The Journal of Neuroscience, March 15, 1997, 17(6):1950–1958

Post-Transcriptional Regulation of the GAP-43 Gene by Specific Sequences in the 3′ Untranslated Region of the mRNA

Kao-Chung Tsai,[1] Victor V. Cansino,[1] Douglas T. Kohn,[1] Rachael L. Neve,[2] and Nora I. Perrone-Bizzozero[1]

[1]Departments of Biochemistry and Neuroscience and Cancer Center, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131, and [2]McLean Hospital, Departments of Psychiatry and Genetics, Harvard Medical School, Boston, Massachusetts 02115

We have shown previously that GAP-43 gene expression during neuronal differentiation is controlled by selective changes in mRNA stability. This process was found to depend on highly conserved sequences in the 3′ untranslated region (3′ UTR) of the mRNA. To map the sequences in the GAP-43 3′ UTR that mediate this post-transcriptional event, we generated specific 3′ UTR deletion mutants and chimeras with the β-globin gene and measured their half-lives in transfected PC12 cells. Our results indicate that there are two distinct instability-conferring elements localized at the 5′ and 3′ ends of the GAP-43 3′ UTR. Of these destabilizing elements, only the one at the 3′ end is required for the stabilization of the mRNA in response to treatment with the phorbol ester TPA. This 3′ UTR element consists of highly conserved uridine-rich sequences and contains specific recognition sites for two neural-specific GAP-43 mRNA-binding proteins. Analysis of the levels of mRNA and protein derived from various 3′ UTR deletion mutants indicated that all mutants were translated effectively and that differences in gene expression in response to TPA were attributable to changes in GAP-43 mRNA stability. In addition, the phorbol ester was found to affect the binding of specific RNA-binding proteins to the 3′ UTR of the GAP-43 mRNA. Given that, like the GAP-43 mRNA, its degradation machinery and the GAP-43 mRNA-binding proteins are expressed primarily in neural cells, we propose that these factors may be involved in the post-transcriptional regulation of GAP-43 gene expression during neuronal differentiation.

Key words: GAP-43; post-transcriptional regulation; gene expression; neuronal differentiation; mRNA stability; RNA-binding proteins; PC12 cells

The growth-associated protein GAP-43 is expressed in neurons primarily during the development and regeneration of neural connections (for review, see Benowitz and Routtenberg, 1987; Skene, 1989). After synaptogenesis, GAP-43 expression declines sharply in most neuronal populations except for those in specific association areas in the neocortex and limbic system (Benowitz et al., 1988; Neve et al., 1988). GAP-43 is known to participate both in mechanisms of axonal pathfinding during neural development and in the regulation of neurotransmitter release and synaptic plasticity in mature synapses (Dekker et al., 1989; Aigner and Caroni, 1993; Ivins et al., 1993; Meberg et al., 1995; Strittmatter et al., 1995). Given the important biological properties of this protein, it is of great interest to define the mechanisms that control the developmental pattern and regional variations in GAP-43 gene expression. Findings by several laboratories clearly indicate that neural specificity in the expression of this gene is controlled by elements in the promoter region (Nevidi et al., 1992; Verhaagen et al., 1993; Starr et al., 1994; Vanselow et al., 1994). A second level of control of GAP-43 gene expression is mediated by post-transcriptional mechanisms (Federoff et al., 1988; Perrone-Bizzozero et al., 1991, 1993). The induction of the GAP-43 mRNA during brain development and nerve regeneration does not correlate well with its rate of transcription (Perrone-Bizzozero et al., 1991). Furthermore, in undifferentiated PC12 cells, the gene is transcribed constitutively, although the steady-state levels of the GAP-43 mRNA are very low. The apparent discrepancy between the rates of synthesis and the accumulation of the GAP-43 mRNA in PC12 cells was found to be attributable to the intrinsic instability of the mRNA. In contrast, when cells are induced to differentiate in response to NGF, the GAP-43 mRNA is stabilized selectively via protein kinase C-dependent mechanisms (Perrone-Bizzozero et al., 1993). These results are consistent with the idea that changes in mRNA stability play a crucial role in the control of GAP-43 gene expression.

To define the molecular basis for the control of GAP-43 mRNA stability, we began to examine the *cis*- and *trans*-acting factors involved in this process. We found that the GAP-43 3′ UTR is highly conserved and contains major determinants for mRNA instability (Kohn et al., 1996a). Unlike such determinants in other post-transcriptionally regulated mRNAs (Shaw and Kamen, 1986; Shyu et al., 1991), these sequences do not contain AUUUA motifs but exhibit multiple U-rich stretches. We recently have shown that these U-rich regions contain recognition sites for three brain-specific RNA-binding proteins (Kohn et al., 1996a). The finding that their binding activity and the levels of the GAP-43 mRNA are correlated spatially and temporally further suggests that these proteins may be involved in the post-transcriptional regulation of GAP-43 gene expression (Kohn et al., 1996a).

Received Oct. 31, 1996; revised Dec. 23, 1996; accepted Jan. 13, 1997.

This work was supported in part by National Institutes of Health (Grants NS30255 and HD24236), the American Paralysis Association (Grant PBI-9006), and dedicated Health Research Funds of the University of New Mexico School of Medicine. K-C. T. was supported by the Tri-Service General Hospital, Taipei, Taiwan. We thank Dr. Richard Burry for his gift of the PC12–N36 cell line and Dr. D. A. Melton for supplying the pSP64T β-globin UTR vector used in these studies.

Correspondence should be addressed to Dr. Nora I. Perrone-Bizzozero, Department of Biochemistry, BMSB Room 249, University of New Mexico School of Medicine, Albuquerque, NM 87131-5221.

Dr. Tsai's present address: Department of Physical Medicine and Rehabilitation, Tri-Service General Hospital, Taipei, Taiwan, Republic of China.

Dr. Kohn's present address: School of Physical Therapy, Ohio University, 119 Convocation Center, Athens, OH 45701.

In this study we sought to investigate the specific elements in the 3′ UTR of the GAP-43 mRNA that contribute to its post-transcriptional regulation. Analysis of the half-life of several 3′ UTR deletion mutants and chimeras with the β-globin gene revealed that a U-rich element in the 3′ end of the GAP-43 3′ UTR controls both the intrinsic instability of the mRNA in undifferentiated PC12 cells and the specific stabilization of the mRNA in response to phorbol ester treatment. This region also contains recognition sites for two brain-specific GAP-43 mRNA-binding proteins. Our results suggest that RNA protein interactions between these proteins and their cognate sequences in the GAP-43 3′ UTR may contribute to the control of GAP-43 gene expression during neuronal differentiation.

MATERIALS AND METHODS

Cell culture and transfection studies

As in previous studies, mRNA stability assays were performed in the GAP-43-deficient cell clone PC12–N36 (Kohn et al., 1996a). This line does not express detectable levels of the GAP-43 mRNA or protein (Perrone-Bizzozero et al., 1994). PC12–N36 cells were transfected with GAP-43 cDNAs in the expression vector pMEP4 (Invitrogen, San Diego, CA) by electroporation. Briefly, cells were resuspended in 1× HeBS buffer (137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM dextrose, and 20 mM HEPES, pH 7.05) containing 20 μg of recombinant plasmid DNA and 380 μg of salmon testis DNA as carrier. The cell suspension was electroporated under 250 V and 960 μF in a Gene Pulser apparatus (Bio-Rad, Richmond, CA). Then cells were plated in complete medium for 24 hr, and the selection was initiated with 100 μg/ml hygromycin-B for 2 weeks, followed by 150 μg/ml thereafter. Once cells were selected, they were used for mRNA decay experiments as described below. To compare the variations of mRNA stability in different cell lines, we also transfected constructs into the human neuroblastoma cell line SH-SY5Y and the monkey kidney cell line COS-7. SH-SY5Y and COS-7 cells were cultured in Eagle's minimal essential medium. The complete medium included 10% fetal calf serum, 2 mM L-glutamine, 75 U/ml penicillin, and 75 μg/ml streptomycin.

Plasmid preparation

Deletion mutants and point mutations. To investigate the sequences in the 3′ UTR that regulate GAP-43 mRNA stability, we generated several 3′ UTR deletion mutants and chimeras with a rat full-length GAP-43 cDNA clone (GA11B; Neve et al., 1987). *Bst*YI digestion of this clone created three fragments—A, C, and B (in 5′ to 3′ orientation). Fragment A contains the GAP-43 5′ UTR coding region and 62 bases of the 3′ UTR downstream of the stop codon. C contains 114 base pairs (bp) of the 3′ UTR downstream of A and is followed by B (225 bp) (see Fig. 1). These fragments were ligated to create the A, AC, and AB constructs in pGEM3Z (Promega, Madison, WI) and subsequently were digested with *Eco*RI/*Bam*HI and cloned into the *Pvu*II site of pMEP4. The Δ3′ UTR construct was prepared from the GA11B GAP-43 cDNA by deleting its 3′ UTR at the *Nsp*I site. Re-ligation of this plasmid created a new stop codon 12 bases downstream of the *Nsp*I site. The construct D40 was made by deleting 21 bp 5′ and 19 bp 3′ of the stop codon in the GAP-43 cDNA. The U3 construct was created by PCR-mediated site-directed mutagenesis (Neve and Neve, 1995), in which five U residues were replaced by A residues in three A/GUUUG/C stretches within the B fragment (^{939}AUUUGUUUC to AUAUGUAUC, ^{965}GUUUUUG to GUUAUUG and ^{1000}GUUUUUUG to GUAUAUUG; see Fig. 1). D40 and U3 were cloned into the *Spe*I/*Sal*I sites of pBluescript II SK$^+$ (pBSKII$^+$; Stratagene, La Jolla, CA), digested with *Xba*I and *Xho*I, and cloned into the *Nhe*I/*Xho*I sites in pMEP4. The construct containing the coding region of rabbit β-globin gene was prepared from pRSV-globin (Gorman et al., 1983) by digestion with *Hin*dIII and *Bgl*II. This fragment then was cloned into pMEP4.

Chimeras. Several chimeras of the rat GAP-43 3′ UTR and the β-globin gene were prepared. The C1 chimera contains the coding region of rabbit β-globin and the 3′ UTR of GAP-43. Trans-PCR (Neve and Neve, 1995) was used to delete the GAP-43 coding region and introduce *Hin*dIII/*Bgl*II sites between the 5′ and 3′ UTRs. The coding region of β-globin (from pRSV-globin) was ligated to the *Hin*dIII/*Bgl*II site, after which the C1 chimera was released from pBSKII$^+$ and cloned into *Xho*I/*Nhe*I-digested pMEP4. In addition, two chimeric constructs were prepared by linking the rabbit β-globin coding region with either the B fragment (globin-B), or the C fragment (globin-C), of GAP-43 3′ UTR. To generate these chimeras, we excised the GAP-43 3′ UTR from the C1 chimera in pBSKII$^+$ by digestion with *Bgl*II and *Cla*I. Then the B and C fragments were cloned into the *Bgl*II/*Cla*I sites of this construct to create globin-B or globin-C in pBSKII$^+$. These chimeric cDNAs were excised out and cloned into the *Xho*I/*Nhe*I sites of pMEP4 as described above. The C2 chimera contained the coding region of GAP-43 flanked by the β-globin 5′ and 3′ UTRs from pSP64T (a gift from Dr. D. A. Melton, Harvard University, Cambridge, MA; Krieg and Melton, 1984). The plasmid was digested with *Bgl*II to separate the β-globin 5′ and 3′ UTRs, after which the coding region of GAP-43 was inserted into the *Bgl*II site. The resulting construct then was digested with *Hin*dIII/*Bam*HI and cloned into the same restriction sites of the expression vector pMEP4.

mRNA stability analysis

Studies on the stability of the various mRNAs were performed as described by Kohn et al. (1996a). Once cultures reached 75–85% confluency, cells were induced for 16 hr in the presence of 5 μM $CdCl_2$. Exposure to this metal ion causes an 8- to 10-fold activation of the human metallothionein-II_A promoter in the pMEP4 vector (Richards et al., 1984). After induction, cadmium was washed out, and cells were collected at various time points. In some studies, cultures were treated with various agents, including 12-*O*-tetradecanoylphorbol-13-acetate (TPA; 160 nM), polymyxin B (2000 U/ml), and nerve growth factor (NGF; 100 ng/ml). Samples containing 15 μg of cytosolic RNA were processed for Northern blot analysis as described by Perrone-Bizzozero et al. (1993). Blots were probed with labeled cDNAs for GAP-43, β-globin (Gorman et al., 1983), or glyceraldehyde-3-phosphate dehydrogenase (G3PD). The intensity of the mRNA band was determined by densitometry with the FotoAnalyst system (Fotodyne, New Berlin, WI) within the linear range of response. Optical densities of the GAP-43 bands were corrected by those of G3PD. As we have shown previously (Perrone-Bizzozero et al., 1993), the decay of the GAP-43 mRNA follows an exponential function, $M_o = M_t e^{-\lambda t}$ (in which $\lambda = ln2/T_{1/2}$). The half-life ($T_{1/2}$) of the various GAP-43 transcripts was calculated from the plot of relative mRNA levels versus time of decay by using linear regression analysis.

Western blot analysis

Western blots were used to verify that the mRNAs derived from the various GAP-43 3′ UTR deletion mutants were translated effectively into GAP-43 protein. For these studies, total RNA and protein were extracted from permanently transfected PC12–N36 with the Tri reagent (Sigma, St. Louis, MO), and samples were processed for Northern and Western blot analysis. Aliquots containing 50 μg of protein were separated on 10% polyacrylamide gels and electrotransferred onto polyvinylidene fluoride membranes. GAP-43 levels were detected by a polyclonal antibody to rat GAP-43 as previously described (Sower et al., 1995).

In vitro *RNA-binding studies*

Analyses of the interactions between brain cytosolic proteins and different subregions of the GAP-43 3′ UTR were performed as described by Kohn et al. (1996a). Briefly, the GAP-43 3′ UTR and the B fragment were subcloned into pGEM3Z and used to generate ^{32}P-labeled sense RNA by *in vitro* transcription with SP6 and T7 RNA polymerases. *In vitro* RNA-binding studies were performed with 0.5 ng of labeled RNA (5×10^4 cpm) and 50 μg of brain cytosolic protein (S100 fraction). RNA protein complexes were cross-linked by irradiation for 30 min at 4°C at 4 cm from a UV lamp (Sylvania G30T8, 254 nm). UV cross-linked complexes were digested with RNase A, and labeled proteins were analyzed by 10% polyacrylamide gel electrophoresis and autoradiography. In some experiments, protein extracts were prepared from control and TPA-induced PC12 cells and used in RNA-binding assays as indicated above.

RESULTS

The main determinants of GAP-43 mRNA instability are localized in the 3′ UTR

We previously showed that the GAP-43 3′ UTR is highly conserved in evolution and that this region seems to control mRNA turnover via its specific interactions with three brain-specific RNA-binding proteins (Kohn et al., 1996a). To characterize the role of specific sequences within the GAP-43 3′ UTR in mRNA

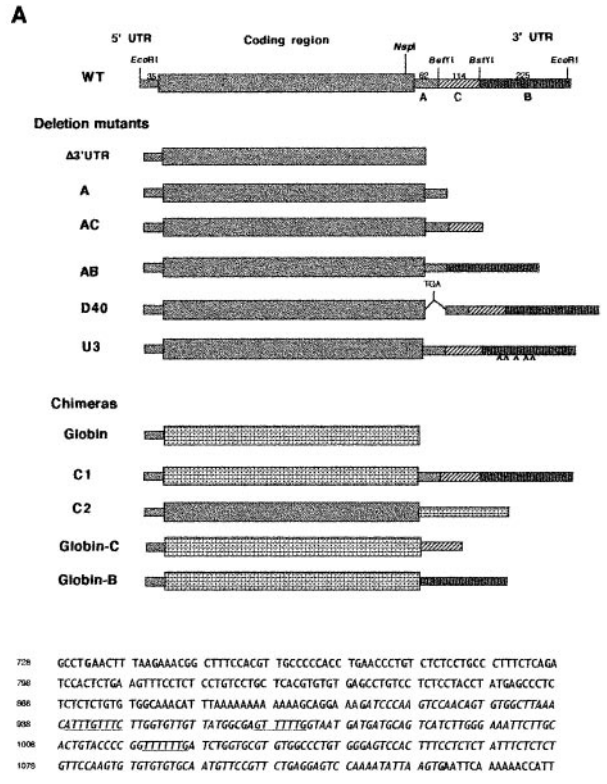

Figure 1. Schematic representation of the wild-type GAP-43 cDNA and of several 3′ UTR deletion mutants and chimeras. *A*, The restriction map shown in the wild-type construct (*WT*) corresponds to the full-length rat GAP-43 cDNA (GA11B; Neve et al., 1987) that was used to generate the various constructs. Segments represent the different fragments created by restriction enzyme digestion. Chimeras were generated by a combination of these fragments with the β-globin coding region. *B*, Sequence of the GAP-43 3′ untranslated region (3′ UTR) of the GAP-43 cDNA. The sequences in the B region are indicated in *italics*, and the three regions in U3, including five point mutations, are *underlined*.

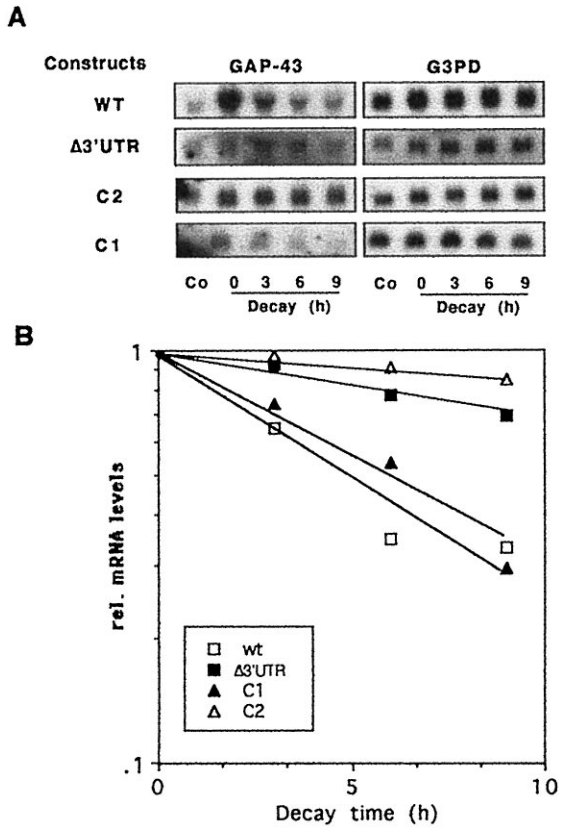

Figure 2. Role of the GAP-43 3′ UTR on mRNA stability. PC12–N36 cells were transfected with a rat full-length GAP-43 cDNA construct (*WT*), a deletion mutant without the 3′ UTR (*Δ3′ UTR*), or chimeras with the β-globin cDNA (*C1* and *C2*; see Fig. 1). Cells were incubated with cadmium to induce high levels of transfected mRNAs. For mRNA decay studies, RNAs were isolated from cells harvested at the time points indicated after removal of cadmium. Control lanes (*Co*) show the basal levels of the transfected mRNA in the absence of the metal. *A*, Northern blots from representative mRNA decay experiments: analysis was performed with 15 μg of cytosolic RNA. The same membrane was probed for GAP-43 first and then reprobed for G3PD. *B*, mRNA decay curves. GAP-43 mRNA levels were determined by densitometry, corrected by those of G3PD, and expressed relative to those before the decay phase (*time 0*).

stability, we generated a series of 3′ UTR deletion mutants and chimeras of the GAP-43 and β-globin cDNAs in the expression vector pMEP4 (Fig. 1). The stability of these constructs was tested in transfected PC12–N36 cells (Perrone-Bizzozero et al., 1994). This GAP-43-deficient PC12 cell line enables the determination of the stability of transfected GAP-43 transcripts without the interference of the endogenous mRNA (Kohn et al., 1996a). Northern blots from representative mRNA decay studies are shown in Figures 2*A* and 3. The use of an inducible promoter allows for the determination of mRNA turnover without disturbing the intracellular environment with transcription inhibitors (Chen et al., 1995; Kohn et al., 1996a). Although the metallothionein promoter exhibits a small level of basal activity (control lanes, Figs. 2–4), on stimulation with cadmium there is an 8- to 10-fold increase in the rate of transcription and mRNA accumulation (time 0 lanes, Figs. 2–4). After cadmium induction, mRNA decay rates are examined in the absence of the metal ion. Under these conditions the transfected full-length GAP-43 mRNA decayed with a half-life of 5 hr (*WT*, Fig. 2*B* and Table 1), consistent with the stability of the endogenous mRNA (Perrone-Bizzozero et al., 1993). On deletion of the 3′ UTR, the half-life of the mRNA increased threefold (Δ3′ UTR mutant, Fig. 2), indicating that this region contains the main determinants for mRNA instability. The destabilizing effect of these sequences was confirmed by addition of the GAP-43 3′ UTR to the β-globin mRNA. The globin mRNA is very stable, with reported half-life values ranging from 17 to >50 hr (Aviv et al., 1976; Volloch and Housman, 1981). However, ligation of the GAP-43 3′ UTR to the β-globin coding region (C1 construct, Fig. 2) made this otherwise stable mRNA become unstable, with a half-life of ~5 hr similar to the half-life of wild-type GAP-43 mRNA (Table 1). Finally, the opposite effect was observed on ligation of the 3′ UTR of globin to the GAP-43 coding sequence (C2 chimera). Although deletion of the 3′ UTR (Δ3′ UTR) significantly increased the stability of the GAP-43 mRNA, the half-life of the mRNA became even longer ($T_{1/2}$ = 19 hr) when the GAP-43 coding region was linked to the 3′ UTR of β-globin (Fig. 2, Table 1).

Two *cis*-acting elements in the 3′ UTR regulate GAP-43 mRNA instability

Having demonstrated the destabilizing effect of the whole GAP-43 3′ UTR, we sought to map the instability determinants by using a series of 3′ UTR deletion mutants and chimeras with the β-globin gene. Initial studies involved the analysis of different fragments derived from the GAP-43 3′ UTR (Fig. 1). Combina-

Table 1. Half-lives of the GAP-43 mRNA and various 3′ UTR mutants and chimeras

Construct	Mean ± SD	*p* value
WT	4.99 ± 0.42 (n = 9)	
A. 3′ UTR mutants		
Δ3′ UTR	14.58 ± 1.97 (n = 6)	$p < 0.001$*
A	8.50 ± 1.01 (n = 5)	$p < 0.02$*
AC	9.55 ± 0.38 (n = 5)	$p < 0.01$*
AB	6.55 ± 0.81 (n = 8)	NS
D40	4.44 ± 0.61 (n = 5)	NS
U3	5.77 ± 0.53 (n = 5)	NS
B. Chimeras		
Globin	10.50 ± 1.51 (n = 13)	
C1	5.53 ± 0.52 (n = 10)	$p < 0.01$**
C2	19.35 ± 4.14 (n = 5)	$p < 0.01$*
Globin-C	14.60 ± 2.08 (n = 4)	$p < 0.05$**
Globin-B	5.93 ± 1.50 (n = 7)	$p < 0.01$**

*p values relative to WT; **p values relative to globin. NS, Nonsignificant.

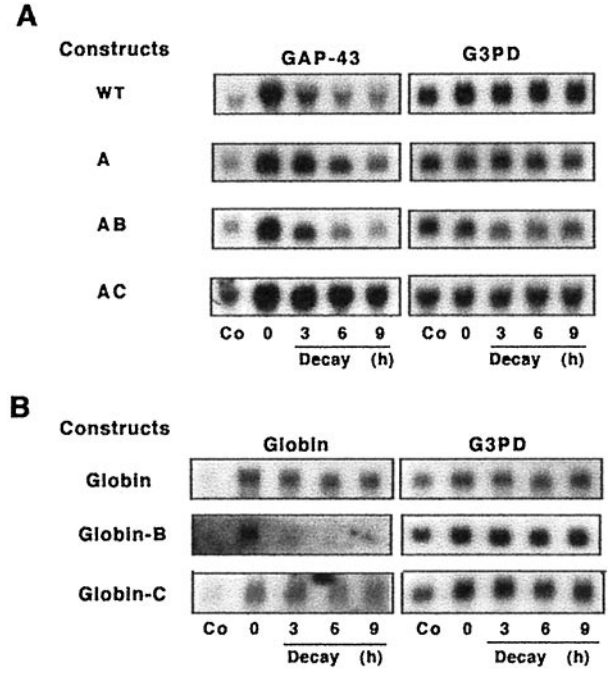

Figure 3. Analysis of the stability of the different GAP-43 3′ UTR deletion mutants and chimeras with the β-globin cDNA in transfected cells. PC12–N36 cells were transfected with various constructs in the expression vector pMEP4, and mRNA decay studies were performed as indicated in Figure 2. Northern blots show the rate of decay of different GAP-43 3′ UTR deletion mutants and chimeras. Blots were probed with either GAP-43 (*A*) or β-globin (*B*) cDNAs and reprobed with G3PD to control for RNA loading. The structure of each construct is shown in Figure 1.

tions of these fragments (A, AB, and AC) were cloned into the expression vector pMEP4, and the stability of the corresponding mRNAs was tested in transfected PC12–N36 cells. Among these constructs, A was found to decay with a half-life of 8.5 hr (Fig. 3*A*, Table 1), which was significantly shorter than that of the mRNA lacking its 3′ UTR (Δ3′ UTR construct) but longer than that of the full-length mRNA (*WT*). Addition of the C fragment to the 3′ end of A did not change the half-life significantly ($T_{1/2}$ = 9.5 hr, Table 1). In contrast, addition of the B fragment shortened the half-life of the resulting AB mRNA to a level comparable to that of the wild-type GAP-43 mRNA ($T_{1/2}$ = 6.5 hr, Table 1). These results indicate that there are at least two *cis*-acting instability-conferring elements within the GAP-43 3′ UTR. One element is ~60 nucleotides (nt) long and is localized at the 5′ end of the GAP-43 3′ UTR (A fragment). The other determinant is 250 nt long and resides at the terminal 3′ end of the 3′ UTR (B fragment). It also is worth noting that fragment C contains a single AUUUA motif adjacent to a poly(A) stretch. Unlike other AU motifs (Shaw and Kamen, 1986), this element is not present in a U-rich region and does not confer instability to the GAP-43 mRNA.

To investigate further the destabilizing effect of the B fragment, we created a chimeric construct by linking the B fragment to the β-globin coding region; this was designated globin-B (Fig. 3*B*). To control for specificity, we also ligated the C fragment to the β-globin coding region (globin-C). Addition of the B fragment to the β-globin coding region significantly decreased the stability of the globin transcript ($T_{1/2}$ = 6 hr, Table 1), confirming the role of this region as a destabilizing element in the GAP-43 mRNA. In contrast, the globin-C mRNA showed a slightly longer half-life ($T_{1/2}$ = 14.5 hr) than that of the β-globin coding region ($T_{1/2}$ = 10.5 hr), suggesting that the C fragment has a stabilizing, rather than destabilizing, effect on the mRNA.

Recent studies indicate that the function of certain instability-conferring elements in the 3′ UTRs depends on the presence of stretches of three or more adjacent U residues within them (Chen et al., 1995; Zubiaga et al., 1995). Given that the destabilizing B fragment contains several U-rich stretches and that these motifs are highly conserved (Kohn et al., 1996a), subsequent studies examined the role of these motifs in GAP-43 mRNA stability. Five U residues within three U-rich stretches in the B fragment were mutated to A by site-directed mutagenesis to generate the U3 mutant (Fig. 1). When tested in mRNA decay studies, the U3 mRNA showed a half-life of ~6 hr, similar to that of the wild-type GAP-43 mRNA (Fig. 4). Thus, although the three A/GUUUG/C motifs within the B fragment were reasonable candidates to mediate the destabilizing effect of this region, mutations in these elements did not affect the half-life of the mRNA. These results are in agreement with a recent finding that similar point mutations in GUUUUUG motifs in the *c-jun* mRNA do not affect mRNA stability (Peng et al., 1996) and suggest that other regions within the B fragment mediate this effect.

It has been shown that the stop codon and its flanking sequences may affect the half-life of mRNA (Belgrader et al., 1994). To test whether these sequences contribute to the instability of the A region, we generated the D40 mutant. This construct contained a deletion of 21 nucleotides upstream and 19 downstream of the stop codon, resulting in the net loss of 40 nucleotides within the A region (Fig. 1). The half-life of the D40 construct was similar to that of the wild-type mRNA in transfected PC12–N36 cells ($T_{1/2}$ = 4.5 hr, Table 1), suggesting that this region does not contribute to the intrinsic instability of the GAP-43 mRNA in undifferentiated PC12 cells. These results are in contrast with a recent study in which the region surrounding the stop codon in the GAP-43 mRNA was found to control GAP-43 mRNA stability in NGF-induced PC12 cells [Nishizawa and Okamoto (1994); see Discussion].

In summary, the results of our initial characterization of the determinants of GAP-43 mRNA stability indicated that the main instability determinants are localized in the 3′ UTR. As shown in Figure 5 and Table 1, the presence or absence of these elements

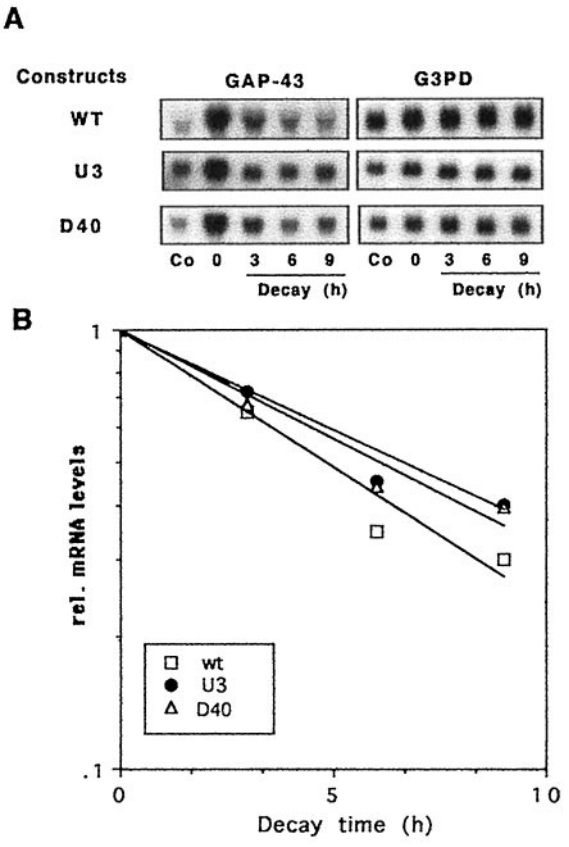

Figure 4. Effect of three U-rich regions in GAP-43 3′ UTR and the region surrounding the stop codon on GAP-43 mRNA stability. *A*, Northern blots show the decays of the wild-type and mutant *U3* and *D40* constructs. *B*, mRNA decay curves indicate that neither the five U by A replacements in *U3* nor the deletion of 40 nt in *D40* has any effect in the rate of decay of the mRNA.

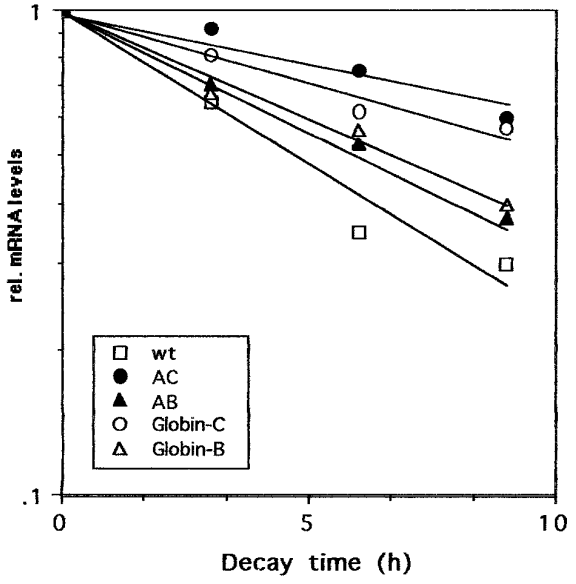

Figure 5. Analysis of the stability of the wild-type GAP-43 mRNA and several 3′ UTR mutants in PC12–N36 cells. mRNA decay assays were performed in stably transfected PC12–N36 cells, as described in Figure 2. The relative levels of the GAP-43 mRNA at different decay times were calculated by densitometric analysis of Northern blots and were corrected by the levels of G3PD in the same sample. The plot shows representative decay curves for the wild-type GAP-43 mRNA (*WT*), the *AC*, *AB*, and *A* mutants, and the *Globin-B* and *Globin-C* chimeras.

results in a threefold variation in the half-life of the GAP-43 mRNA. These GAP-43 3′ UTR sequences were also equally effective when they were linked to the β-globin coding sequence, suggesting that their function is independent of the particular coding region to which they are attached. On the basis of these findings, we propose that the A and B regions of the GAP-43 3′ UTR contain the main determinants for the instability of the mRNA in undifferentiated PC12 cells.

Role of 3′ UTR sequences in the TPA-mediated stabilization of the GAP-43 mRNA

We showed previously that NGF and the phorbol ester TPA induce GAP-43 gene expression in PC12 cells by causing selective changes in the stability of the mRNA (Perrone-Bizzozero et al., 1991, 1993). To begin to identify the specific mRNA sequences mediating this response, we tested the effect of TPA on the induction and stability of the full-length GAP-43 mRNA in transfected PC12–N36 cells. As shown in Figure 6*A*, both TPA and NGF caused the induction of the transfected GAP-43 mRNA, with the phorbol ester showing a more robust induction. Thus, as shown for the endogenous GAP-43 mRNA, the transfected mRNA exhibits a more rapid and powerful induction with TPA than with NGF, although both agents use the same signal transduction pathway to elicit this response (Perrone-Bizzozero et al., 1993). With regard to the mechanism of this induction, we found that the half-life of the transfected mRNA was prolonged threefold by the phorbol ester (Fig. 6*B*) and was decreased significantly by the protein kinase C inhibitor polymyxin B (Fig. 6*A*). Addition of the transcription inhibitor DRB did not affect the response to TPA, but in agreement with previous studies (Perrone-Bizzozero et al., 1991; Chen et al., 1995), DRB was found to increase the half-life of the mRNA in control cells. Thus, the transfected GAP-43 mRNA was found to behave similarly to the endogenous mRNA in that it was stabilized by PKC activators and transcription inhibitors and destabilized by PKC inhibitors (Perrone-Bizzozero et al., 1991, 1993).

To map the *cis*-acting elements that contribute to the response to TPA, we evaluated GAP-43 mRNA and protein levels in PC12–N36 cells transfected with the 3′ UTR deletion mutants A, AC, and AB (Fig. 7*A,B*). Comparison of cadmium-induced and basal levels of expression of these constructs indicated that, first, all of the constructs produced mRNAs that were translated adequately into GAP-43 protein and, second, the accumulation of GAP-43 protein paralleled that of the mRNA of each construct and responded as expected to cadmium stimulation. Analysis of the levels of GAP-43 protein and mRNA in the presence of TPA revealed that the phorbol ester caused a threefold induction in GAP-43 expression in cells transfected with the AB construct. In contrast, phorbol ester treatment had no effect on the expression of the A construct, whereas it decreased the expression of the AC mRNA (Fig. 7*C*). These effects are not likely to be exerted at the level of translation, because both GAP-43 mRNA and protein levels were affected similarly by TPA. Comparison of the response of the A, AC, and AB mutants to phorbol ester treatment indicates clearly that the specific elements responsible for the TPA-mediated stabilization of the GAP-43 mRNA are contained within the B fragment.

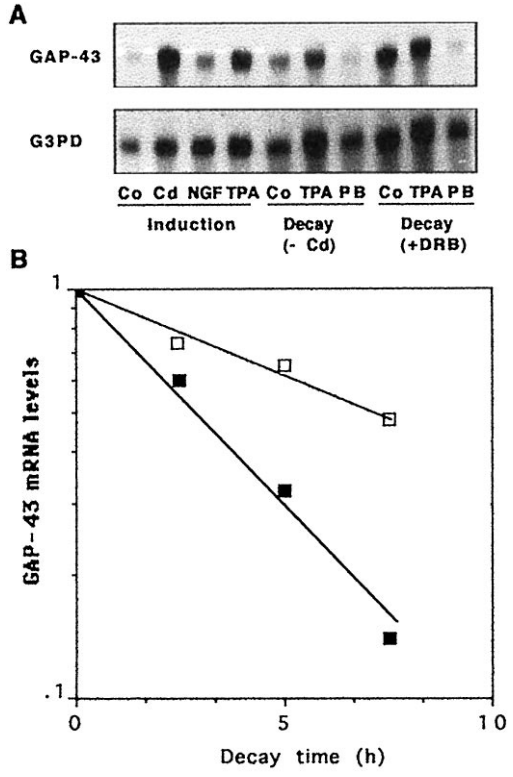

Figure 6. TPA causes the induction and stability of the GAP-43 mRNA in transfected PC12–N36 cells. *A*, Northern blots demonstrate the levels of induction and stability of the transfected wild-type GAP-43 mRNA in PC12–N36 cells. For the induction phase, cells were treated for 16 hr in the presence of NGF (*NGF*; 100 ng/ml), TPA (*TPA*; 160 nM), or $CdCl_2$ (*Cd*; 5 μM). For the decay phase, after cadmium induction, cells were washed out of the metal ion and incubated for 6 hr in the presence (*+DRB*) or absence of DRB (60 μM) and *TPA* (160 nM) or polymyxin B (*PB*; 2000 U/ml). *B*, Cells were induced for 16 hr with either Cd^{2+} (*filled squares*) or TPA and Cd^{2+} (*open squares*), and mRNA decay assays were performed in the presence or absence of 160 nM TPA. The relative half-lives of the mRNA were calculated as described in Materials and Methods.

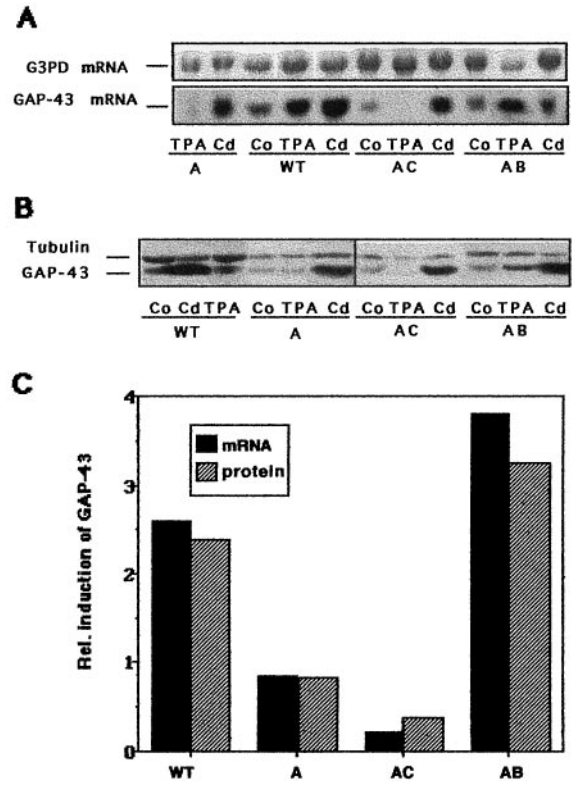

Figure 7. Effect of TPA on GAP-43 mRNA and protein levels in PC12–N36 cells transfected with different 3′ UTR mutants. *A*, *B*, Northern (*A*) and Western (*B*) blots show GAP-43 mRNA and protein levels in permanently transfected control cells (*Co*) or in cells induced for 16 hr with cadmium (*Cd*) or phorbol ester (*TPA*), as described in Figure 6. The levels of G3PD in Northern blots and tubulin in Western blots are presented as controls for gel loading. *C*, Relative induction of the GAP-43 mRNA and protein in TPA-treated PC12–N36 cells. The levels of induction of each construct represent the mean of at least three independent experiments.

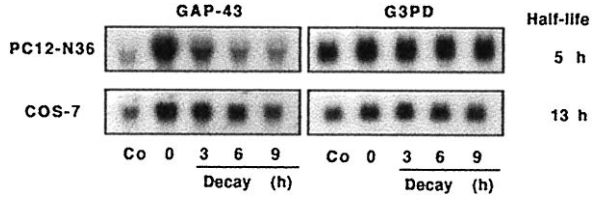

Figure 8. Comparative analysis of the half-life of the GAP-43 mRNA in COS-7 cells. Northern blots show the decay of the transfected wild-type GAP-43 mRNA in *PC12–N36* cells and non-neural *COS-7* cells. The half-life of the mRNA was found to be ~2.5-fold shorter in the neural lines than in COS-7 cells.

Trans-acting factors for the control of GAP-43 mRNA stability

Like transcriptional regulation, the control of mRNA stability is thought to involve both *cis*- and *trans*-acting factors (Malter et al., 1989; Brewer, 1991). We previously identified potential *trans*-acting factors affecting GAP-43 mRNA stability (Kohn et al., 1996a). These are three developmentally regulated RNA-binding proteins that interact with highly conserved polypyrimidine-rich sequences in the GAP-43 3′ UTR. Because both the GAP-43 mRNA and these RNA-binding proteins are selectively localized to the nervous system (Kohn et al., 1996a), we hypothesized that the factors controlling GAP-43 mRNA stability are neuronal-specific. To test this idea, we compared the stability of the mRNA in neuronal and non-neuronal cells (Fig. 8). When transfected into PC12 cells, the GAP-43 mRNA exhibited a significantly faster turnover rate ($T_{1/2}$ = 5 hr) than it did when transfected into non-neuronal COS-7 cells ($T_{1/2}$ = 13 hr, Fig. 8). A similar increase in mRNA stability was observed when the AB and AC mutants were transfected into COS-7 cells (data not shown). Analysis of the stability of the GAP-43 mRNA in another neural cell line, the human neuroblastoma SH-5ySy, indicated that the mRNA decayed with a half-life similar to that observed in PC12 cells (Perrone-Bizzozero et al., 1994). Because the GAP-43 mRNA binding proteins are expressed selectively in brain and neural cell cultures (Kohn et al., 1996a) (see also Fig. 9 below), our results are consistent with the idea that prolonged half-life of the GAP-43 mRNA in non-neural COS-7 cells may be attributable to the absence of one or more of the neuronal-specific GAP-43 mRNA-binding proteins.

Given that the B region mediates the increased stability of the GAP-43 mRNA in the presence of TPA, RNA-binding proteins interacting with these sequences are potential candidates for *trans*-acting factors involved in this process. Thus, subsequent experiments examined the interactions of sequences in the B

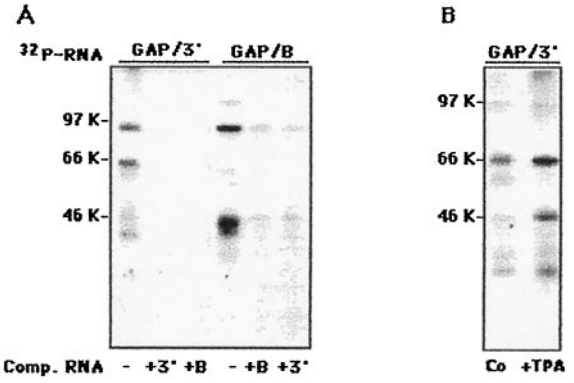

Figure 9. Detection of GAP-43 mRNA-binding proteins by UV cross-linking experiments. *A*, ^{32}P-labeled RNAs containing the entire GAP-43 3′ UTR (*GAP/3′*) or the B region (*GAP/B*) were synthesized *in vitro*, as described by Kohn et al. (1996a). RNAs (0.5 ng, 5×10^4 cpm) were incubated with 50 μg of brain S100 protein for 10 min at 4°C. To test for binding specificity, we incubated reactions in the presence or absence of a 100-fold excess of the corresponding cold competitor RNA (*GAP/3′* or *GAP/B*). After UV irradiation, RNA protein complexes were analyzed in 10% polyacrylamide gels. *B*, RNA-binding reactions were performed with ^{32}P-labeled *GAP/3′* RNA and cytosolic extracts derived from control PC12 cells (*Co*) or cells induced for 16 hr with 160 nM TPA (*+TPA*). Gels were exposed to film for 3–7 d at −80°C.

fragment or the entire GAP-43 3′ UTR with brain cytosolic proteins (Fig. 9). Of the three major GAP-43 mRNA-binding proteins in brain extracts, the 90 and 45 kDa proteins were found to interact preferentially with the B fragment. Competition experiments revealed that, although the B fragment did not show a direct interaction with the 65 kDa RNA-binding protein, an excess of this region was as effective as the entire GAP-43 3′ UTR in competing for the formation of all three RNA protein complexes (Fig. 9*A*). In contrast to the 65 kDa protein, the 45 and 90 kDa proteins display a preferential affinity for poly(U) while showing no significant binding to poly(C) (Kohn et al., 1996a). Therefore, our results suggest that U-rich sequences within the B region participate in these RNA protein interactions.

To begin to examine the role of these proteins in the stabilization of the GAP-43 mRNA in response to TPA, we performed RNA-binding assays with extracts derived from control and TPA-treated PC12 cells. As shown in Figure 9*B*, TPA altered the pattern of RNA protein interactions by decreasing the binding of a 60 kDa species and increasing the binding of those of 40, 45, and 65 kDa to the GAP-43 3′ UTR. These results suggests that regulated RNA protein interactions between these proteins and specific U-rich sequences in the 3′ UTR may contribute to the control of GAP-43 mRNA stability during neuronal differentiation.

DISCUSSION

Differences in the rates of degradation of messenger RNAs play a significant role in the control of gene expression in eukaryotic cells (for review, see Sachs, 1993; Beelman and Parker, 1995). Within the nervous system, mRNA stability mechanisms are known to regulate the expression of a number of important neuronal proteins such as growth-associated proteins (e.g., GAP-43; Perrone-Bizzozero et al., 1991, 1993), cytoskeletal elements (e.g., neurofilament proteins; Schwartz et al., 1995), neurotransmitter biosynthetic enzymes and receptors (Haddock et al., 1989; Czyzyk-Krzeska et al., 1994), and the amyloid precursor protein (Zaidi and Malter, 1994). After the discovery of the post-transcriptional control of the GAP-43 gene, the 3′ UTR of the mRNA was proposed to mediate this regulatory mechanism. First, the GAP-43 3′ UTR is highly conserved across different species, from human to goldfish, with a level of conservation that matches or exceeds that of the coding region (Perrone-Bizzozero et al., 1991; Kohn et al., 1996a). Second, these sequences contain putative instability-conferring sequences that resemble AU-rich elements described in other mRNAs with a fast turnover (Chen et al., 1995; Peng et al., 1996). Third, the GAP-43 3′ UTR contains recognition sites for the binding of specific brain cytosolic proteins (Kohn et al., 1996a). Like the GAP-43 mRNA, these proteins are regulated developmentally and localized to the nervous system, suggesting that they may participate in the post-transcriptional regulation of the GAP-43 gene. In this study we present direct evidence that elements in the 3′ UTR of GAP-43 mRNA are involved in the control of mRNA stability. On deletion of the 3′ UTR (Δ3′ UTR), the resulting GAP-43 mRNA was found to be three times more stable than the wild-type mRNA. Not only did deletion of these sequences have a stabilizing effect on the GAP-43 mRNA, but also addition of the GAP-43 3′ UTR to the β-globin coding region destabilized this normally stable mRNA. Conversely, the 3′ UTR of β-globin was found to stabilize the GAP-43 coding region (C2 chimera) even beyond the level observed for the Δ3′ UTR construct. Thus, analyses of the stability of deletion mutants and chimeras indicate clearly that the intrinsic instability of the GAP-43 mRNA in PC12 cells is attributable to sequences localized in its highly conserved 3′ UTR.

With regard to the precise mechanisms that control differential mRNA turnover, recent evidence indicates that this process is extremely complex and that multiple *cis*- and *trans*-acting factors are involved. In addition, several studies have revealed that a number of variables need to be considered in the interpretation of mRNA stability data, including the effect of translation on mRNA turnover (Savant-Bhonsale and Cleveland, 1992; Nishizawa and Okamoto, 1994; Chen et al., 1995). For example, minor changes in mRNA sequence, because of the introduction of point mutations or specific deletions, may affect mRNA stability indirectly via a perturbation of the RNA secondary structure that leads to changes in translation efficiency (Weiss and Liebhaber, 1994). In view of this potential problem, we examined the ability of different 3′ UTR deletion mutants used in this study to generate GAP-43 protein in transfected cells and found that all of them were translated effectively. Thus, these results suggest that differences in the stability of the various transcripts are not the result of translational effects. Furthermore, because translation imposes a series of structural requirements on mRNA molecules, our results suggest that the differences in mRNA decay rates observed here are not likely to be attributable to major alterations in the overall structure of the mRNA but rather to specific changes within limited regions in the 3′ UTR. Finally, comparison of the structural and functional properties of specific subregions of the GAP-43 3′ UTR reveals that the overall length of the 3′ UTR or the location of specific elements relative to the stop codon does not have a direct effect in mRNA stability. As shown in Figure 1 and Table 1, it is the presence of specific regions in the 3′ UTR, rather than their size and specific location, that determines the half-life of the GAP-43 mRNA.

Using deletion mutation analysis, we identified two different instability-conferring sequences in the 3′ UTR of the GAP-43 mRNA. One is a 60 nt region at the 5′ end of the 3′ UTR. Removal of this element increases the half-life of the mRNA twofold. Although this element contributes to the intrinsic insta-

bility of the mRNA in undifferentiated PC12 cells, it is not sufficient to stabilize the mRNA in response to phorbol ester treatment. These results are in contrast with a recent report in which this region was found to mediate the stabilization of the mRNA in response to NGF (Nishizawa and Okamoto, 1994). Although the reasons for this discrepancy are unclear, it should be noted that there are major differences in the transfection protocols and cell lines used in that study and in the use of transcription inhibitors for the determination of mRNA turnover rates. The other determinant of GAP-43 mRNA stability identified in our study is localized in a 250 nt U-rich region in the 3′ end of the GAP-43 3′ UTR. Not only did deletion of this element result in the stabilization of the mRNA, but also addition of these sequences to the coding region of β-globin significantly decreased the half-life of the chimeric transcript. Although this element contains several highly conserved U-rich stretches (A/GUUUG/C), replacement of the middle U with A in three of the U-rich stretches did not change the half-life of the transcripts, suggesting that additional sequences in this region may contribute to its destabilizing effect. In fact, we recently have found a 25 nt U-rich region downstream of these motifs that contains a recognition site for a neuronal-specific elav-like RNA-binding protein [Chung et al. (1997); also see below].

Phorbol esters such as 12-*O*-tetradecanoyl-phorbol-12-acetate (TPA) are known to exert a highly pleiotropic response in a variety of cell types both *in vivo* and *in vitro* (Nishizuka, 1986). Several lines of evidence indicate that phorbol esters can mimic many of the responses of neural cells to growth factors, such as the induction in GAP-43 expression and neurite outgrowth by NGF (Montz et al., 1985; Perrone-Bizzozero et al., 1993). There is also evidence that TPA can affect gene expression regulation at the post-transcriptional level by causing specific changes in mRNA stability (Iwai et al., 1991; Saceda et al., 1991; Perrone-Bizzozero et al., 1993). In this study we investigated the possibility that the TPA-induced stabilization of the GAP-43 mRNA (Perrone-Bizzozero et al., 1993) could be mediated via specific sequences within the GAP-43 3′ UTR. We found that the 250 nt B region in the GAP-43 3′ UTR contains an element that confers responsiveness to TPA; the mRNA containing this region was unstable in uninduced cells and stabilized in the presence of TPA. This increase in GAP-43 mRNA stability resulted in an enhanced expression of GAP-43 protein in the cells. Thus, the B region in the GAP-43 3′ UTR can mimic major regulatory features of the entire GAP-43 mRNA.

To characterize further the mechanisms by which sequences in the B region exert this effect, we examined their interaction with specific RNA-binding proteins. Of the three GAP-43 mRNA-binding proteins identified in brain cytosolic extract, those that have the highest specificities for poly(U) RNA (Kohn et al., 1996a) were found to bind this 250 nt U-rich region. One is a 90 kDa molecular weight RNA-binding protein, the activity of which is dependent on protein phosphorylation (Kohn et al., 1996a). The other is a 45 kDa protein that recently was identified as a member of the elav family of neuronal-specific RNA-binding proteins (Robinow et al., 1988; Szabo et al., 1991; Kim and Baker, 1993; King et al., 1994). These proteins are involved in the development of the nervous system and are thought to regulate specific gene expression via post-transcriptional mechanisms (King et al., 1994; Chung et al., 1996). Analysis of RNA protein interactions indicated that there is an increased binding of the low molecular weight GAP-43 mRNA-binding proteins in TPA-induced PC12 cells. In contrast, we found decreased binding of a 60 kDa neural-specific protein. These results suggest that these GAP-43 mRNA-binding proteins may function as *trans*-acting factors for the post-transcriptional regulation of GAP-43 gene expression. In fact, this *elav*-like protein is enriched in brain polysomes, the same subcellular fraction that contains the GAP-43 mRNA degradation machinery (Kohn et al., 1996b), further suggesting a role of this factor in mRNA turnover. Characterizing the function and regulation of these RNA-binding proteins will help clarify not only the fundamental basis for the control of GAP-43 mRNA stability but also the role of this post-transcriptional mechanism in the expression of this and other important neural-specific genes during neuronal differentiation.

REFERENCES

Aigner L, Caroni P (1993) Depletion of 43 kDa growth-associated protein in primary sensory neurons leads to diminished formation and spreading of growth cones. J Cell Biol 123:417–429.

Aviv H, Voloch Z, Bastos R, Levy S (1976) Biosynthesis and stability of globin mRNA in cultured erythroleukemic friend cells. Cell 8:495–503.

Beelman CA, Parker R (1995) Degradation of mRNA in eukaryotes. Cell 81:179–183.

Belgrader P, Cheng J, Zhou X, Stephenson LS, Maquat LE (1994) Mammalian nonsense codons can be *cis* effectors of nuclear mRNA half-life. Mol Cell Biol 14:8219–8228.

Benowitz LI, Routtenberg A (1987) A membrane phosphoprotein associated with neural development, axonal regeneration, phospholipid metabolism, and synaptic plasticity. Trends Neurosci 10:527–532.

Benowitz LI, Apostolides PJ, Perrone-Bizzozero NI, Finklestein SP, Zwiers H (1988) Anatomical distribution of the growth-associated protein GAP-43/B-50 in the adult rat brain. J Neurosci 8:339–352.

Brewer G (1991) An A+ U-rich element RNA binding factor regulates *c-myc* mRNA stability *in vitro*. Mol Cell Biol 8:1697–1708.

Chen CYA, Xu N, Shyu AB (1995) mRNA decay mediated by two distinct AU-rich elements from *c-fos* and granulocyte-macrophage colony-stimulating factor transcripts: different deadenylation kinetics and uncoupling from translation. Mol Cell Biol 15:5777–5788.

Chung S, Jiang L, Cheng S, Furneaux H (1996) Purification and properties of HuD, a neuronal RNA-binding protein. J Biol Chem 271:11518–11524.

Chung S, Eckrich M, Perrone-Bizzozero NI, Kohn DT, Furneaux H (1997) Elav-like proteins bind to a conserved regulatory element in the 3′ UTR of the GAP-43 mRNA. J Biol Chem, in press.

Czyzyk-Krzeska MF, Furnari BA, Lawson EE, Milhorn DE (1994) Hypoxia increases rate of transcription and stability of tyrosine hydroxylase mRNA in pheochromocytoma (PC12) cells. J Biol Chem 269:760–764.

Dekker LV, De Graan PNE, Oestriecher AB, Versteeg DHG, Gispen WH (1989) Inhibition of noradrenaline release by antibodies to B-50 (GAP-43). Nature 34:274–276.

Federoff HJ, Grabczyk E, Fishman MC (1988) Dual regulation of GAP-43 gene expression by nerve growth factor and glucocorticoids. J Biol Chem 263:19290–19295.

Gorman C, Padmanabhan R, Howard BH (1983) High efficiency DNA-mediated transformation of primate cells. Science 221:551–553.

Haddock JR, Wang H, Malbon CC (1989) Agonist-induced destabilization of the β-adrenergic receptor mRNA. J Biol Chem 264:19928–19933.

Ivins KJ, Neve KA, Feller DJ, Fidel SA, Neve RL (1993) Antisense GAP-43 inhibits the evoked release of dopamine from PC12 cells. J Neurochem 60:626–633.

Iwai Y, Bickel M, Pluznik DH, Cohen RB (1991) Identification of sequences within the murine granulocyte-macrophage colony-stimulating factor mRNA 3′-untranslated region that mediate mRNA stabilization induced by mitogen treatment of EL-4 thymoma cells. J Biol Chem 266:17959–17965.

Kim YJ, Baker BS (1993) The *Drosophila* gene *rbp9* encodes a protein that is a member of a conserved group of putative RNA binding proteins that are nervous system-specific in both flies and humans. J Neurosci 13:1045–1056.

King PH, Levine TD, Fremeau RT, Keene JD (1994) Mammalian homologs of *Drosophila* ELAV localized to a neuronal subset can bind *in vitro* to the 3′ UTR of mRNA encoding the Id transcriptional repressor. J Neurosci 14:1943–1952.

Kohn DT, Tsai K-C, Cansino VV, Neve RL, Perrone-Bizzozero NI (1996a) Role of highly conserved pyrimidine-rich sequences in the 3′ untranslated region of the GAP-43 mRNA in mRNA stability and RNA-protein interactions. Mol Brain Res 36:240–250.

Kohn DT, Tsai K-C, Cansino VV, Perrone-Bizzozero NI (1996b) Developmental regulation of GAP-43 mRNA stability *in vitro*. Soc Neurosci Abstr 22:1710.

Krieg PA, Melton DA (1984) Functional messenger RNAs are produced by SP6 *in vitro* transcription of cloned cDNAs. Nucleic Acids Res 12:7057–7070.

Malter JS (1989) Identification of an AUUUA-specific messenger RNA binding protein. Science 246:664–666.

Meberg PJ, Valcourt EG, Routtenberg A (1995) Protein F1/GAP-43 and PKC gene expression patterns in hippocampus are altered 1–2 hr after LTP. Mol Brain Res 34:343–346.

Montz HPM, Davis GE, Skaper SD, Manthorpe M, Varon S (1985) Tumor-promoting phorbol diester mimics two distinct neurotrophic factors. Dev Brain Res 23:150–154.

Neve RL, Neve KA (1995) Receptor expression in mammalian cells. In: Receptor Molecular Biology, Vol 25, Methods in Neurosciences (Sealfon SC, ed), pp 163–174. San Diego: Academic.

Neve RL, Perrone-Bizzozero NI, Finklestein S, Zwiers H, Bird E, Kurnit D, Benowitz LI (1987) The neuronal growth-associated protein GAP-43 (B-50, F1): specificity, developmental regulation, and regional expression of the human and rat genes. Mol Brain Res 2:177–183.

Neve RL, Finch EA, Bird ED, Benowitz LI (1988) Growth-associated protein GAP-43 is expressed selectively in associative regions of the adult human brain. Proc Natl Acad Sci USA 85:3638–3642.

Nevidi E, Basi GS, Virag Akey I, Skene JHP (1992) A neural-specific GAP-43 core promoter located between unusual DNA elements that regulate its activity. J Neurosci 12:691–704.

Nishizawa K, Okamoto H (1994) Mutation analysis of the role for the carboxy-terminus encoding region in NGF-induced stabilization of GAP-43 mRNA. Biochem Biophys Res Commun 205:1380–1385.

Nishizuka K (1986) Studies and perspectives on protein kinase C. Science 246:664–666.

Peng SSY, Chen CCA, Shyu AB (1996) Functional characterization of a non-AUUUA AU-rich element from the *c-jun* proto-oncogene mRNA: evidence for a novel class of AU-rich elements. Mol Cell Biol 16:1490–1499.

Perrone-Bizzozero NI, Neve RL, Irwin N, Lewis SE, Fischer I, Benowitz LI (1991) Post-transcriptional regulation of GAP-43 mRNA levels during neuronal differentiation and nerve regeneration. Mol Cell Neurosci 2:402–409.

Perrone-Bizzozero NI, Cansino VV, Kohn DT (1993) Post-transcriptional regulation of GAP-43 gene expression in PC12 cells through PKC-dependent stabilization of the mRNA. J Cell Biol 120:1263–1270.

Perrone-Bizzozero NI, Tsai K-C, Cansino VV, Romero L, Burry RW (1994) Post-transcriptional control of GAP-43 mRNA in transfected cells. J Neurochem 62[Suppl]:S14C.

Richards RI, Heguy A, Karin M (1984) Structural and functional analysis of the human metallothionein-I_A gene: differential induction by metal ions and glucocorticoids. Cell 37:263–272.

Robinow S, Campos A, Yao K, White K (1988) The *elav* gene product of *Drosophila*, required in neurons, has three RNP consensus motifs. Science 42:1570–1572.

Saceda M, Knabbe C, Dickson RB, Lippman ME, Beonzert D, Lindsey RK, Gottardis MM, Martin MB (1991) Post-transcriptional destabilization of estrogen receptor mRNA in MCF-7 cells by 12-*O*-tetradecanoylphorbol-13-acetate. J Biol Chem 266:17809–17814.

Sachs AB (1993) Messenger RNA degradation in eukaryotes. Cell 74:413–421.

Savant-Bhonsale S, Cleveland DW (1992) Evidence for instability of mRNAs containing AUUUA motifs mediated through translation-dependent assembly of a >20S degradation complex. Genes Dev 6:1927–1939.

Schwartz ML, Bruce J, Shneidman PS, Schlaepfer WW (1995) Deletion of 3′-untranslated region alters the level of mRNA expression of a neurofilament light subunit transgene. J Biol Chem 270:26364–26369.

Shaw G, Kamen R (1986) A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell 46:659–667.

Shyu AB, Belasco JG, Greenberg ME (1991) Two distinct destabilizing elements in the *c-fos* message trigger deadenylation as a first step in rapid mRNA decay. Genes Dev 5:221–231.

Skene JHP (1989) Axonal growth-associated proteins. Annu Rev Neurosci 12:127–156.

Sower AC, Bird ED, Perrone-Bizzozero NI (1995) Increased levels of GAP-43 protein in schizophrenic brain tissues demonstrated by a novel immunodetection method. Mol Chem Neuropathol 24:1–11.

Starr RG, Lu B, Federoff HJ (1994) Functional characterization of the rat GAP-43 promotor. Brain Res 638:211–220.

Strittmatter SM, Fankhauser C, Huang PL, Mashimo H, Fishman MC (1995) Neuronal pathfinding is abnormal in mice lacking the neuronal growth cone protein GAP-43. Cell 80:445–452.

Szabo A, Dalmau J, Manley G, Rosenfeld M, Wonf E, Henson J, Posner JB, Furneaux HM (1991) Hud, a paraneoplastic encephalomyelitis antigen, contains RNA-binding domains and is homologous to elav and sex-lethal. Cell 67:325–333.

Vanselow J, Grabczyk E, Ping J, Baetscher M, Teng S, Fishman M (1994) GAP-43 transgenic mice: dispersed genomic sequences confer a GAP-43-like expression during development and regeneration. J Neurosci 14:499–510.

Verhaagen J, Koster JG, Schrama LH, Eggen BJL, van der Neut R, Gispen WH, Destree OHJ (1993) The rat B-50 (GAP-43) promoter directs neural-preferred expression of a reporter gene in *Xenopus laevis* embryos. Neurosci Res Commun 13:83–90.

Volloch V, Housman D (1981) Stability of globin mRNA in terminally differentiating murine erythroleukemic cells. Cell 23:509–514.

Weiss IM, Liebhaber SA (1994) Erythroid specific determinants of α-globin mRNA stability. Mol Cell Biol 14:8123–8132.

Zaidi SHE, Malter JS (1994) Amyloid precursor protein mRNA stability is controlled by a 29-base element in the 3′-untranslated region. J Biol Chem 269:24007–24013.

Zubiaga A, Belasco J, Greenberg M (1995) The nonamer UUAUUUAUU is the key AU-rich sequence motif that mediates mRNA degradation. Mol Cell Biol 15:2219–2230.