indicating that the lack of receptor protein was due to impaired translational elongation or termination. These data suggest that translational efficiency is a key mechanism for regulating $\alpha_{2C}$-AR expression and associated signaling events.

The response of the cell to hormones/neurotransmitters is an integrated process that involves varying numbers of molecules. Several factors interact to engineer a specific cell response to a particular hormone. The cell-specific and developmentally regulated expression of entities involved in the signaling process is a key component in this process, allowing different cells to respond to the same hormone but with dramatically different results depending on the receptor subtype expressed and/or the cell phenotype. To maintain signaling specificity and diversity in higher organisms, the system has evolved such that the components of the signaling pathway are expressed as isoforms or closely related molecules subserving similar but distinct functions. The preceding thought is particularly evident for cell-signaling events initiated through heptahelical membrane receptors coupled to heterotrimeric guanine nucleotide-binding proteins. For example, the adrenergic signaling system includes two agonists (norepinephrine and epinephrine) that interact to varying degrees with nine different receptors. Signaling by this system is tightly regulated by mechanisms involving the expression and turnover of members of the adrenergic receptor family. Regulatory mechanisms influence receptor gene transcription, receptor mRNA stability, receptor trafficking, and posttranslational events such as receptor phosphorylation.

The $\alpha_2$ subfamily of adrenergic receptors consists of three distinct proteins that differ in their ligand recognition properties, tissue distribution, signaling efficiency, and regulation (1, 2). Heterologous expression of the three $\alpha_2$-AR1 subtypes in various cells indicates that the three subtypes also exhibit different trafficking patterns within the cell (3, 4) and are selectively phosphorylated by receptor kinases (5). The $\alpha_{2A/D}$-AR subtype is widely distributed in both peripheral tissues and within the central nervous system, whereas in the rat the $\alpha_{2B}$-AR is found primarily in the kidney, liver, and neonatal lung. The rat $\alpha_{2C}$-AR is primarily expressed in the central nervous system, and recently we identified cis elements in the 5' upstream region of the rat $\alpha_{2C}$-AR important for cell type-specific transcription of the receptor gene (6). In contrast to the $\alpha_{2A/D}$-AR, there is an apparent dissociation between $\alpha_{2C}$-AR protein expression and receptor mRNA observed in discrete areas of the central nervous system and the NG108-15 neuroblastoma x glioma cell hybrid (7–10), suggesting that translation of the $\alpha_{2C}$-AR mRNA is a regulated event. To address this possibility, we compared the relationship between mRNA and protein expression following ectopic expression of the $\alpha_{2C}$-AR and $\alpha_{2A/D}$-AR in two cell lines. We report that the 3'-untranslated region of the $\alpha_{2C}$-AR impedes translational processing of the receptor mRNA.

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*These abbreviations used are: AR, adrenergic receptor; 3'-UTR, 3'-untranslated region; nt, nucleotide(s); MSV, murine sarcoma virus.

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NIH-3T3 fibroblasts were maintained in a monolayer culture in Dulbecco’s modified Eagle’s medium at 37 °C under 95% atmosphere of air and 5% CO2. Tissue culture supplies were obtained from JRH Biosciences (Lenexa, KS). Rauwolscine was obtained from Santa Cruz Biochemical Corp. Restriction enzymes and DNA sizing markers were supplied from New England Biolabs Inc. (Beverly, MA). Tissue culture and radioisotope supplies were obtained from JRI Biosciences (Lenexa, KS). Rauwolscine was obtained from Atomergic Chemicals Corp. (Farmindale, NY). RNA isolation kits were obtained from Stratagene (La Jolla, CA).

**Cell Culture, Membrane Preparations, and Radioligand Binding—**

NIH-3T3 fibroblasts were transfected with a eukaryotic expression vector which contained both the MSV long terminal repeat and upstream of the SV40 polyadenylation fragment (2,493 nt) were inserted into the expression vector 3′ to the translational termination codon. To generate α2C-AR constructs were restricted with 3′-UTR, the 1,299-nt segment was subcloned into the EcoRI-No1 restriction sites of pGEM7 (restriction sites at nt 137 of the protein coding region) and 64 nt 3′ to the translational stop codon, the 3′-UTR construct was generated by a three-component ligation (1) pGEM7 containing the α2C-AR (nt 1–782 of the protein coding region), (II) a fragment of α2C-AR (nt 782–1353 of the protein coding region plus 64 nt 3′ to the translational stop codon), and (III) the α2C-AR 3′-UTR from nt 113 to 552 following the translational stop codon. pGEM7.α2C-AR (receptor gene inserted 5′ to EcoRI and 3′ to BamHI in the polylinker) was digested with KpnI and HindIII to remove the last 586 nt of the coding region and the 3′-UTR to generate component I. To generate component II, pGEM7.α2C-AR was digested with NcoI (restriction sites at nt 644 and at nt 643 3′ to the translational stop codon), and then digested with KpnI, yielding component II. To generate component III, we took advantage of an 3′-UTR construct was sequenced by the dideoxy chain termination method. The secondary structure of the α2C-AR gene consisting of the 1374-nt coding region and a 552-nt segment of the gene sequence 3′ to the translational stop codon was insert into the HindIII cloning site of the pMYSV.neo vector as described above. Segments of the various constructs were sequenced by the dyeoxy chain termination method.

NIH-3T3 fibroblasts were transfected with a calcium phosphate precipitate containing 16 µg of expression vector and 4 µg of a plasmid encoding neomycin resistance or with 20 µg of plasmid when the vector containing both the receptor construct and the neomycin resistance cassette was used. Transfected cells were selected for their resistance to the antibiotic G418 (0.5 mg/ml) and harvested by gentle scraping of the plate. Cells were pelleted and resuspended in lysis buffer (50 mM Tris•HCl, pH 8.0, 100 mM NaCl, 5 mM MgCl2, and 0.5% Nonidet P-40). The homogenate was centrifuged at 10,000 × g, and the supernatant was used to prepare cytoplasmic RNA. Cytoplasmic and total cellular RNA was isolated using the Stratagene RNeasy isolation kit according to the manufacturer’s instructions. Isolated RNA was subjected to electrophoresis on 1% agarose, 3% formaldehyde gels followed by transfer to a nylon filter (Hybond-N) by pressure blotting. The filter was then baked for 2 h at 80 °C in a vacuum oven and prehybridized in phosphate buffer containing 0.5 M NaH2PO4, pH 7.2, 1% bovine serum albumin, 7% SDS, 1 mM EDTA at 65 °C for 1 h before being added probe as described previously (6, 15). Radiolabeled probes were prepared by random priming using the receptor gene segment as a template. The stability of receptor mRNA was determined by hybridizing cells at different times after the blockade of transcription with actinomycin D (5 µg/ml) (15). RNA blots were then hybridized with the appropriate probe as described above. mRNA degradation rate was calculated after densitometric scanning of the autoradiographs.

**Analysis of Ribosomal Distribution of Receptor mRNA**—Polysomes were isolated as described previously (16, 17). Monolayer cultures of NIH-3T3 transfectants were washed with Hanks’ balanced salt solution at 4 °C (5.4 mM KCl, 0.3 mM NaH2PO4, 0.4 mM KH2PO4, 4.2 mM NaHCO3, 1.5 mM CaCl2, 0.5 mM MgCl2, 0.6 mM MgSO4, 137 mM NaCl, 5.6 mM KCl, 0.02% sodium azide, 0.01% cycloheximide, and 2 µl/mg RNasin. 150 µl of 10% Tween 80, 5% deoxycholate was added to the homogenate, the intact nuclei and mitochondria were removed by centrifugation, and the supernatant was loaded onto 15–50% linear sucrose gradients. The gradients were spun at 4 °C at 35,000 rpm in an SW 41 rotor for 2 h and then displaced upward through a modified 0.5-mm flow cell in an ISCO fractionator set to continuously monitor absorbance at 254 nm. Each fraction was extracted with phenol:chloroform, and the RNA was precipitated. The RNA pellet was dissolved in water and denatured by 50% formamide, 8% sodium dodecyl sulfate, 1× SSC, 1× Denhardt’s, and incubated at 68 °C for 15 min, and −µg of RNA was blotted directly onto a nylon membrane in a slot-blot apparatus. The blot was hybridized as described above. Following the removal of bound receptor subtype probe, the blot was hybridized with a nick-translated probe derived from rat 288 RNA to provide controls for sample loading.

**Secondary Structure of RNA**—The secondary structure of the α2C-AR 3′-UTR was determined by the use of a genetic algorithm, which is able to simulate RNA folding pathways. The essential features of the algorithm involve mutations and crossovers in the population of solutions, with subsequent processing of the fittest solutions to generate new solutions as described previously (18, 19). At every algorithm iteration, the population of structures was expanded via the mutation/crossover process and subsequently diminished to that of the original population by fitness criteria. A particular analysis was considered completed when the free energy was not improved after a chosen number of repetitions. The program MFOOLD in the University of Wisconsin GCG sequence analysis package was used for energy-minimum calculations. The analysis was achieved using the IBM programming language in the program STAR.

**RESULTS**

**Receptor Expression in NIH-3T3 Fibroblasts and DDT, MF-2 Cells**—A stable transfection system was used to evaluate the role of the 3′-UTR in regulating expression of the α2C-AR. A fragment of the rat α2C-AR gene consisting of the 1374-nt coding region and a 552-nt segment of the gene sequence 3′ to the translational stop codon was inserted into an expression vector downstream of the pMSV promoter and upstream of an SV40 polyadenylation cassette (11) (Fig. 1A). The α2C-AR gene construct was introduced into NIH-3T3 fibroblasts by calcium phosphate transfection, and G418-resistant clones were evaluated for receptor expression by radioligand binding assays and RNA blot analysis. Only 14% of the drug-resistant clones expressed α2C-AR protein, whereas 90% of the clones expressed receptor mRNA (Fig. 1, B and C). Similar results were obtained in three different transfections in which a total of >100 individual clones were screened for receptor expres-
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FIG. 1. Expression construct and transfection efficiency for the α<sub>2c</sub>-AR in NIH-3T3 fibroblasts and DDT<sub>1</sub>MF-2 cells. NIH-3T3 fibroblasts and DDT<sub>1</sub>MF-2 cells were stably transfected with the receptor subtype gene fragment (A) as described under "Experimental Procedures." G418-resistant clonal transfectants were screened for receptor subtype expression by radioligand binding assays using the α<sub>2c</sub>-AR-selective ligand [3H]RX821002 at saturating concentrations (~20 nM), and the results are expressed as fmol of receptor/mg of membrane protein (B). α<sub>2c</sub>-AR transfectants were further evaluated for gene expression by RNA blot analysis (C). Total RNA was prepared from selected transfectants and processed as described under "Experimental Procedures." The RNA blot was hybridized with a random-primed probe generated from the gene insert contained in the expression vector. The asterisks above the lanes in C indicate that the RNA was prepared from a transfectant expressing α<sub>2c</sub>-AR protein as determined in radioligand binding assays. The lines to the right of each RNA blot indicate the migration of 28S and 18S rRNA. The results are representative of the data obtained from similar studies in which 100 individual clones were screened for receptor expression in each cell type.

The dissociation between mRNA and expressed protein for the α<sub>2c</sub>-AR mRNA was not greater than that in clones lacking receptor protein (Fig. 1C). Similar results were obtained when NIH-3T3 fibroblasts were transfected with a receptor construct in which the drug resistance cassette was inserted into the receptor expression vector. The dissociation between α<sub>2c</sub>-AR protein and mRNA was also observed following stable transfection of DDT<sub>1</sub>MF-2 cells derived from hamster smooth muscle, indicating that the failure to process the receptor mRNA is not restricted to a fibroblast cell line (Fig. 1, B and C).

The dissociation between mRNA and expressed protein for the α<sub>2c</sub>-AR was not observed in similar experiments using constructs encoding the α<sub>2b/D</sub>-AR subtype. A fragment of the α<sub>2b/D</sub>-AR gene consisting of the 1350-nt coding region and a 1140-nt segment of the gene sequence 3′ to the translational stop codon was inserted into the pMSV expression vector and introduced into NIH-3T3 fibroblasts and DDT<sub>1</sub>MF-2 cells as described above (Fig. 2A). Approximately 95% of the α<sub>2b/D</sub>-AR transfectants expressed receptor protein, suggesting that the α<sub>2c</sub>-AR and α<sub>2b/D</sub>-AR mRNAs are processed with different efficiencies by the two cell lines (Fig. 2B). The two receptor constructs contained identical 5′ upstream regions derived from the vector and exhibited 64% nucleotide sequence identity in the protein coding region. The two receptor constructs encoded proteins that exhibited 55% overall homology. A major difference between the two constructs was the gene segment 3′ to the translational stop codon, and subsequent studies focused on the influence of this region on α<sub>2c</sub>-AR expression.

α<sub>2c</sub>-Adrenergic Receptor Expression Using Truncated Constructs—Sequence analysis of the 3′-UTR of the α<sub>2c</sub>-AR identified a polyadenylation signal AAUAAA at nt 469 (Fig. 3). The sequence of the genomic clone in this region was identical to that of a rat α<sub>2c</sub>-AR cDNA (20). The α<sub>2c</sub>-AR 3′-UTR sequence from nt 1 to 221 is rich in GC nt (67%), whereas the GC content decreases to 40% from nt 222 to 481. To determine if the 3′-UTR of the α<sub>2c</sub>-AR influenced receptor expression, NIH-3T3 cells were transfected with α<sub>2c</sub>-AR gene constructs in which the 3′-UTR was progressively truncated to 470, 182, 143, and 74 nt 3′ to the translational stop codon (Fig. 4A). In contrast to the limited expression of the original receptor construct, ~50 (nt 182), 80 (nt 143), and 100% (nt 74) of the cells transfected with α<sub>2c</sub>-AR constructs in which the 3′-UTR was truncated expressed receptor protein (Fig. 4B). In terms of the number of clones expressing receptor protein, the α<sub>2c</sub>-AR.3′-UTR-182 transfectants were intermediate relative to the α<sub>2c</sub>-AR.3′-UTR-143 and the α<sub>2c</sub>-AR.3′-UTR-470 transfectants. Photoaffinity labeling of the expressed α<sub>2c</sub>-AR with the α<sub>2c</sub>-AR photoprobe 125I-AzRAU and radioligand binding studies indicated that the receptor exhibited the ligand recognition properties and M<sub>r</sub> expected of an α<sub>2c</sub>-AR (13, 14).2 The expression of receptor in the 3′-truncated constructs but not in the α<sub>2c</sub>-AR.3′-UTR-470 or the α<sub>2c</sub>-AR.3′-UTR-552 was independent of the relative levels of receptor mRNA (Fig. 4C). Truncation of the 3′-UTR to nt 470 removed the polyadenylation signal in the receptor gene sequence, and the α<sub>2c</sub>-AR.3′-UTR-470 transfectants were similar to the α<sub>2c</sub>-AR.3′-UTR-552 transfectants in that ~90% of the clones expressed receptor message but not receptor protein (Fig. 4B). These data indicated that the presence of a polyadenylation site in addition to that in the expression vector did not account for the observed lack of mRNA processing and also suggest that there is no long range inter-

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action of this region with the 5′ region of the transcript. These data indicated that a segment of the α2C-AR 3′-UTR from nt 183 to 470 (3′ to the translational stop codon) regulated the processing of the receptor transcript.

Computer simulation of RNA folding in the 3′-UTR generates a relatively stable secondary structure (Fig. 5). The most stable structural elements of the α2C-AR 3′-UTR are the hairpins from nt 13–90, 135–237, and the long branched hairpin between nt 260 and 450. The 3′-UTR also contains a motif (nt 469–476, UUUUUUAA) similar to sequences UUAUUUAU associated with message instability. Relative to the results of receptor expression in Fig. 4, the hairpin from nt 13–90 is apparently not involved in the inhibition of translational processing of the α2C-AR mRNA, as receptor expression was observed with the p0α2C-AR.3′-UTR-143 construct. As the most efficient processing of the receptor mRNA occurs with the p0α2C-AR.3′-UTR-74 and the p0α2C-AR.3′-UTR-143 constructs, the stable hairpin from nt 135–237 may contribute to the observed results. However, the p0α2C-AR.3′-UTR-182 construct also expressed receptor protein in ~50% of the transfecants, suggesting that the large branched hairpin between nt 260–450 also played a role in the translational processing of the receptor message.

Cellular Localization and Stability of α2C-AR Transcripts—The processing of transcripts involves several steps including capping, polyadenylation, splicing, transport out of the nucleus, and movement through various populations of ribosomes in the cytoplasm. The role of the 3′-UTR in these events was addressed by determining the distribution of full-length and truncated mRNA species within the cell and the relative stability of the different α2C-AR transcripts. The apparently poor processing of the full-length versus truncated mRNA may reflect a failure of the full-length transcript to move out of the nucleus and associate with a translationaly active population of ribosomes in the cytoplasm. This issue was addressed by comparing the relative amounts of α2C-AR mRNA in the cytosol in the transfecants that expressed the receptor protein with those that did not. Analysis of cytosolic versus total cellular α2C-AR mRNA indicated that a portion of the mRNA species generated from the p0α2C-AR.3′-UTR-74 and p0α2C-AR.3′-UTR-552 constructs were both found in the cytosol (Fig. 6). Thus, the failure of the p0α2C-AR.3′-UTR-552 to be processed into receptor protein was not due to the lack of potential mRNA access to the translational machinery. Fig. 6 also indicates that the lack of receptor protein in the p0α2C-AR.3′-UTR-552 transfecants was not due to lower amounts of receptor mRNA relative to those observed in p0α2C-AR.3′-UTR-74 transfecants.

Once in the cytosol, the pα2C-AR.3′-UTR-552 mRNA underwent translational initiation as indicated by the presence of the mRNA in the polysome complex of translationaly active ribosomes (Fig. 7). These data suggest that the presence of the 3′-UTR segment in pα2C-AR.3′-UTR-552 impedes the movement of the ribosome along the mRNA and that removal of the 3′-UTR segment between nt 74 and 552 removes this constraint. The failure to complete the translational processing of the mRNA was not associated with any differences in the relative stabilities of the full-length and truncated mRNAs (Fig. 8). The stability of the α2C-AR mRNA species was determined following the transcription block with actinomycin D and compared with that of β-actin as an internal control for RNA loading. Analysis of the degradation rate of receptor mRNA in p0α2C-AR.3′-UTR-74 and p0α2C-AR.3′-UTR-552 transfecants revealed a similar t1/2 (~6 h) for both species, indicating that the 3′-UTR segment from nt 74 to 552 did not influence mRNA stability (Fig. 8C). These data indicated that the α2C-AR 3′-UTR was interfering with translational processing of the α2C-AR mRNA. To determine if the 3′-UTR of the α2C-AR could regulate translation of a heterologous mRNA, we generated a
construct in which the 3'-UTR of the α2C-AR was substituted for the 3'-UTR of the α2A/D-AR (Fig. 9). The segment of the α2C-AR 3'-UTR appended to the α2A/D-AR contained the portion that apparently impeded translation of the α2C-AR mRNA (Figs. 4 and 9A). NIH-3T3 cells were transfected with the α2A/D-AR/α2C-AR-3'-UTR construct, and receptor expression was compared with that obtained in parallel transfections with the wild-type α2A/D-AR construct indicated in Fig. 2. The pα2A/D-AR/α2C-AR-3'-UTR and pα2A/D-AR transfectants behaved similarly in terms of receptor expression (Fig. 9). All of the six clonal cell lines examined from each transfection expressed receptor protein as determined in radioligand binding assays (Fig. 9). RNA blot analysis indicated that similar amounts of α2A/D-AR/α2C-AR-3'-UTR and α2A/D-AR mRNA were expressed in the two series of transfections.

**DISCUSSION**

The processing of mRNA to the mature protein is subject to regulation at several steps including translational initiation, elongation, and termination (21–23). These events are influenced by several factors and often involve cis elements in the 5'- and 3'-untranslated regions of the mRNA that are recognized by specific RNA-binding proteins and participate in mRNA masking, message stabilization, and/or movement of messages among different ribosome populations within the cell (24–33). The regulation of protein expression at a translational level has evolved to play significant roles in various aspects of cell-signaling events. One of the first points of regulation in the translation process is translational initiation and the association of the mRNA with polysomes via the 43 S complex. This step is rate-limiting for the translation of most mRNA molecules, primarily due to stoichiometric issues concerning the factors required to form the translational initiation complex. One of the best understood examples of translational regulation involves the iron-responsive elements present in the 5'-UTR of ferritin mRNA and their influence on translational initiation (30). Translational initiation is also influenced by the 3'-UTR as indicated by the masking of maternal mRNAs. The masking of maternal mRNAs in *Xenopus* involves the binding of proteins to specific sequences in the 3'-UTR of the mRNA, resulting in a translational block. At appropriate stages of development, the mRNA species are unmasked with subsequent expression of the protein. These and other observations related to posttranscriptional editing of the poly(A) tail and its influence on translation indicate that there is a possibility of physical interplay between the 5'- and 3'-untranslated regions of mRNAs. Such an interaction of these two domains may be an important component of translational regulation. The 3'-UTR is also an important determinant of stability for several mRNA species (e.g., β-tubulin, β2-AR) and plays a key role in the segregation of specific mRNAs within the cell (21, 31, 32, 34).

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**Fig. 5. Predicted structure of the 3'-UTR of the α2C-AR mRNA.** mRNA folding pathways were simulated using a genetic algorithm to generate the displayed secondary structure as described under "Experimental Procedures." The sites of truncated constructs described in Fig. 4 are indicated by the boxed numbers. The polyadenylation signal is underlined.
A second point of regulation occurs as the translational machinery searches for the start codon positioned in the most favorable context for initiation of protein synthesis. Once translation is initiated, elongation proceeds at varying rates for different messages, eventually terminating at the stop codon through the action of the release factor and the subsequent dissociation of the peptide from the ribosome. The elongation process is engineered through the action of elongation factors, and it is fairly rapid, incorporating 4–6 amino acids per s. The rate of the elongation process is also potentially subject to regulation, although the mechanisms involved in such regulation are poorly understood. A decrease in elongation rate (i.e., translational stalling) may result in an increased amount of mRNA associated with the polysome fraction in the cytosol, as it is not efficiently processed through the translation process.

In contrast, mRNAs that are elongated at normal rates would spend less time in the polysome complex. Thus, there are several points during translation at which the processing of a particular mRNA can be specifically regulated. Depending upon the type of translational regulation, a situation could exist where there is detectable mRNA but the corresponding protein is absent. Such is the situation for the $\alpha_{2C}$-AR mRNA.

The translational processing of $\alpha_{2C}$-AR mRNA appears to be a regulated event, and this regulation involves a 278-nt segment in the 3'5'-UTR of the $\alpha_{2C}$-AR. The importance of this segment in the processing of the $\alpha_{2C}$-AR mRNA is indicated by the expression of the protein following removal of this domain. As the protein coding region is identical in the truncated construct, it is not possible to explain the observed data based on...
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Among the three \( \alpha_2 \)-AR subtypes, the \( \alpha_{2A/D} \)-AR has the widest tissue distribution in the rat as determined by both analysis of mRNA and radioligand binding studies. \( \alpha_{2A/D} \)-AR mRNA is found in kidney, liver, pancreas, adipocytes, vascular smooth muscle cells, and RIN-5A\( \) pancreatic beta cells. Each of these tissues also expresses the receptor protein. In peripheral tissues and within the central nervous system, the distribution of \( \alpha_{2A/D} \)-AR mRNA correlates with receptor expression as determined by in situ hybridization, immunoblotting, and radioligand binding (37–39). In contrast to the \( \alpha_{2A/D} \)-AR, the expression of the rat \( \alpha_{2B} \)-AR and \( \alpha_{2C} \)-AR is more restricted. \( \alpha_{2C} \)-AR mRNA and protein are primarily found in the central nervous system, although low levels are detected by in situ hybridization in the kidney (7, 38, 39). The distribution of \( \alpha_{2C} \)-AR and \( \alpha_{2A/D} \)-AR mRNA and immunoreactivity in the central nervous system is discussed by Rosin et al. (7) and Talley et al. (37). The distribution of \( \alpha_{2C} \)-AR mRNA within the central nervous system is not entirely consistent with the receptor distribution defined by immunohistochemistry. In contrast to the close relationship between mRNA and detectable protein for the \( \alpha_{2A/D} \)-AR, there are selected sites within the central nervous system (islands of Calleja, nucleus accumbens, superior and inferior colliculus, caudate putamen) in which there is a dissociation between \( \alpha_{2C} \)-AR mRNA and detectable protein as determined by either immunohistochemistry or radioligand binding. A dissociation between \( \alpha_{2C} \)-AR mRNA and detectable protein is also observed in the neuroblastoma \( \times \) glioma cell line NG108-15. Although transcripts encoding the \( \alpha_{2B} \)-AR and \( \alpha_{2C} \)-AR are identified in NG108-15 cells (6–10), receptor purification and radioligand binding studies indicate expression of the \( \alpha_{2C} \)-AR but not the \( \alpha_{2C} \)-AR protein (10, 40). The presence of \( \alpha_{2C} \)-AR mRNA, but the absence of receptor protein, is exactly the situation observed in the present studies. Precise interpretation of receptor mRNA versus protein distribution in the rat central nervous system can be complicated by potential neuronal transport of mRNA and/or proteins. However, the dissociation between the \( \alpha_{2C} \)-AR mRNA and receptor protein contrasts with the close relationship between mRNA and protein for other membrane receptors in the central nervous system and suggests that translation may be an important point of regulation for the expression of the \( \alpha_{2C} \)-AR. The data presented in the present manuscript are consistent with this possibility and suggest that the 3'-UTR of the \( \alpha_{2C} \)-AR mRNA exerts a strong influence on translational processing of the receptor message.

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REFERENCES

1. Hieble, J. P., Bondindell, W. E., and Ruffolo, R. R., Jr. (1995) J. Med. Chem. 38, 3415–3444
2. Ruffolo, R. R., Jr., Bondindell, W., and Hieble, J. P. (1995) J. Med. Chem. 38, 3681–3716
3. von Zastrow, M., Link, R., Daunt, D., Barsh, G., and Kohlbik, B. (1993) J. Biol. Chem. 268, 763–766
4. Wozniak, M., and Limbird, L. E. (1996) J. Biol. Chem. 271, 5017–5024
5. Jewell-Metz, E. A., and Liggett, S. B. (1996) J. Biol. Chem. 271, 18082–18087
6. Saulnier-Blanche, J. S., Yang, Q., Sherlock, J. D., and Lanier, S. M. (1996) Mol. Pharmacol. 50, 1432–1442
7. Rosin, D. L., Taylor, E. M., Lee, A., Stornetta, R. L., Gaylinn, B. D., Guynet, P. G., and Lynch, K. R. (1996) J. Comp. Neurol. 372, 135–165
8. Lorenz, W., Lomasney, J. W., Collins, S., Regan, J. W., Caron, M. G., and Lefkowitz, R. J. (1990) Mol. Pharmacol. 38, 599–603
9. Hu, G., Quermit, L. A., Downing, L. A., and Charness, M. E. (1993) J. Biol. Chem. 268, 23441–23447
10. Wilson, A. L., Seibert, K., Brandson, S., Cragoe, E. J., Jr., and Limbird, L. E. (1991) Mol. Pharmacol. 39, 481–486
11. Duiz, E., and Lanier, S. M. (1992) J. Biol. Chem. 267, 24045–24052
12. Lanier, S. M., Downing, S., Duiz, E., and Homcy, C. J. (1991) J. Biol. Chem. 266, 10470–10478
13. Duiz, E., Couprie, I., Downing, S., and Lanier, S. M. (1992) J. Biol. Chem. 267, 9844–9851
14. Couprie, I., Duiz, E., and Lanier, S. M. (1992) J. Biol. Chem. 267, 9852–9867
15. Hamadzic, D., Duiz, E., Sherlock, J. D., and Lanier, S. M. (1995) Annu. Rev. Physiol. 60, 162–171
16. Craig, N. (1973) J. Cell. Physiol. 82, 133–150
17. Ivettz, C. T., Twuworth, W. J., Cooper, G., and McDermott, P. J. (1995) J. Biol. Chem. 270, 21950–21957
18. Van Batenburg, E., Gultyaev, A. P., and Pleij, C. W. A. (1994) J. Theor. Biol. 174, 269–280
19. Gultyaev, A. P., Van Batenburg, F. H. D., and Pleij, C. W. A. (1995) Mol. Biol. 230, 37–51
20. Voigt, M., McCune, S. K., Kanterman, R. Y., and Felder, C. C. (1991) FEBS Lett. 278, 45–50
21. Hofland, R., Hesketh, J. E., and Pryme, I. F. (1996) Annu. Rev. Biochem. Cell Biol. 29, 1089–1105
22. Jacobson, A., and Peltz, S. W. (1996) Annu. Rev. Biochem. 65, 683–739
23. Kuruk, M. (1992) Annu. Rev. Cell Biol. 8, 197–225
24. Ross, J. (1995) Microbiol. Rev. 59, 423–450
25. Kim-Ha, J., Kerr, K., and Macdonald, P. M. (1995) Cell 81, 403–412
26. Dubnau, J., and Struhl, G. (1996) Nature 380, 694–699
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27. Sonenberg, N. (1994) Curr. Opin. Genet. Dev. 4, 310–315
28. Hannan, A. J., Henke, R. C., Weinberger, R. P., Sentry, J. W., and Jeffrey, P. L. (1996) Neuroscience 72, 889–900
29. Elizabeth, R. G., and Lehmann, R. (1994) Nature 369, 315–318
30. Müllner, E. W., Neupert, B., and Kuhn, L. C. (1989) Cell 58, 373–382
31. Wormington, M. (1993) Cell Biol. 5, 950–954
32. Tholanikunnel, B. G., Granneman, J. G., and Malbon, C. C. (1995) J. Biol. Chem. 270, 12787–12793
33. Sommerville, J., and Ladomery, M. (1996) FASEB J. 10, 435–443
34. Ferrandon, D., Elpick, L., Nüsslein-Volhard, C., and St. Johnston, D. (1994) Cell 79, 1221–1232
35. Theodorakis, N. G., Banerji, S. S., and Morimoto, R. I. (1988) J. Biol. Chem. 263, 14579–14585
36. Goldspink, P. H., Thomason, D. B., and Russell, B. (1996) Am. J. Physiol. 40, H2584–H2590
37. Talley, E. M., Rosin, D. L., Lee, A., Guyenet, P. G., and Lynch, K. R. (1996) J. Comp. Neurol. 372, 111–134
38. Scheinin, M., Lomasney, J. W., Hayden-Hixson, D. M., Schambra, U. B., Caron, M. G., Lefkowitz, R. J., and Fremeau, R. T., Jr. (1994) Mol. Brain Res. 21, 133–149
39. Nicholas, A. P., Pieribone, V., and Hokfelt, T. (1993) J. Comp. Neurol. 328, 575–594
40. Bylund, D. B., and Ray-Prenger, C. (1989) J. Pharmacol. Exp. Ther. 251, 640–644