Posttranscriptional Regulation of GAP-43 Gene Expression in PC12 Cells through Protein Kinase C-dependent Stabilization of the mRNA

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Abstract. We have previously shown that nerve growth factor (NGF) selectively stabilizes the GAP-43 mRNA in PC12 cells. To study the cellular mechanisms for this post-transcriptional control and to determine the contribution of mRNA stability to GAP-43 gene expression, we examined the effects of several agents that affect PC12 cell differentiation on the level of induction and rate of degradation of the GAP-43 mRNA. The NGF-mediated increase in GAP-43 mRNA levels and neurite outgrowth was mimicked by the phorbol ester TPA, but not by dibutyryl cAMP or the calcium ionophore A12783. Downregulation of protein kinase C (PKC) by high doses of phorbol esters or selective PKC inhibitors prevented the induction of this mRNA by NGF, suggesting that NGF and TPA act through a common PKC-dependent pathway. In mRNA decay studies, phorbol esters caused a selective 6-fold increase in the half-life of the GAP-43 mRNA, which accounts for most of the induction of this mRNA by TPA. The phorbol ester-induced stabilization of GAP-43 mRNA was blocked by the protein kinase inhibitor polymyxin B and was partially inhibited by dexamethasone, an agent that blocks GAP-43 expression and neuronal differentiation in PC12 cells. In contrast, the rates of degradation and the levels of the GAP-43 mRNA in control and TPA-treated cells were not affected by cycloheximide treatment. Thus, changes in GAP-43 mRNA turnover do not appear to require continuous protein synthesis. In conclusion, these data suggest that PKC activity regulates the levels of the GAP-43 mRNA in PC12 cells through a novel, translation-independent mRNA stabilization mechanism.

The growth-associated protein (GAP), GAP-43 (also called B-50, Fl, pp46 and neuromodulin), is a neural-specific phosphoprotein that is especially prominent in neuronal growth cones and synapses (Meiri et al., 1986; Skene et al., 1986), where it is localized to the internal surface of the plasma membrane (Gispen et al., 1985; DiFiglia et al., 1990). GAP-43 is expressed in neurons primarily during the development and regeneration of neural connections (for reviews see Skene, 1989; Benowitz and Perrone-Bizzozero, 1991). In primary neuronal cultures and in NGF-induced PC12 cells, GAP-43 expression correlates with the onset of neuronal differentiation as seen by the elongation of neurites and localization of the protein in the growth cones (Perrone-Bizzozero et al., 1986; Meiri et al., 1986; van Hoof et al., 1989). GAP-43 is not only a substrate of protein kinase C (PKC) (Aloyo et al., 1983; Nelson and Routenberg, 1985) but is also known to bind calmodulin in the absence of calcium, releasing it either in the presence of Ca++ (Andreasen et al., 1983) or upon PKC-dependent phosphorylation (Alexander et al., 1987). In addition, GAP-43 was found to regulate the function of G,, a major guanine nucleotide binding protein in growth cones (Strittmatter et al., 1990). Thus, while the precise function of this protein is not completely understood, its molecular properties suggest that GAP-43 is likely to participate in signal transduction mechanisms at the nerve-terminal membrane. Recent studies using specific antibodies to block the function of this protein or antisense RNA to block its expression demonstrated that GAP-43 is required for neurotransmitter release (Dekker et al., 1990; Neve et al., 1992; Ivins et al., 1993). Similar experiments using a neuroblastoma cell line or primary neuronal cultures showed that the protein is also necessary for neurite outgrowth (Shea et al., 1991; Benowitz and Perrone-Bizzozero, 1991; Neve et al., 1993). Thus, this evidence indicates that GAP-43 is essential for both growth cone and synaptic function.

With regards to the regulation of the GAP-43 gene, several neural systems the expression of this protein was found to correlate with the induction of its mRNA (Skene et al., 1986; Basi et al., 1987; Karns et al., 1987; Neve et al., 1987, 1988; Hoffman, 1989). GAP-43 mRNA levels, however, do not appear to be directly controlled by transcriptional activation. During brain development and nerve regeneration, the rate of transcription of the GAP-43 gene does not correlate with the observed 10-20-fold increase in the mRNA levels.
mRNA. We found that phorbol esters and NGF induce GAP-43 mRNA levels in PC12 cells via PKC-dependent pathways. Thus, GAP-43 gone expression in PC12 cells appear to be controlled by a novel mRNA stabilization mechanism involving changes in PKC-dependent phosphorylation of pre-existing cellular proteins.

Materials and Methods

PC12 Cell Cultures

PC12 cells (Greene et al., 1986) were cultured on poly-l-lysine-coated 90-mm culture dishes (Falcon) in RPMI 1640 media containing 10% serum (7.5% donor horse and 2.5% FCS, Sigma Immunochemicals, St. Louis, MO). For induction experiments, cells were grown to 50% confluency and treated with NGF (75-NGF, 100 ng/ml, Sigma Immunochemicals), dibutyryl cAMP (1 mM), the calcium ionophore A23187 (5 μM), or phorbol esters (1.6 μM-16 μM). Stock solutions of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and of the α and δ isomer of phorbol-12,13-didecanoate were prepared at 1.6 mM in DMSO. Control cells for these experiments were treated with equivalent amounts of DMSO. For the inhibition experiments, before the addition of NGF or TPA, cells were pretreated for 30 min with cycloheximide (0.5 μg/ml), sphingosine (5 μM), polyoxyn B (2,000 U/ml) or the protein kinase inhibitor H7 (50 μM). The conditions for sphingosine, polyoxyn B and H7 used here were based upon previous studies in which these agents were found to selectively inhibit PKC (Mazzu et al., 1982; Hall et al., 1988; Mizuno et al., 1989). The protein kinase inhibitor H7 (1-(5-isoquinolinylsulfonyl)-2-methylpiperazine) was purchased from Boehringer-Mannheim Corp. (Indianapolis, IN) and the activators of second messenger systems and other inhibitors were from Sigma Immunochemicals.

Protein Phosphorylation Assays

To study the effect of TPA on protein phosphorylation, PC12 cells were preincubated for 1.5 h in phosphate-free RPMI 1640 (GIBCO BRL, Gaithersburg, MD) containing 32P-orthophosphate (10 μCi/ml, NEN,Boston, MA). Half of the cultures were then exposed to 160 nM TPA for 30 min while the remaining cells were treated with equal amounts of DMSO. After labeling, cells were washed and resuspended in ice-cold PBS. Proteins were then solubilized and separated in 10% SDS–polyacrylamide gels. Phosphorylation of specific proteins was determined as previously described (Perrone-Bizzozero and Benowitz, 1987).

Northern Blots

Cytoplasmic RNAs were isolated in NP-40 containing buffer according to Sambrook et al., (1989). Aliquots containing 15 μg of total RNA were electrophoresed in denaturing agarose-formaldehyde gels and transferred onto nylon membranes (Pall Biotrans, ICN Radiochemicals, Irvine, CA) as previously described (Perrone-Bizzozero et al., 1991). RNA blots were hybridized against cDNA clones labeled by the random primers protocol (Feinberg and Vogelstein, 1983). The cDNA probes for GAP-43 (GallB1), glyceraldehyde-3-phosphate-dehydrogenase (G3PDH), 28S rRNA and actin were a gift from Dr. Rachael L. Neve (Harvard Medical School, Cambridge, MA) and her preparation and use are described elsewhere (Neve et al., 1987; 1988). After hybridization with GAP-43 cDNA, blots were exposed against x-ray film (X-OMAT; Eastman Kodak Co., Rochester, NY) for 2–7 d. To correct for small differences in RNA loading in different lanes, blots were stripped according to the manufacturer’s protocols and re-probed with actin cDNA. Quantitative analysis of the autoradiograms was performed on a gel scanner system (Fotodyne, Inc., New Berlin, WI) using film exposures within the linear range of response. Optical densities of the GAP-43 band were corrected by those of actin and expressed relative to the values of control cells.

mRNA Decay Experiments

For these studies, PC12 cells were cultured for 16 h in phosphate-free RPMI 1640 (GIBCO BRL) containing 32P-orthophosphate (10 μCi/ml, NEN,011, Dupont/NEN), either in the presence or absence of TPA (160 μM). Under these conditions, ~2.5 × 104 cpm/μg RNA were incorporated in cytoplasmic RNAs from control and TPA-treated cells, indicating that phorbol ester treatment did not affect the specific activity of the nucleotide pool available for RNA synthesis in these cells. After the labeling period, the media was replaced by RPMI media containing an excess of nonradioactive phosphate and additional phorbol ester in the case of TPA-treated cultures. At the indicated periods after the chase, cells were harvested and cytoplasmic RNA isolated as indicated above. Linearized cDNAs for GAP-43, G3PD, and 28S rRNA were loaded onto nylon membranes using a dot blot apparatus (GIBCO BRL). Equal amounts of labeled RNAs (2.5 × 104 cpm/μl) were hybridized against filters containing an excess of nonradioactive cDNAs as previously described (Perrone-Bizzozero et al., 1991). The radioactivity associated with each filter was determined by liquid scintillation counting. The results were corrected by background (pUC18 vector alone) and expressed relative to the values obtained just before starting the chase (time zero).

Results

Phorbol Esters Increase GAP-43 mRNA Levels and Process Outgrowth in PC12 Cells

NGF is known to exert its effects via several signal transduction pathways (Cremins et al., 1986; Doherty et al., 1988; Machida et al., 1991). To determine which of these participate in the induction of GAP-43 mRNA, we analyzed the effects of NGF and various activators of second messenger systems on GAP-43 mRNA levels and process outgrowth in PC12 cells. As shown in Fig. 1, GAP-43 mRNA levels were increased 5–10-fold not only by exposure of the cells to NGF but also by treatment with the phorbol ester TPA. Stimulation of PC12 cells with dibutyryl cyclic AMP resulted in a small increase in GAP-43 mRNA levels, while the calcium ionophore A23187 had no effect. The combination of cAMP and TPA treatments resulted in an mRNA induction equivalent to that of TPA alone, indicating that these agents do not have an additive effect. Since none of these treatments affected the amounts of actin mRNA in these cells (Fig. 1A), we used the levels of this mRNA to correct for differences in total RNA loaded onto the gel. When the amounts of GAP-43 mRNA were corrected in this way and expressed
Figure 1. Effect of various activators of second messenger systems on GAP-43 mRNA levels in PC12 cells. PC12 cells were induced with the different activators for 16 h. Cytoplasmic RNAs were isolated and analyzed as described in Materials and Methods. (A) Northern blots probed with labeled cDNAs for GAP-43 and actin. The conditions are: control cells (Co), 100 ng/ml NGF (NGF), 160 nM TPA (TPA), 1 mM dibutyryl cAMP (cAMP), 5 μM calcium ionophore A23187 (Ca), and 160 nM TPA and 1 mM dibutyryl cAMP (TPA+cAMP). (B) Quantitation of GAP-43 mRNA levels by densitometry. Levels were corrected by those of actin and expressed relative to the values of untreated cells, TPA treatment resulted in a 12.5-fold increase in the levels of this mRNA (Fig. 1 B). Thus, under these conditions, the phorbol ester was the most effective agent in inducing GAP-43 mRNA levels in these cells.

Since phorbol esters are potent activators of PKC (Nishizuka, 1986), these results suggest that GAP-43 mRNA levels are regulated by this protein kinase system. To examine this issue further, we defined the time course and dose-response curves for the effect of TPA (Fig. 2). After TPA stimulation, GAP-43 mRNA levels reached a peak at ~16 h and slowly decreased after 24 h of treatment (Fig. 2 A), a time course similar to that obtained for the induction of this mRNA by NGF (Perrone-Bizzozero et al., 1991). Moreover, this time course also agrees with the temporal accumulation of the GAP-43 protein in differentiating PC12 cells (van Hooff et al., 1989; Yankner et al., 1990). As shown in Fig. 2 B, GAP-43 mRNA levels were significantly elevated even by exposure to relatively low doses of the phorbol ester and decreased at high doses of TPA. These high doses of TPA were not toxic to the cultures since they did not affect cell number and viability (not shown) or their ability to express actin mRNA (see Fig. 4). In fact, the dose-response curve for the effect of TPA on GAP-43 mRNA levels is consistent with the effect of phorbol esters on PKC activity, since downregulation of the kinase occurs at high doses of TPA (Matthies et al., 1987). Furthermore, as for the activation of PKC, only phorbol esters in the beta conformation were able to induce GAP-43 mRNA levels, while an inactive 4-α-isomer was completely ineffective (Fig. 2 B).

At the concentrations of phorbol ester used to induce GAP-43 mRNA levels in these cells, we observed that short-term TPA treatment stimulated the phosphorylation of an 80-kD protein (Fig. 2 C). Given its apparent molecular weight, this protein is likely to be equivalent to MARCS, the major 80-kD substrate of PKC in a wide variety of neural and non-neural tissues (Seykora et al., 1991). In addition to its effects on GAP-43 mRNA levels and protein phosphorylation, TPA was found to promote neurite outgrowth in PC12 cells (Fig. 3), indicating that phorbol esters can mimic several of the actions of NGF in these cells. Comparison of the time course of these two agents on neuronal differentiation revealed that TPA has a more rapid effect than NGF on the initial onset of neurite outgrowth, but with longer times of exposure their effects become indistinguishable (data not shown).

Activation of PKC Is Required for the Induction of GAP-43 Gene Expression

Having established that activators of PKC induce GAP-43 mRNA levels in PC12 cells, a second series of experiments was performed to determine whether the effect of NGF on GAP-43 gene expression was also mediated by this signal transduction pathway. As shown in Fig. 4, NGF did not enhance GAP-43 mRNA levels in PC12 cells in which PKC had previously been downregulated by high doses of TPA (Matthies et al., 1987 and Fig. 2 B). This result suggests that NGF and TPA use a common PKC-dependent pathway to induce GAP-43 mRNA levels in these cells. In agreement with this hypothesis, pretreatment of the cultures with selective inhibitors of PKC such as polymyxin B, sphingosine, and H7 treatment on protein phosphorylation. PC12 cells were incubated for 30 min in the presence of 160 nM TPA and protein phosphorylation was determined as described in Materials and Methods. Arrow indicates the migration of an 80-kD phosphoprotein.
Figure 5. Effects of protein kinase inhibitors on GAP-43 mRNA levels in NGF-treated PC12 cells. PC12 cells were cultured for 16 h in RPMI containing 100 ng/ml NGF, by itself or in the presence of the following protein kinase inhibitors: H7 (50 μM), sphingosine (5 μM), and polymyxin B (2,000 U/ml). The conditions used for downregulation of PKC by high doses of phorbol esters (TPA high) are the same as in Fig. 4. GAP-43 mRNA levels were calculated by Northern blot analysis as described in Fig. 1. The results represent the mean ± SD for at least two separate experiments.

Phorbol Esters Cause a Selective Stabilization of the GAP-43 mRNA

We have previously shown that NGF increases the stability of the GAP-43 mRNA in PC12 cells (Perrone-Bizzozero et al., 1991). To explore the role of PKC in the stabilization of this mRNA, we measured the half-life of GAP-43 mRNA in control and TPA-treated cells. As shown in Fig. 6, the mRNAs decayed following a simple exponential function: 

\[ M = M_0 e^{-\lambda t} \]

where \( M \) is the amount of mRNA at time \( t \), \( M_0 \) is the amount of mRNA at \( t = 0 \), and \( \lambda = (\ln 2)/t_{1/2} \). Using this equation, the half-life \( t_{1/2} \) of the GAP-43 mRNA in unstimulated cells was estimated to be 5.5 h, a value intermediate between that of very unstable (e.g., \( t_{1/2} < 1 \) h) and stable (e.g., \( t_{1/2} \approx 24 \) h) mammalian mRNAs (Shaw and Kamen, 1986; Wilson and Treisman, 1988). In contrast, the mRNA for the housekeeping enzyme G3PD was found to be very stable \( t_{1/2} \approx 24 \) h, Fig. 6). Upon TPA treatment, the GAP-43 mRNA was selectively stabilized \( t_{1/2} = 30 \) h, Fig. 6 A), whereas the turnover of the G3PD mRNA was not affected (Fig. 6 B). These results and the finding that TPA does not affect the degradation of the 28S ribosomal RNA (not shown) suggest that the phorbol ester exerts a highly selective effect on GAP-43 mRNA turnover rather than a generalized stabilization of cellular RNAs. Furthermore, analysis of the relative rates of accumulation and turnover of the GAP-43 mRNA indicates that the sixfold increase in the half-life of the GAP-43 mRNA can account for most of the 12.5-fold accumulation of this mRNA in the presence of TPA, leaving only a minor control component (twofold change) to alternative mechanisms. Thus, GAP-43 mRNA levels in PC12 cells appear to be regulated primarily by a PKC-dependent mRNA stabilization.

Destabilization of the GAP-43 mRNA by Polymyxin B and Dexamethasone

To further evaluate the contribution of mRNA stabilization to the levels of induction of the GAP-43 mRNA and to begin studying the cellular mechanisms responsible for this control, we examined the effects of two agents that inhibit GAP-43 expression on the half-life and steady-state levels of this mRNA in TPA-treated PC12 cells (Figs. 7 and 8, and Table I). We first determined the effects of polymyxin B, since as shown in Fig. 5, this PKC inhibitor was able to inhibit the NGF-mediated increase in GAP-43 mRNA levels. Treatment of TPA-stimulated cells with polymyxin B led to a 70%
the text. The conditions are: control (Co), 160 nM TPA (TPA), 0.5 μg/ml cycloheximide (+CHM), 1 μM dexamethasone (+DEXA), and 2,000 U/ml polymyxin B (+Poly.B). Northern blots for GAP-43 and actin were prepared as described in Fig. 1.

Figure 8. Effects of various treatments on GAP-43 mRNA levels in PC12 cells. PC12 cells were preincubated for 30 min with the various agents and then cultured for 16 h in the presence of TPA and/or inhibitors as described in the text. The conditions are: control (Co), 160 nM TPA (TPA), 0.5 μg/ml cycloheximide (+CHM), 1 μM dexamethasone (+DEXA), and 2,000 U/ml polymyxin B (+Poly.B). Northern blots for GAP-43 and actin were prepared as described in Fig. 1.

The expression of the growth-associated protein GAP-43 constitutes one of the critical events during both neuronal development and nerve regeneration. In a wide variety of neuronal pathways, the levels of expression of this protein were found to change 20-100-fold during the period of axonal development.
growth (Skene and Willard, 1981a,b; Benowitz et al., 1981, 1983). Thus, it is of great interest to identify the mechanism(s) responsible for such regulation. Although in several neural systems, increased GAP-43 mRNA levels were found to precede the expression of this protein, the level of GAP-43 transcription did not correlate well with the induction of the mRNA (Federoff et al., 1988; Perrone-Bizzozero et al., 1991). Furthermore, in NGF-treated PC12 cells, GAP-43 mRNA levels were found to be controlled in part by changes in the rate of degradation of the mRNA (Perrone-Bizzozero et al., 1991), indicating that posttranscriptional mechanisms also play an important role in regulating the expression of this protein. In this study, we found that NGF and phorbol esters induce GAP-43 mRNA levels in PC12 cells by a signal transduction pathway involving the activation of PKC. This induction was found to correlate with the selective stabilization of the GAP-43 mRNA, which in turn was dependent on PKC activity but independent of protein synthesis. Thus, GAP-43 mRNA levels during neuronal differentiation seem to be regulated by a novel mRNA stabilization mechanism which does not require ongoing translation.

NGF is known to generate cellular responses via a variety of signal transduction pathways, including those requiring the activation of PKA, PKC, and other protein kinase systems (Cremins et al., 1986; Doherty et al., 1988; Machida et al., 1991; Kaplan et al., 1991). In the case of GAP-43, several lines of evidence indicate that the induction of the mRNA is mediated by the activation of PKC. First, the phorbol ester TPA, a potent activator of this kinase, was very effective in inducing GAP-43 mRNA levels and neurite outgrowth in PC12 cells (Figs. 1-3). High doses of TPA, which are known to downregulate PKC, also decreased the levels of GAP-43 mRNA (Figs. 2-5). In cells in which PKC was downregulated by high doses of phorbol ester, NGF was no longer able to stimulate GAP-43 gene expression. Finally, several protein kinase inhibitors, with high selectivity for PKC, prevented NGF from inducing GAP-43 mRNA levels in PC12 cells. Since these effects were not influenced by inhibition of protein synthesis, it appears that PKC-dependent phosphorylation of pre-existing proteins is sufficient for controlling GAP-43 mRNA levels during neuronal differentiation.

In agreement with our findings, TPA was found to increase GAP-43 gene expression in the human neuroblastoma cell line SH-SY5Y (Bjellman et al., 1990) and to a lesser extent in a different PC12 cell clone (Costello et al., 1990). Although the specific response to phorbol esters is known to depend on the cell type, these agents have been shown to induce a number of neuronal characteristics both in primary neuronal cultures and a variety of neural lines such as PC12 cells and neuroblastoma. These include neuronal survival (Montz et al., 1985), process outgrowth (End et al., 1982) and the expression of several differentiation-specific proteins (Doherty et al., 1988; Pennypacker et al., 1989; Vyas et al., 1990; Machida et al., 1991). Furthermore, inhibition of PKC by sphingosine was found to block neurite outgrowth in NGF-induced PC12 cells (Hall et al., 1988), suggesting that activation of this kinase is essential for neuronal differentiation.

PKC is known to regulate gene expression by acting at both transcriptional and posttranscriptional levels. Phorbol esters directly activate transcription of a variety of genes, including those of the proto-oncogenes c-fos and c-myc (Greenberg and Ziff, 1984). This activation was shown to depend on the presence of phorbol ester responsive elements (TRE) upstream of the promoter region (Angel et al., 1987). A combination of transcriptional and posttranscriptional regulation was found to mediate the activation of the tyrosine hydroxylase gene by phorbol diesters (Vyas et al., 1990). In contrast, the influence of PKC activity on GAP-43 gene expression appears to be controlled mainly at the posttranscriptional level. Several observations support this conclusion. The rate of transcription of the GAP-43 gene in PC12 cells does not appear to be affected by either NGF (Federoff et al., 1988; Perrone-Bizzozero et al., 1991) or phorbol ester treatment (Perrone-Bizzozero, N. I., and V. V. Cansino, unpublished observations), although both treatments significantly increase GAP-43 mRNA levels in the cells. In addition, analysis of the 5' flanking region of the GAP-43 gene failed to identify TRE consensus sequences (Nevidi et al., 1992). Finally, in this study, we found that changes in mRNA turnover can account for most of the observed accumulation of GAP-43 mRNA in TPA-induced PC12 cells. The opposite effect was observed with PKC inhibitors; i.e., they destabilized the GAP-43 mRNA and reduced its accumulation in the cells. Thus, PKC-dependent mRNA turnover seems to be the primary point of control for GAP-43 expression during process outgrowth.

Unlike transcriptional control, the molecular mechanisms for posttranscriptional regulation are poorly understood. For several fast-turning over mRNAs, it has been found that the length of the poly(A) tail and the presence of instability-conferring sequences within the 3' untranslated region (3'UTR) greatly influence the rate of degradation of the mRNAs (Wilson and Treisman, 1988; Brewer and Ross, 1988). One of such instability-conferring sequences is the AU-rich element (ARE) found in the 3'UTR of several protooncogene and cytokine mRNAs (Shaw and Kammen, 1986). Degradation of these short-lived mRNAs also requires continuous translation, since inhibition of protein synthesis results in a dramatic stabilization of the mRNAs (Shaw and Kammen, 1986; Wilson and Treisman, 1988). Other determinants of mRNA instability are the stem–loop structures at the 3'UTR of histone mRNAs (Pandey and Marzluff, 1987) and the iron-responsive element (IRE) in the transferrin receptor mRNA (Müller and Kühn, 1988). Finally, in a few

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**Table I. Effects of Several Agents on the Relative Half-lives and Levels of the GAP-43 mRNA in PC12 Cells**

| Condition          | Half-life (h) | Relative levels |
|--------------------|--------------|----------------|
| Control            | 5 ± 1        | (1)            |
| TPA                | 30 ± 3       | 12.5 ± 2.5     |
| TPA + CHM          | 45 ± 9       | 13.4 ± 2.7     |
| TPA + DEXA         | 18 ± 3       | 5.8 ± 1.5      |
| TPA + Poly. B      | 10 ± 2       | 6.8 ± 1.7      |
| Co + CHM           | 6 ± 1        | 1.4 ± 0.5      |

Values represent the mean ± standard deviation of the mean for at least two independent experiments. The half-lives and relative levels of the GAP-43 mRNA correspond to the data shown in Figs. 7 and 8.
cases, the instability determinants were found to lie within the coding sequences of the mRNAs. For example, the first 13 nucleotides in the coding region of the β-tubulin mRNAs are responsible for the autoregulation of β-tubulin gene expression (Yen et al., 1987). Another example of this type of instability determinants are those present in the coding region of c-fos (Shyu et al., 1988). Thus, in the latter case, there are at least two separate instability conferring sequences located at different sites in the mRNA molecule. In the case of GAP-43, the half-life of this mRNA seems to be affected by the presence of the poly(A)-binding protein (Berstein and Ross, 1989). Although the nature of the mechanism that regulates GAP-43 mRNA stability is still unclear, we have recently found that cytosolic proteins from brain are able to bind specific sequences in the 3’UTR of GAP-43 mRNA (Kohn, D. T., and N. I. Perrone-Bizzozero, manuscript in preparation). This observation and the high sequence conservation in the 3’UTR of the GAP-43 mRNA suggest that specific RNA–protein interactions within this region may contribute to the stabilization of this mRNA during neuronal differentiation. The possibility that these interactions are indeed regulated by PKC activity is currently under investigation.

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