MOLECULAR AND CELLULAR BIOLOGY, Dec. 1990, p. 6769–6774
0270-7306/90/126769-06$02.00/0

Vol. 10, No. 12

Pip92: A Short-Lived, Growth Factor-Inducible Protein in BALB/c 3T3 and PC12 Cells

CATHERINE H. CHARLES, JEFFREY S. SIMSKE, TIMOTHY P. O'BRIEN, AND LESTER F. LAU*

Department of Genetics, University of Illinois College of Medicine, 808 South Wood Street, Chicago, Illinois 60612

Received 6 June 1990/Accepted 10 September 1990

***pip92* is a cellular immediate-early gene inducible by serum growth factors in fibroblasts. It is also induced in the rat pheochromocytoma cell line PC12 by agents that cause proliferation, neuronal differentiation, and membrane depolarization. We show that the *pip92*-encoded polypeptide is a proline-rich protein of 221 amino acids, has an extremely short half-life, and is localized in the cytoplasm. We hypothesize that Pip92 plays a role in mediating the cellular responses to a variety of extracellular signals.**

Polypeptide growth factors regulate a variety of cellular processes, including growth and differentiation. In cultured mouse fibroblasts, one of the initial cellular responses to serum growth factors is the transcriptional activation of a set of specific genes even in the absence of de novo protein synthesis. Among these immediate-early genes are those thought to encode regulatory proteins that mediate the growth response. Consistent with this hypothesis, there is now a growing list of known or putative nuclear transcription regulators encoded by immediate-early genes, including members of the Fos and Jun families, Myc, and several zinc finger-containing proteins (reviewed in references 3, 14, and 21). In addition to nuclear regulators, one might also expect the induction of cytoplasmic regulatory proteins upon growth factor stimulation. These proteins may regulate signal transduction, protein synthesis, and the activities of key biosynthetic and metabolic enzymes (18). While recent studies on immediate-early genes have focused primarily on those encoding potential nuclear regulators, the only cytoplasmic proteins encoded by immediate-early genes identified thus far are cytoskeletal proteins, including actin, tropomyosin, and a putative actin-associated protein (3). We report here the characterization of a growth factor-inducible immediate-early gene, *pip92*, that encodes a highly unstable cytoplasmic protein. We hypothesize that Pip92 (proline-rich induced protein) plays a role in mediating the cellular responses to a variety of extracellular signals.

***pip92* expression and cDNA sequence.** *pip92* is an immediate-early gene that is expressed during the G_0/G_1 transition (originally called 3CH92 in reference 12). Its mRNA is accumulated rapidly and transiently as quiescent fibroblasts are stimulated with serum, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), or 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA). Transcription of *pip92* is activated within 2 min of serum stimulation, reaches a peak level by 10 min, and is attenuated to low levels in 1 to 2 h (13). Its mRNA is accumulated rapidly during liver regeneration following partial hepatectomy (16), suggesting that its expression plays a role in cell growth in living animals as well as in cultured fibroblasts.

A nearly full-length *pip92* cDNA clone was isolated from a BALB/c 3T3 cell cDNA library (13). Primer extension analysis showed mRNA cap sites at 7, 8, and 12 nucleotides (nt) upstream of the cDNA (not shown). The 5′-most 12 nt that the cDNA lacks were deduced from the *pip92* genomic sequence; a TATA sequence occurs 25 to 28 nt upstream of the 5′ most cap site (Fig. 1). Sequence analysis (22) of the *pip92* cDNA reveals an mRNA of 1515 nt, excluding the poly(A) tail. The *pip92* mRNA has a short half-life of about 10 to 20 min (13). However, its 3′ untranslated region, though A+U rich, lacks the AUUUA sequence thought to play a role in rapid mRNA degradation (23). Recently, a cDNA (*chx1*) corresponding to a cycloheximide-superinducible mRNA in activated T lymphocytes was isolated (6); its cDNA sequence is nearly identical to that of *pip92*.

pip92 is inducible by TPA, suggesting that protein kinase C (PKC) substrates play a role in its activation (12). To ascertain whether PKC activity is obligatory for *pip92* activation, we utilized the observation that chronic treatment of fibroblasts with TPA abolishes PKC activity (20). BALB/c 3T3 cells were treated with TPA for 24 h to down-regulate PKC as described previously (17) and were compared with untreated cells for responsiveness to various inducing agents (Fig. 2A). An RNase protection assay (17) was devised to detect the *pip92* mRNA; T7 RNA polymerase transcription of a *Sac*I-*Nco*I cDNA fragment cloned into the pGEM4 vector (Promega) yielded a 187-nt antisense riboprobe that protected the *pip92* mRNA from nucleotides 382 to 529. The *pip92* mRNA accumulated in naive cells in response to TPA but not in cells pretreated with TPA, indicating that PKC activity was effectively down-regulated. In contrast, cells pretreated with TPA still responded to stimulation by serum, PDGF, and FGF, indicating that *pip92* can be induced through PKC-independent pathways. This observation appears to be general for immediate-early genes (15, 17). The levels of *pip92* mRNA induction by FGF and PDGF were lower in TPA-pretreated cells, suggesting that these growth factors may act through the additive or synergistic action of both PKC-dependent and -independent pathways to induce *pip92*.

Whereas either cholera toxin, which elevates cyclic AMP levels (4), or isobutylmethylxanthine, a phosphodiesterase inhibitor (5), alone had little effect on *pip92* expression, the combination of the two agents induced a notable increase in *pip92* mRNA (Fig. 2A). These results suggest that *pip92* can be activated through a cyclic AMP-dependent pathway. Although the B subunit of cholera toxin may also raise

* Corresponding author.

```
                                        TATATTAAACTAGCGCGCTTGCTGCCCTGCGCCCGAACGT
                                                                   GCGCCCGAACGTCTAGCAGAGTAC

  25 CTGCTGCTGTAAGCTTGTCGTCTGGGCTGCACCGCCCGTCTTAACCCATTCTCGACTTAACTACTCTCGTCGAACAAGC

 104 ATG GAA GTA CAG AAA GAA GCG CAG CGC ATC ATG ACT CTG TCG GTA TGG AAG ATG TAC CAC
     Met Glu Val Gln Lys Glu Ala Gln Arg Ile Met Thr Leu Ser Val Trp Lys Met Tyr His  20

 164 TCT CGC ATG CAG CGA GGT GGC TTG CGA CTC CAC CGG AGT CTG CAG CTA TCC CTC GTT ATG
     Ser Arg Met Gln Arg Gly Gly Leu Arg Leu His Arg Ser Leu Gln Leu Ser Leu Val Met  40

 224 CGC AGC GCT CGA GAG CTC TAC CTC TCA GCC AAG GTA GAA GCC CAC CAG CCC GAG TTC CCG
     Arg Ser Ala Arg Glu Leu Tyr Leu Ser Ala Lys Val Glu Ala His Gln Pro Glu Phe Pro  60

 284 CCA TCC CGC AGG GCT CTT GAC CCT CGC CTG CAC CCG CCG CGG GAA GCC GAA GTT GCA GTG
     Pro Ser Arg Arg Ala Leu Asp Pro Arg Leu His Pro Pro Arg Glu Ala Glu Val Ala Val  80

 344 GAA GTA GCG TCC CCC GAA GCC GTG CAG CCT CCG GAG CCC ATG GAT ACG CAA GAG GAA GTG
     Glu Val Ala Ser Pro Glu Ala Val Gln Pro Pro Glu Pro Met Asp Thr Gln Glu Glu Val 100

 404 CTG CGA GTC CAG GAG ACC CCT GCG CTC TGT GAC CCG CCC CCC GCT AGA GTC AGC CGC AAG
     Leu Arg Val Gln Glu Thr Pro Ala Leu Cys Asp Pro Pro Pro Ala Arg Val Ser Arg Lys 120

 464 CGC CGG AGC AGC AGC GAT TTG AGC GAC AGT AGT GAT GCC GGA CTG GTA CCA AGC AAG AAG
     Arg Arg Ser Ser Ser Asp Leu Ser Asp Ser Ser Asp Ala Gly Leu Val Pro Ser Lys Lys 140

 524 GCC CGT CTA GAA GAG GTG GAG GGG GAG GCG ACG TCG GAG GTT CCC GAT CGC CTG CAG CTT
     Ala Arg Leu Glu Glu Val Glu Gly Glu Ala Thr Ser Glu Val Pro Asp Arg Leu Gln Leu 160

 584 CCT CCG GCA CAA AGC GAA GGT GCC TTC CCT AAC CTC GCC CGC GTC CTC CAA AGG CGC TTC
     Pro Pro Ala Gln Ser Glu Gly Ala Phe Pro Asn Leu Ala Arg Val Leu Gln Arg Arg Phe 180

 644 TCC AGT CTC CTG AAC TGT GGA CCC GCC GTG CCC CCG CCG ACG CCC CCC ACG TGC GAG GCC
     Ser Ser Leu Leu Asn Cys Gly Pro Ala Val Pro Pro Pro Thr Pro Pro Thr Cys Glu Ala 200

 704 AAG CCA GCC TGC CGC CCG GCC GAC AAT ATG CTC AAC GTG CTG GTG CGA GCT GTG GTG GCC
     Lys Pro Ala Cys Arg Pro Ala Asp Asn Met Leu Asn Val Leu Val Arg Ala Val Val Ala 220

 764 TTC TGA GAGCTCTGGTGGCTTCTTTCGAGCGGCGCCACCGGAGCGGAGAACGCACACCCGAGGCGAAGGCCGGCGGG
     Phe TER                                                                        221

 841 GGCCGTGAAGAAGAGCCGCGGCCCGAGCTGCCGAGAGGCCAGGGCAAGGACTGAGGAGCGAGGGGCGCGGGCGCCTTCT

 920 CCCAGACGTGCGTCCATAGGTGCTATTAAAGGACTGTCCCTTCCTTGGCTTGGAGAAGGGACACCTAGATCTTGAATCT

 999 CAGGGTCGAACTCTCTAGGGGCCAGGCTGCCCTTTCAAGGCCGTTTCACTACCATTCGCGTTTCGGCCCCTACAAGTGG

1078 GCACGCTTGTGCAAGCGGTCAGAGTTGCGTCATGGGACAGACGCGGGTGCTTCCTGTTGCCTTGCGTGGGTGTGGGGCC

1157 TGGGAGGAGGCCAGGGTGTGGACCCGCCCTAGGGACTGGGAAGTGACTTGAGTCACCTCGCCCCCACAGGCTGCTGTGG

1236 GTGAGCCTGAACTGAACCAATCAAATCTGCGCAGAGTTGAAGTGGCTGGAGACCCCGGGACTGGTCAACCTAGATGATC

1315 GCCTGGCGTGGACCACCGCGGGACGGGTGGGCCGCTGGTCGTAGTTGCTGCCGTAGACACAGCTTCTTCGGGCAGGAAA

1394 GAAAATTTTTTTTTTACCAGCGTGTTTAAGAAAGTCTGTTTACTTTTCCCACGGTGGGTTGTTTAATTAGCAACTACCT

1473 GGAGTTTTACAATGTCAGCTAGGAAaataaaGACCATCGGTGT(A)n
```

FIG. 1. Nucleotide sequence and the encoded amino acid sequence of the *pip92* cDNA. Numbers at the left refer to the first nucleotides on the lines, and numbers at the right refer to the last amino acids on the lines. The 5′-most nucleotides deduced from the genomic sequence are shown in italics. The top line shows the upstream genomic TATA sequence. The polyadenylylation signal is shown in lowercase letters, and the terminal (A)n denotes the poly(A) tail.

intracellular calcium levels (8), the calcium ionophore A23187 does not appear to affect *pip92* expression to any significant extent.

We determined the tissue distribution of *pip92* mRNA by an RNase protection assay (Fig. 2B). *pip92* mRNA was found to be present in many tissues, most abundantly in the testis, ovary, uterus, lung, and intestine.

The rat pheochromocytoma cell line PC12 is a suitable

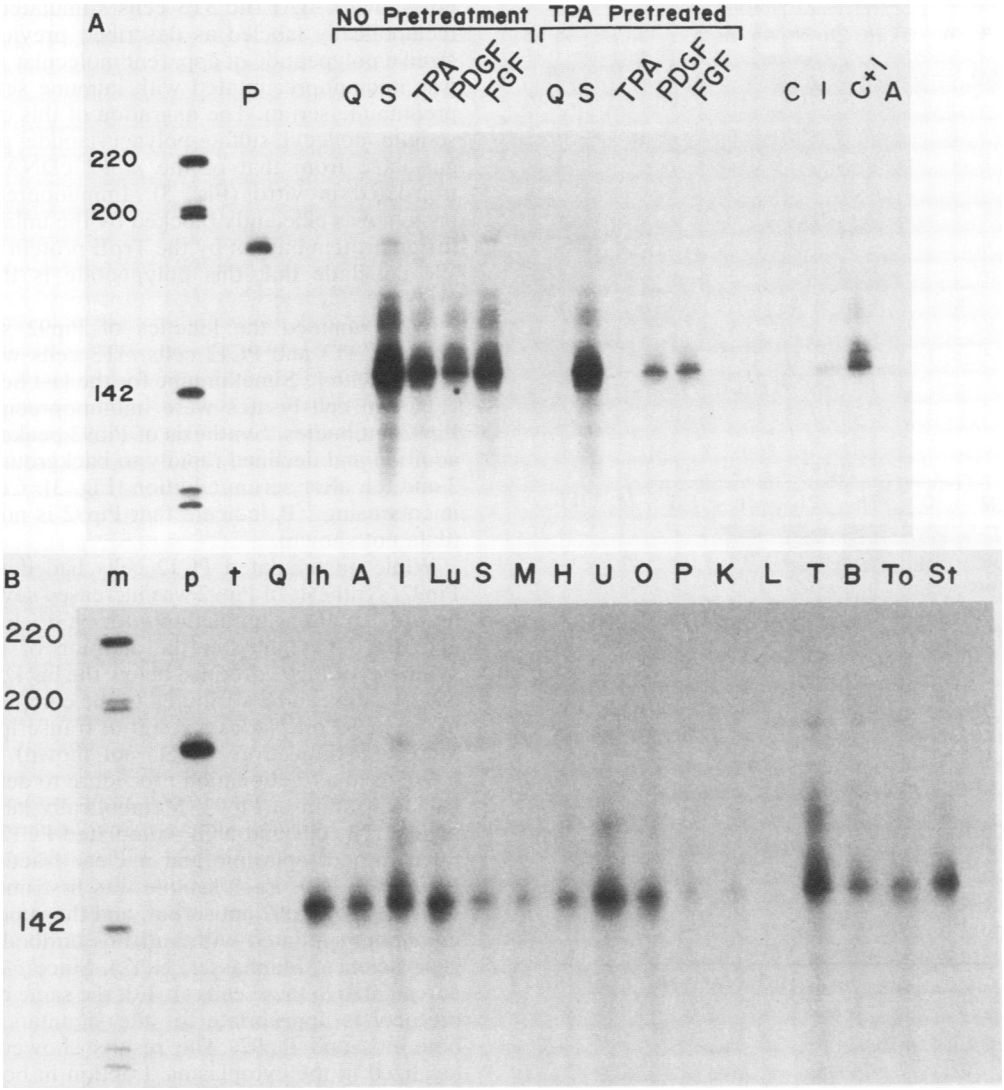

FIG. 2. (A) Induction of *pip92* mRNA through multiple pathways. Shown are results of an RNase protection assay of *pip92* mRNA in cells stimulated with various agents, with or without TPA pretreatment as indicated. Cells were treated as described previously (17). P, Probe alone (1/2,000 of the amount used in each reaction). Total RNA samples (10 μg) were isolated from either quiescent 3T3 cells (Q) or cells treated with serum (S), TPA, PDGF, FGF, cholera toxin (C) and isobutylmethylxanthine (I), alone or in combination (C+I), and A23187 (A). Numbers of nucleotides of size markers are given on the left. (B) Tissue distribution of *pip92* mRNA. Total RNA isolated from various adult BALB/c mouse tissues was subjected to RNase protection analysis. Protections with 10 μg of yeast tRNA (t), quiescent 3T3 cell RNA (Q), and 1-h serum-stimulated cell RNA (1h; 1/10 of sample loaded) show specificity of the assay. Tissue RNA samples (10 μg) were from the following sources: A, adrenal gland; I, intestine; Lu, lung; S, spleen; M, skeletal muscle; H, heart; U, uterus; O, ovary; P, pancreas; K, kidney; L, liver; T, testis; B, brain; To, tongue; and St, stomach. m, Size markers (lengths are given in nucleotides on the left); p, probe alone.

model with which to examine the effects of diverse extracellular signals, since it can be induced to undergo divergent cellular responses by different agents (9). Upon addition of nerve growth factor (NGF), these cells initially undergo cell division and then differentiate into sympathetic neuron-like cells and cease to proliferate. Epidermal growth factor (EGF) stimulates proliferation but not differentiation (2). In addition, PC12 cells have excitable membranes that can be depolarized by either specific neurotransmitters or elevated levels of extracellular KCl (9). Consistent with the observation that *pip92* is transcriptionally activated in PC12 cells upon stimulation by NGF, EGF, or KCl (1), we found that *pip92* mRNA was present in unstimulated cells and accumulated to peak levels within 30 min to 1 h after stimulation by each of these agents (not shown).

The Pip92 protein. The *pip92* cDNA encodes a protein of 221 amino acids with a predicted molecular weight of 24,502 and a calculated isoelectric point of 8.29. This protein is proline rich, is highly hydrophilic, and contains no potential N-linked glycosylation site. Pip92 is not related to any protein of known function, as judged by comparison of its sequence with those in protein sequence data bases. To generate antibodies against Pip92, a cDNA fragment encoding amino acids 25 to 142 (nt 177 to 530) was fused downstream of the *Escherichia coli trpE* gene in the pATH-2 vector (7). This construct directs the inducible synthesis of a TrpE-Pip92 fusion protein, which was used to immunize New Zealand White rabbits (10). The antiserum raised against this antigen, but not the preimmune serum, immunoprecipitated the Pip92 polypeptide translated in vitro (data

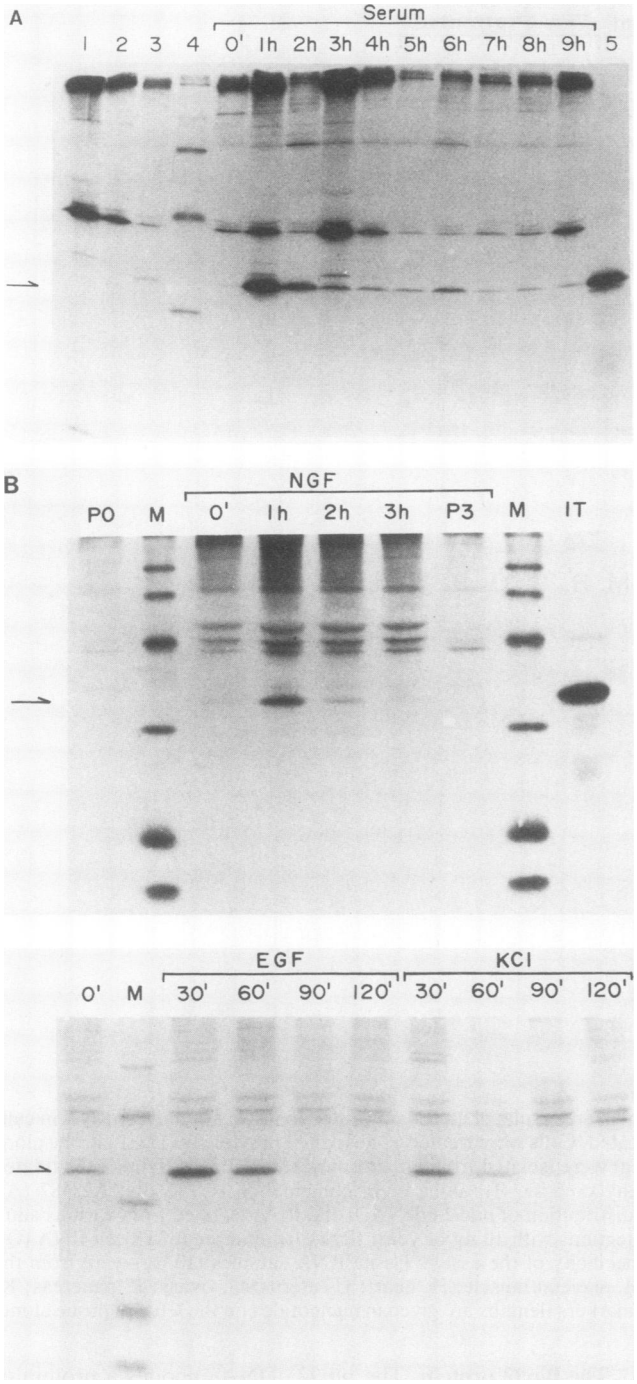

FIG. 3. Synthesis of Pip92 in BALB/c 3T3 and PC12 cells. (A) Quiescent BALB/c 3T3 cells were serum stimulated for the indicated times as described previously (17). The cells were metabolically labeled with [^{35}S]methionine during the last hour of stimulation, and cell lysates were immunoprecipitated as described elsewhere (10) with anti-Pip92 antiserum. Extracts from cells stimulated with serum for 2 h (lane 1) and logarithmically growing cells (lane 2) were immunoprecipitated with preimmune serum, and log-phase cells were immunoprecipitated with immune serum (lane 3). Lane 4, ^{14}C-labeled molecular weight markers; lane 5, in vitro translation product of the *pip92* sense RNA. (B) Synthesis of Pip92 in PC12 cells. PC12 cells were stimulated with NGF, EGF, or KCl for the indicated times as described previously (1) and metabolically labeled with [^{35}S]methionine during the last 15 min of stimulation. Cell lysates were immunoprecipitated with anti-Pip92 antiserum. IT, In vitro translation product of *pip92*. Preimmune serum was used to immunoprecipitate lysates from unstimulated cells (PO) and cells stimulated with NGF for 3 h (P3). M, ^{14}C-labeled molecular weight markers. Arrows point to the Pip92 bands. ', Minutes.

not shown). BALB/c 3T3 cells stimulated with serum and metabolically labeled as described previously (17) synthesized a polypeptide of apparent molecular weight 33,000 that was immunoprecipitated with immune serum but not with preimmune serum. The migration of this cellular protein on sodium dodecyl sulfate-polyacrylamide gels was indistinguishable from that of the *pip92* cDNA-encoded protein translated in vitro (Fig. 3). Immunoprecipitation of this protein was efficiently blocked by the unlabeled TrpE-Pip92 fusion protein but not by the TrpE protein (data not shown). We conclude that this polypeptide is the *pip92*-encoded protein.

We examined the kinetics of Pip92 synthesis in both BALB/c 3T3 and PC12 cells. 3T3 cells were metabolically labeled with [^{35}S]methionine for the last hour before harvest (17), and cell lysates were immunoprecipitated with anti-Pip92 antibodies. Synthesis of Pip92 peaked 1 h after serum addition and declined rapidly to background levels between 2 and 8 h after serum addition (Fig. 3). Cell-labeling experiments using $^{32}P_i$ indicate that Pip92 is not phosphorylated (data not shown).

While unstimulated PC12 cells had detectable levels of Pip92, synthesis of Pip92 was increased severalfold within 30 min to 1 h after stimulation with NGF, EGF, or KCl (Fig. 3B). By 2 to 3 h after the addition of these agents, the synthesis of Pip92 dropped below the background level. This observation can be attributed to the complete disappearance of the *pip92* mRNA as a result of transcriptional repression following stimulation (1; data not shown).

We used a fractionation procedure to determine the cellular localization of Pip92. Metabolically labeled serum-stimulated 3T3 cells and NGF-stimulated PC12 cells were separated into cytoplasmic and nuclear fractions as described previously (19). Each sample was first immunoprecipitated with the anti-Pip92 antiserum, and the supernatant was then immunoprecipitated with anti-Fos antibodies (Ab-2; Oncogene Science, Manhasset, N.Y.). Since *pip92* and c-*fos* are coregulated in these cells (1, 13), the same metabolic labeling protocol is appropriate for the simultaneous detection of both Fos and Pip92. The results showed that Pip92 was localized in the cytoplasmic fraction in both 3T3 and PC12 cells (Fig. 4A). As expected, Fos was found predominantly in the nuclear fraction with an apparent molecular weight of approximately 62,000, indicating that the fractionation procedure is valid.

A pulse-chase experiment was carried out to determine the half-life of Pip92. BALB/c 3T3 and PC12 cells were stimulated for 30 min with serum and NGF, respectively, and then metabolically labeled with [^{35}S]methionine for 5 min and chased for various times in medium without label (Fig. 4B). In both cell types, Pip92 was degraded extremely rapidly, with an estimated half-life of 5 to 10 min. The short half-life of the *pip92*-encoded mRNA and protein are consistent with its gene product playing a regulatory role.

Although initially isolated as a gene inducible by mitogens in fibroblasts, *pip92* is also inducible by agents that cause disparate cellular responses in PC12 cells. Responsiveness to a broad range of stimuli has also been observed for other immediate-early genes, in particular those that encode nuclear regulators (24). It is thus possible that such genes

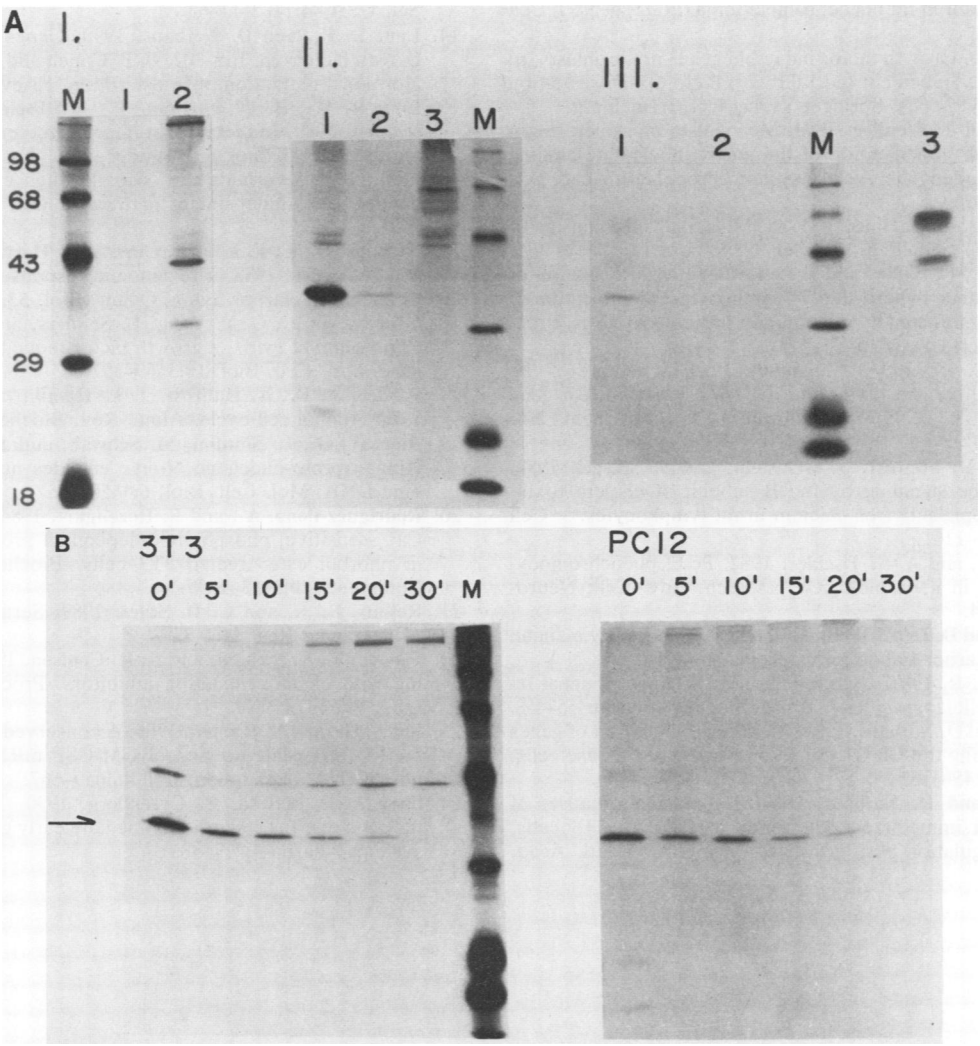

FIG. 4. (A) Subcellular fractionation. (I) PC12 cells metabolically labeled with [^{35}S]methionine for 30 min during NGF stimulation were separated into nuclear (lane 1) and cytoplasmic (lane 2) fractions and immunoprecipitation with anti-Pip92 antiserum (II) BALB/c 3T3 cells stimulated with serum for 1 h were metabolically labeled with [^{32}S]methionine and separated into cytoplasmic (lane 1) and nuclear fractions (lane 3). The nuclear fraction was washed to remove cytoplasmic contaminants (lane 2). These samples were immunoprecipitated with anti-Pip92 antiserum. (III) The samples shown in panel II were immunoprecipitated with anti-Fos antibodies after precipitation with anti-Pip92 antiserum. The lower-molecular-weight band in the nuclear fraction (lane 3) is likely to be c-Jun coprecipitated with Fos. M in each case represents molecular weight markers; the approximate molecular weight of each polypeptide is indicated on the left in thousands. (B) Degradation of Pip92. BALB/c 3T3 cells and PC12 cells were pulse-labeled for 5 min with [^{35}S]methionine after a 30-min stimulation with serum and NGF, respectively, and chased for the indicated times (in minutes). Cell lysates were immunoprecipitated with anti-Pip92 antiserum. M, Molecular weight markers.

encode components of overlapping pathways that mediate the cellular responses to a variety of extracellular signals (1, 11, 24).

Little is known about the potential role of immediate-early gene-encoded proteins as cytoplasmic regulators. While the activities of a number of cytoplasmic kinases are rapidly activated upon growth factor stimulation, these proteins appear to be largely regulated by posttranslational modification (14). The cytoplasmic localization and short half-life of Pip92 protein raise the intriguing possibility that Pip92 is a regulatory protein that functions in the cytoplasm.

Nucleotide sequence accession number. The nucleotide sequence reported here has been deposited in the EMBL and GenBank nucleotide sequence data bases under accession number M33756.

This work was supported by grants from the National Institutes of Health (Public Health Service grants RO1 CA46565 and RO1 CA52220) and the March of Dimes Birth Defects Foundation. C.H.C. is a National Science Foundation predoctoral fellow. L.F.L. is the recipient of an American Cancer Society Junior Faculty Research Award and is a Pew Scholar in the Biomedical Sciences.

LITERATURE CITED

1. **Bartel, D. P., M. Sheng, L. F. Lau, and M. E. Greenberg.** 1989. Growth factors and membrane depolarization activate distinct programs of early response gene expression: dissociation of *fos* and *jun* induction. Genes Dev. 3:304–313.
2. **Boonstra, J., W. H. Moolenaar, P. H. Harrison, P. Moed, P. T. van der Saag, and S. W. de Laat.** 1983. Ionic responses and growth stimulation induced by nerve growth factor and epider-

mal growth factor in rat pheochromocytoma (PC12) cells. J. Cell Biol. **97**:92–98.
3. **Bravo, R.** 1989. Growth factor inducible genes in fibroblasts, p. 324–343. *In* A. Habenicht (ed.) Growth factors, differentiation factors, and cytokines. Springer-Verlag KG, Heidelberg.
4. **Cassel, D., and T. Pfeuffer.** 1978. Mechanism of cholera toxin action: covalent modification of the guanyl nucleotide-binding protein of the adenylate cyclase system. Proc. Natl. Acad. Sci. USA **75**:2669–2673.
5. **Cohen, P., and M. D. Houslay.** 1985. Molecular mechanisms of transmembrane signalling. Elsevier, New York.
6. **Coleclough, D., L. Kuhn, and I. Lefkovits.** 1990. Regulation of mRNA abundance in activated T lymphocytes: identification of mRNA species affected by inhibition of protein synthesis. Proc. Natl. Acad. Sci. USA **87**:1753–1757.
7. **Dieckmann, C. L., and A. Tzagoloff.** 1985. Assembly of the mitochrondrial membrane system: CBP6, a yeast nuclear gene necessary for synthesis of cytochrome b. J. Biol. Chem. **260**: 1513–1520.
8. **Dixon, S. J., D. Stewart, S. Grinstein, and S. Spiegel.** 1987. Transmembrane signaling by the B subunit of cholera toxin: increased cytoplasmic free calcium in rat lymphocytes. J. Cell Biol. **105**:1153–1161.
9. **Greene, L. A., and A. S. Tischler.** 1982. PC12 pheochromocytoma cultures in neurobiological research. Adv. Cell. Neurobiol. **3**:373–414.
10. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y.
11. **Herschman, H. R.** 1989. Extracellular signals, transcriptional responses and cellular specificity. Trends Biochem. Sci. **14**:455–458.
12. **Lau, L. F., and D. Nathans.** 1985. Identification of a set of genes expressed during the G0/G1 transition of cultured mouse cells. EMBO J. **4**:3145–3151.
13. **Lau, L. F., and D. Nathans.** 1987. Expression of a set of growth-related immediate early genes in BALB/c 3T3 cells: coordinate regulation with c-*fos* or c-*myc*. Proc. Natl. Acad. Sci. USA **84**:1182–1186.
14. **Lau, L. F., and D. Nathans.** 1990. Genes induced by serum growth factors, p. 165–202. *In* P. Cohen and J. G. Foulkes (ed.), Hormonal regulation of transcription. Elsevier, Amsterdam.
15. **Lim, R. W., B. C. Varnum, T. G. O'Brien, and H. R. Herschman.** 1989. Induction of tumor promoter-inducible genes in murine 3T3 cell lines and tetradecanoyl phorbol acetate-nonproliferative 3T3 variants can occur through protein kinase C-dependent and -independent pathways. Mol. Cell. Biol. **9**:1790–1793.
16. **Nathans, D., L. F. Lau, B. Christy, S. Hartzell, Y. Nakabeppu, and K. Ryder.** 1988. The genomic response to growth factors. Cold Spring Harbor Symp. Quant. Biol. **53**:893–900.
17. **O'Brien, T. P., G. P. Yang, L. Sanders, and L. F. Lau.** 1990. Expression of *cyr61*, a growth factor-inducible immediate early gene. Mol. Cell. Biol. **10**:3569–3577.
18. **Pardee, A. B., R. Dubrow, J. L. Hamlin, and R. F. Kletzien.** 1978. Animal cell cycle. Annu. Rev. Biochem. **47**:715–750.
19. **Ramsay, G., L. Stanton, M. Schwab, and J. M. Bishop.** 1986. Human proto-oncogene N-*myc* encodes nuclear proteins that bind DNA. Mol. Cell. Biol. **6**:4450–4457.
20. **Rodriguez-Pena, A., and E. Rozengurt.** 1984. Disappearance of Ca^{2+}-sensitive, phospholipid-dependent protein kinase activity in phorbol ester-treated 3T3 cells. Biochem. Biophys. Res. Commun. **120**:1053–1059.
21. **Rollins, B. J., and C. D. Stiles.** 1989. Serum-inducible genes. Adv. Cancer Res. **53**:1–32.
22. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
23. **Shaw, G., and R. Kamen.** 1986. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell **46**:659–667.
24. **Sheng, M., and M. E. Greenberg.** 1990. The regulation and function of c-fos and other immediate early genes in the nervous system. Neuron **4**:477–485.