PKC Plays a Critical Role during Stromelysin Promoter Activation by Platelet-derived Growth Factor through a Novel Palindromic Element

(Received for publication, October 19, 1993, and in revised form, January 4, 1994)

Laura Sanz‡‡, Edurne Berra††, Maria M. Municio‡‡, Isabel Dominguez‡‡, Jose Lozano‡‡, Terje Johansen‡‡‡, Jorge Moscat++‡, and Maria T. Diaz-Meco++‡‡

From the ‡Centro de Biología Molecular "Severo Ochoa," Consejo Superior de Investigaciones Científicas-Universidad Autonoma de Madrid, Canto Blanco, 28049 Madrid, Spain and the ++Department of Biochemistry, Institute of Medical Biology, University of Tromso, 9037 Tromso, Norway

Stromelysin is a metalloproteinase with the widest substrate specificity that plays a critical role in the induction of the metastatic phenotype in cancer cells. The mechanisms whereby growth factors and oncogenes control stromelysin expression are beginning to be characterized. We have recently demonstrated that protein kinase C isoforms down-regulated by chronic exposure to phorbol esters are not involved in stromelysin gene expression in response to platelet-derived growth factor, ras oncogene, and phosphatidylycholine-hydrolyzing phospholipase C. We also identified a region in the stromelysin promoter, distinct from the 12-O-tetradecanoylphorbol-13-acetate-responsive element, responsible for the promoter activity in response to these stimuli. In this paper, we further characterize that promoter fragment and demonstrate that the region encompassing nucleotides −1218 to −1202, including the palindromic sequence ACTAGT, is necessary and sufficient for the control of stromelysin gene expression. The involvement of α-protein kinase C but not of c-ras in the stimulation of stromelysin promoter activity in response to platelet-derived growth factor is also demonstrated here. All these data suggest the existence of a bifurcation downstream of ras in the signaling mechanisms leading to stromelysin expression and DNA synthesis.

Regulation of gene transcription is associated with cell growth and tumor transformation (1). At the first level, the so-called nuclear proto-oncogenes (c-fos, c-jun, c-myc; Refs. 2 and 3) play critical roles as tertiary messengers in the transcriptional regulation of a number of second-phase genes; these are crucial to the maintenance of the normal cell phenotype, as well as to the degree of tumor invasiveness and metastatic potential (4–6). Thus, a number of second-phase genes code for metalloproteinases that are increased in some tumors, and their levels correlate with the metastatic status of several cancer cell lines (7–9). To date, a number of metalloproteinases have been described exhibiting matrix-degrading activity: collagenases, gelatinases, and stromelysins (10–12). Stromelysins, with the widest substrate specificity, can degrade fibronectin, laminin, collagen IV, and diverse proteoglycans (13). The expression of stromelysin or its rat homolog, transin, is subject to dual regulation. Enzyme synthesis is induced by interleukin-1β (14, 15), phorbol esters (16, 17), growth factors (17), and oncogenes (18), whereas it is suppressed by dexamethasone (14, 15) or transforming growth factor-β (19). It would be of great interest to unveil the mechanisms whereby growth factors and oncogenes control the expression of stromelysin.

Recently, we and others have identified a novel required step in these cascades; activation by growth factors and oncogene products of a phospholipase C specific for phosphatidylincholine (PC-PLC) has been shown to be both necessary and sufficient for mitogenic activation (20–28). Furthermore, we recently found that PC-PLC activation, besides governing cell growth and tumor transformation, is also involved in the regulation of stromelysin and, therefore, could be implicated in the appearance of metastatic phenotypes (29). In that study it was also demonstrated, at a gene transcriptional level, that the signaling mechanisms utilized by PDGF/ras p21/PC-PLC differ from those triggered by PMA/PKC. Thus, PDGF, ras p21, and PC-PLC activate stromelysin expression in fibroblasts lacking PMA-sensitive PKCs to an extent similar to that found in cells with normal PKC levels (23, 29). Furthermore, this route does not involve the 12-O-tetradecanoylphorbol-13-acetate-responsive element existing in the stromelysin promoter but utilizes a potentially novel element located in the region encompassing nucleotides −1240 to −1145 of that promoter (29). These results would be consistent with our recent data demonstrating the critical role played by a PMA-unsensitive PKC isotype, termed ζ, in the mitogenic signaling pathways activated by ras p21/PC-PLC (30–33).

In the study presented here we further characterize that promoter region and demonstrate that a fragment located between nucleotides −1218 and −1202, which includes the palindromic sequence ACTAGT, is necessary and sufficient for the control of stromelysin gene expression. Moreover, following PDGF stimulation of fibroblasts, the induction of a factor binding to this region was detected by mobility shift assays. Even

* This work was supported in part by Grants SAF93-0018 and PB90-0074 from Director General de Investigacion Cientifica y Tecnica, Grant C256-91 from Comunidad de Madrid, and Grant SC1*-CT92-0769 from the Science Plan of the European Community. Partial funding for this work was from Glaxo Spain and an institutional grant to the Centro de Biologia Molecular from the Fundacion Ramon Areces. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Fellow from Ministerio de Educacion.
† Fellow from Gobierno Vasco.
‡ Fellow from Fundación Científica de la Asociación Española Contra el Cancer.
‡‡ To whom correspondence should be addressed. Tel.: 34-1-397-8039; Fax: 34-1-397-8344.

1 The abbreviations used are: PDGF, platelet-derived growth factor; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C; DTT, dithiothreitol; SPRE, stromelysin PDGF-responsive element; WT, wild-type; PC-PLC, phosphatidylincholine-phospholipase C; CAT, chloramphenicol acetyltransferase.
more important, we demonstrate here the involvement of PKC in the stimulation of stromelysin promoter activity in response to PDGF.

MATERIALS AND METHODS

Cell Cultures—NIH-3T3 fibroblasts were cultured and maintained as described (29) in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 2 mg/ml-glutamine in standard tissue culture flasks in a humidified air/CO₂ (19:1) incubator at 37 °C. Cells were made quiescent by incubation for 24 h in the presence of serum-free medium supplemented with transferrin (5 μg/ml) and Na₂SO₄ (1 μM).

Plasmid Construction—Plasmid pHBCAT contains 1.3 kilobases of the stromelysin promoter encompassing nucleotides -1303 to -11 (ref. to the transcription initiation site, cloned into the HindIII-BamHI site of the CAT reporter vector pBCCAT3. Plasmids pA63HACAT (nucleotides -1240 to -754, pA78HACAT (nucleotides -1225 to -754), pA89HACAT (nucleotides -1214 to -754), and pA102HACAT (nucleotides -1201 to -764) were constructed following the amplification of corresponding fragments by polymerase chain reaction using the plasmid pHBCAT as template and the following primers: 5'-ATGAAGCTGCTGATCGGGAAG-3' and 5'-ATGTCGACTGATAAGGTACCATTG-3' for pA63HACAT, 5'-ATGGATCCGGATCATATTTTAAACTGATGATCTCT-3' and 5'-ATGTCGACTGACACAGATGATTCT-3' for pA78HACAT, 5'-ATGGATCCGGATCATATTTTAAACTGATGATCTCT-3' and 5'-ATGTCGACTGATAAGGTACCATTG-3' for pA89HACAT, and 5'-ATGTCGACTGACACAGATGATTCT-3' and 5'-ATGTCGACTGATAAGGTACCATTG-3' for pA102HACAT. The polymerase chain reaction fragment for pA63HACAT was digested with HindIII, blunt ended, digested again with PstI, and ligated to pCATβ promoter, which has previously been cut with the same enzymes. Plasmid pCACT contains one copy of the palindromic sequence (nucleotides -1218 to -1202) inserted into the BamHI site of pBCCAT2, which contains the herpes simplex virus thymidine kinase minimal promoter.

Gel Shift Mobility Assays—Nuclear extracts were isolated as previously described (32). Cells at 75% confluence in 100-mm diameter culture dishes were made quiescent by serum starvation for 24 h, after which they were either untreated or stimulated with PDGF (10 ng/ml) for 24 h. Afterward, cells were washed with ice-cold phosphate-buffered saline and scraped into 1 ml of lysis buffer (10 mM Tris, pH 7.9, 150 mM NaCl, 0.1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 5 mM NaF, 1 mM NaVO₄), and allowed to swell on ice for 10 min. Afterward, cells were homogenized with 10 strokes of a glass Dounce homogenizer, and the nuclei were collected by centrifugation at 15,000 g for 15 min. The supernatant was dialyzed against dialysis buffer (20 mM Tris, pH 7.9, 150 mM NaCl, 0.5 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 5 mM NaF, 1 mM NaVO₄), rotated gently at 4 °C for 45 min, and centrifuged at 15,000 g for 15 min. The supernatant was dialyzed against dialysis buffer (20 mM Tris, pH 7.9, 150 mM NaCl, 0.5 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 5 mM NaF, 1 mM NaVO₄) at 4 °C. The dialysate was cleared by centrifugation at 15,000 g for 15 min, and the supernatant was aliquoted and stored at -70 °C. Ten μg of nuclear extracts were preincubated with or without unlabeled competitors for 15 min at room temperature and incubated at room temperature for an additional 30 min with 1 ng of the different 32P-labeled probes (50,000 cpm) in the following gel shift binding buffer (5 μl): 37.5% glycerol, 5 mM MgCl₂, 0.25 mM EDTA, 0.5 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 μg NaF, 0.5 μg NaVO₄, at 4 °C. The dye shift was observed by electrophoresis of the reaction through a 4% polyacrylamide gel in 0.5 x TBE buffer for 2 h and visualized by autoradiography.

Fig. 1. Partial restriction map of stromelysin promoter and gene constructs used in the transient expression assays. At the top of the figure is the 5′-5′ region containing 1.3 kilobases of promoter sequence extending through the first exon (box) and into the second (half-box). The different constructs used in the experiments are shown and described under “Materials and Methods.”

RESULTS

Transient Gene Expression Assays—Previously published results demonstrated that the region encompassing nucleotides -1303 to -754 of the stromelysin promoter is necessary and sufficient for transcriptional activation in response to PDGF and PC-PLC (29). Further deletion analysis of this region revealed that the fragment from -1240 to -1145 is necessary to drive the transcription of a reporter gene linked to a minimal promoter (29). In order to further determine the determinant sequence responsible for the activation of the stromelysin promoter by PDGF, the following plasmids were constructed. pA63HACAT harbors the fragment extending from -1240 to -754 of the stromelysin promoter cloned upstream of an SV40 minimal promoter (plasmid pCATprom). pA78HACAT, pA89HACAT, and pA102HACAT are further deletions of pA63HACAT (see Fig. 1). Results from Fig. 2 demonstrate that the deletion of the transcription initiation site, completely abolishes the stromelysin promoter activity induced by PDGF. There is a palindromic sequence in this region (ACTAGTGTAGC) that we hypothesized could be a critical element responsible for stromelysin promoter activation. Plasmid pCAT was constructed in which a single copy of this element (nucleotides -1218 to -1202) was cloned upstream of the herpes simplex virus TK minimal promoter in a CAT reporter plasmid. Interestingly, results from Fig. 2 demonstrate that this fragment was sufficient to drive CAT synthesis in response to PDGF even more efficiently than the fragment from -1240 to -754 (plasmid pA63HACAT). This is a novel sequence with no obvious similarity to any other enhancer element so far described; from now on, this nucleotide sequence will be termed SPRE for stromelysin PDGF-responsive element.

PDGF-stimulated Fibroblasts Contain Elevated Levels of a Specific SPRE-binding Nuclear Factor—The possible existence of DNA-binding proteins interacting with the SPRE was investigated by gel shift mobility assays. A double-stranded oligonucleotide (WT; nucleotides -1221 to -1203) containing the SPRE sequence was labeled and incubated with nuclear extracts from cells either untreated or treated with PDGF for 24 h. Nucleoprotein complexes were resolved on native poly-
acrylamide gels, and the appearance of retarded bands was detected following autoradiography. Results from Fig. 3 demonstrate that cells treated with PDGF displayed a major band that was not seen in nuclear extracts from untreated cells (Fig. 3, compare lanes 2 and 3). The formation of this DNA-binding complex was abolished by incubation of extracts with an excess of cold oligonucleotide (Fig. 3, lane 4). These results suggest that PDGF induces a nuclear factor that binds to SPRE.

To further demonstrate the critical role played by the palindromic sequence in the binding of the putative nuclear factor, the following series of gel shift mobility assay experiments was carried out. Nuclear extracts from PDGF-treated cells were incubated with an increasing molar excess of either the wild-type double-stranded oligonucleotide (WT) or a deletion mutant (DEL) that lacks the palindromic sequence ACTAGT (see Fig. 4, upper panel) previous to the addition of the labeled WT probe. Results from Fig. 4 demonstrate that the integrity of the palindromic sequence is a requisite for the binding of the nuclear factor to take place. In addition, two mutations that disrupt the palindrome were performed, MUT1 and MUT2 (see Fig. 5, upper panel). Gel shift mobility assay experiments carried out with the corresponding 32P-labeled double-stranded oligonucleotides harboring either mutation and nuclear extracts from PDGF-treated cells demonstrated that both mutations severely impaired binding to the probe; this effect is even more dramatic in the case of MUT1. It is noteworthy that the intensity of the band given by MUT2 when incubated with 10 μg of protein nuclear extracts was similar to that produced by 2 μg of protein nuclear extract incubated with the labeled WT probe.

Involvement of ζPKC in the Regulation of SPRE Enhancer Activity—Stromelysin induction is activated by serum/PDGF and PC hydrolysis (29), and these stimulants utilize ζPKC (30–33) during mitogenic signaling. Therefore, conceivably, this kinase could be involved in the regulation of SPRE by PDGF. To address this possibility, we initially determined whether activation by overexpression of ζPKC leads to the stimulation of SPRE binding activity. Results from Fig. 6 demonstrate that nuclear extracts from ζPKC overexpresser cells (previously described in Refs. 32 and 33) gave dramatically increased levels of SPRE binding activity. The retarded band observed in gel shift mobility assay of nuclear extracts from ζPKC overexpressers is most probably the same as that produced by stimulation with PDGF. In this regard, it is noteworthy that the affinity of the SPRE binding activity from ζPKC overexpresser cells is quite similar to that from PDGF-treated cells (Fig. 7). Thus, a similar competition curve was observed when nuclear extracts from either ζPKC overexpressers or PDGF-treated cells were incubated with increasing concentrations of cold probe prior to the addition of the 32P-labeled WT double-stranded oligonucleotide (Fig. 7). Likewise, binding of the nuclear factor from the ζPKC overexpresser to the labeled WT probe is not competed by an excess (40-fold) of deletion mutant double-stranded oligonucleotide and does not bind either to MUT1 or MUT2 probes (not shown). This behavior is identical to that of nuclear extracts
SpKC and Stromelysin Induction

Mutations in the palindromic sequence ACTAGT impair the binding of the PDGF-induced nuclear factor to the WT oligonucleotide. Different 32P-labeled double-stranded oligonucleotides (1 ng, 50,000 cpm) were incubated either in the absence (lanes 1, 3, and 5) or in the presence of either 10 μg (lanes 2, 4, and 7) or 2 μg (lane 6) of nuclear extracts from PDGF-treated NIH-3T3 fibroblasts. The reactions were performed with the following double-stranded oligonucleotides: MUT1 (lanes 1 and 2), MUT2 (lanes 3 and 4), and WT (lanes 5–7). Essentially identical results were obtained in another three experiments.

FIG. 5. Mutations in the palindromic sequence ACTAGT impair the binding of the PDGF-induced nuclear factor to the WT oligonucleotide. Different 32P-labeled double-stranded oligonucleotides (1 ng, 50,000 cpm) were incubated either in the absence (lanes 1, 3, and 5) or in the presence of either 10 μg (lanes 2, 4, and 7) or 2 μg (lane 6) of nuclear extracts from PDGF-treated NIH-3T3 fibroblasts. The reactions were performed with the following double-stranded oligonucleotides: MUT1 (lanes 1 and 2), MUT2 (lanes 3 and 4), and WT (lanes 5–7). Essentially identical results were obtained in another three experiments.

FIG. 6. Increased levels of SPRE binding activity in nuclear extracts from a 5pKC-overexpresser cell line. 32P-Labeled double-stranded WT oligonucleotide (1 ng, 50,000 cpm) was incubated either in the absence or in the presence of 10 μg of nuclear extracts (10 μg) from either 5pKC overexpresser cells (lane 2), PDGF-treated NIH-3T3 fibroblasts (lane 3), or untreated cells (lane 4). Essentially identical results were obtained in another three experiments.

FIG. 7. Induction of nuclear factor SPRE binding activity by PDGF and 5pKC overexpression. 32P-Labeled double-stranded WT oligonucleotide (1 ng, 50,000 cpm) was incubated in the presence of nuclear extracts (10 μg) from either 5pKC overexpresser cells (insert, ZPKC), or PDGF-treated NIH-3T3 fibroblasts (inset, PDGF), either in the absence or in the presence of different concentrations of cold WT double-stranded oligonucleotide (5-, 15-, and 40-fold molar excess). Autoradiograms from three independent experiments were scanned, and the mean amount of complex formed ± S.D. is represented by arbitrary units. The inset is a representative experiment of another two with similar results.

FIG. 8. Inhibition of SPRE-dependent transactivation by a 5pKC kinase-defective mutant. Subconfluent cultures of NIH-3T3 fibroblasts were transfected with 5 μg of either carrier DNA (control) or plasmids expressing either 5pKC (ZPKCmut) or c-raf (RAFmut) kinase-defective dominant negative mutants together with plasmid palCAT (A) or plasmid pHBCAT (B) as described under Materials and Methods. Cells were either unstimulated (empty bars) or treated with PDGF (10 ng/ml) for 24 h (cross-hatched bars). Results are the means ± standard deviations of three independent experiments with incubations in duplicate.

whether or not c-raf is involved in the regulation of SPRE-dependent promoter activity, a dominant negative mutant of this kinase was transfected along with the palCAT reporter plasmid. RAFmut is a previously described c-raf kinase-defective dominant negative mutant (35). Expression plasmids harboring both kinase mutants (i.e. 5pKCmut and RAFmut) severely impaired cell proliferation when separately transfected into NIH-3T3 fibroblasts (not shown). Interestingly, transfection of
a plasmid expressing RAFmut did not significantly inhibit the PDGF-stimulated SPRE-dependent promoter activity (Fig. 8A). Transfection of the corresponding empty plasmids did not have any effect on this parameter (not shown). Consistent with the notion that SPRE plays a critical role in the regulation of the stromelysin promoter is the fact that transfection of 7PKCmut but not of RAFmut significantly inhibits the stromelysin promoter activity when the pHBCTR reporter plasmid is used instead of pCAT in the transfection assays (Fig. 8B).

**DISCUSSION**

We have previously demonstrated that PC-PLC activation is an important event for stromelysin induction and in the control of PDGF-triggered mitogenic signaling (20–29). Since PC hydrolysis generates diacylglycerol, a logical hypothesis should contemplate PKC as an important intermediary in the activation of stromelysin expression by PDGF/PC-PLC. However, we have already presented evidence that PMA-sensitive PKC subtypes are not required for stromelysin induction in response to these stimuli (which is in very good agreement with the notion that this route is not necessary either for activation of DNA synthesis in Swiss 3T3 fibroblasts (23) or for maturatrion of *Xenopus* oocytes (30)). Transient expression experiments with plasmids harboring different deletions and mutations in the stromelysin promoter region linked to a reporter gene, demonstrated that the 12-O-tetradecanoylphorbol-13-acetate-responsive element located in that promoter is not required to transmit signals generated by PDGF/PC-PLC (29). We defined a region of 750 base pairs located between -1303 and -754, respective to the transcription start site, that is necessary and sufficient for induction of stromelysin by PDGF/PC-PLC.

A careful search in that region reveals the existence of consensus sequences similar or identical to known enhancer elements. Thus, as base pair -1290, there is a sequence very similar to one termed the ras-responsive element that apparently accounts for the inducibility of certain genes by ras (36). However, a deletion in the stromelysin promoter region that removes the 5′-24 base pairs including that element (29) does not affect the ability of PDGF/PC-PLC to activate this promoter. This sequence, therefore, is not important for stromelysin gene induction in response to PDGF/PC-PLC. Another potentially interesting sequence in this zone is the one located at nucleotide -1239 (CACCCTG), identical to the consensus sequence CANNNTG, which has been reported to bind Myc and/or MyoD proteins (37). However, from the results shown in Fig. 1 it is clear that deletion of such an element does not affect stromelysin promoter activity, revealing the lack of importance of that sequence in the induction of stromelysin. Interestingly, a novel region that includes a palindrome sequence was identified in the present study as necessary and sufficient for the activation of the stromelysin promoter in response to PDGF. From the data shown here it is also clear that a nuclear factor is induced by PDGF, which binds to the promoter fragment located between nucleotide positions -1221 and -1203 that includes the palindromic ACTAGT, which is critical for the binding of the nuclear complex. The nature and characterization of that factor is beyond the scope of this paper but clearly indicate the existence of either a completely novel pathway for the transcriptional regulation of stromelysin or, alternatively, classical transcription factors functioning through a novel element. There are precedents for the second possibility. Thus, for example, it has recently been shown the involvement of AP-1 family members as components of the nuclear factor of activated T cells, a critical nuclear factor binding to the interleukin-2 enhancer through an element distinct from the AP-1 binding site (38).

On the other hand, in order to better understand the signaling mechanisms involved in the regulation of stromelysin expression, a series of experiments aimed at identifying critical kinases in this pathway was carried out. From our previously published data (29), it is clear that a classical PMA-sensitive PKC isotype is not involved in the activation of the stromelysin promoter activity. Interestingly, the results depicted here establish the critical role played by 7PKC in the regulation of SPRE-dependent promoter activity in response to PDGF. This, together with recent data demonstrating the involvement of 7PKC in the regulation of nuclear factor c-B (31, 32), permit one to consider 7PKC as a central point in the mechanisms controlling the transcriptional machinery. Another interesting aspect of the results shown here is the fact that c-raf does not appear to be involved in stromelysin gene expression. Both kinases, c-Raf and 7PKC, have been proposed to lie downstream of ras in the mitogenic cascade (30–33, 35). Ras is a potent activator of stromelysin expression (29). Therefore, the results presented in this study suggest the bifurcation of signaling mechanisms downstream of Ras. One mechanism is controlled by 7PKC, whereas the other one is controlled by c-raf. Both are critical for cell proliferation, but only 7PKC is involved in the control of the stromelysin promoter activity. If this model is correct, one could predict that both pathways should converge at some point in the mitogenic cascade. Actually, preliminary data from our laboratory demonstrate that 7PKC, like Raf, phosphorylates and activates mitogen-activated protein kinase kinase, which permits one to propose mitogen-activated protein kinase kinase as the meeting point for both pathways.

**Acknowledgment**—We thank Dr. Ulf Rapp for the c-raf kinase-defective expression plasmid.

**REFERENCES**

1. Hunter, T. and Karin, M. (1992) *Cell* 70, 375–387
2. Angel, P. and Karin, M. (1991) *Biochim. Biophys. Acta* 1072, 129–157
3. Zipursky, S. L. and Watterson, R. (1988) *Cell* 31, 513–514
4. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmody, H. J., Jonat, C., Herrlich, P., and Karin, M. (1987) *Cell* 49, 728–739
5. Schonfeld, A., Herrlich, P., Rahmody, H. J., and Poust, H. (1988) *Cell* 54, 325–334
6. Ostrowski, L. E., Finch, J., Krieg, P., Matrisian, L. P., Gatakan, O., Gonnell, J. P., Phillips, J., Slaga, T. J., Breathnach, R., and Rowden, G. T. (1988) *Mol. Carcinogenesis* 1, 13–19
7. Basset, P., Belloco, J. P., Wolf, C., Soll, I., Hutan, P., Limacher, J. M., Podlusz, L., Chenard, M. P., Rico, M. C., and Chambon, P. (1990) *Nature* 345, 693–704
8. Matrisian, L. M., Braden, G. T., Krieg, P., von Stetten, G., and Breathnach, R. (1986) *Proc. Natl. Acad. Sci. U.S.A.* 83, 9413–9417
9. Wolf, C., Rouyer, N., Lutz, Y., Adda, C., Loriot, M., Belloco, J. P., Cambon, P., and Bassett, P. (1986) *Proc. Natl. Acad. Sci. U.S.A.* 83, 1845–1847
10. Schmid, T. M., Mayne, K., Jeffrey, J. D., and Linsenmayer, T. F. (1989) *J. Biol. Chem.* 264, 4184–4189
11. Gibbs, M. S., Haitjema, K. A., Moyer, R. M. J., Kang, A. H., and Mainsard, C. L. (1986) *J. Biol. Chem.* 261, 2493–2500
12. Murphy, G., McAlpine, C. G., Poll, C. T., and Reynolds, J. J. (1985) *Biochim. Biophys. Acta* 851, 49–56
13. Okada, Y., Nagase, H., and Harris, E. D., Jr. (1986) *J. Biol. Chem.* 261, 14245–14255
14. Saud, J., Quibonos, S., Otani, Y., Nagase, H., Harris, E. D., Jr., and Kurikinen, M. (1988) *J. Biol. Chem.* 263, 6742–6745
15. Quinones, S., Saud, J., Otani, Y., Harris, E. D., Jr., and Kurikinen, M. (1989) *J. Biol. Chem.* 264, 3325–3344
16. Kerr, L. D., Holt, J. T., and Matrisian, L. M. (1996) *Science* 272, 1424–1427
17. McDonnell, S. E., Kerr, L. D., and Matrisian, L. M. (1990) *Mol. Cell. Biol.* 10, 4284–4294
18. Matrisian, L. M., Gneichenhaus, N., Geanel, M. C., and Breathnach, R. (1985) *EMBO J.* 4, 1435–1441
19. Kast, L. D., Miller, D. B., and Matrisian, L. M. (1996) *Cell* 61, 267–274
20. Price, B. D., Morris, J. D. H., Marshall, C. J., and Hall, A. (1989) *J. Biol. Chem.* 264, 16635–16643
21. Ebert, J. H. (1990) *J. Biol. Chem.* 265, 1–4
22. Lopez-Barahona, M. P., Kaplan, P. L., Corneil, M. E., Diaz-Meco, M. T., Larrodera, J., Diaz-Laviada, I., Municio, P. A., M., and Moscat, J. (1990) *J. Biol. Chem.* 265, 9023–9026
23. Larrodera, J., Parnes, M. E., Diaz-Meco, M. T., Lopez-Barahona, M. Diaz-Laviada, I., Guddal, P. J., Hohansen, T., and Moscat, J. (1990) *Cell* 61, 1113–1120
24. Diaz-Laviada, I., Larrodera, P., Diaz-Meco, M. E., Guddal, P. H., and Moscat, J. (1990) *Cell* 61, 1113–1120
25. Domínguez, M. T., Diaz-Meco, J., Lozano, and J. Moscat, unpublished observations.

---

1. Domínguez, M. T., Diaz-Meco, J., Lozano, and J. Moscat, unpublished observations.
25. García de Herreros, A., Dominguez, I., Diaz-Meco, M. T., Graziani, G., Cornet, M. E., Guddal, P. H., Johansen, T., and Moscat, J. (1991) J. Biol. Chem. 266, 6825–6829
26. Dominguez, I., Marshall, M. S., Gibbs, J. B., García de Herreros, A., Cornet, M. E., Graziani, G., Diaz-Meco, M. T., Johansen, T., McCormick, F., and Moscat, J. (1991) EMBO J. 10, 3215–3220
27. Diaz-Meco, M. T., Dominguez, I., Sanz, L., Munícuo, M. M., Berra, E., Cornet, M. E., and Moscat, J. (1992) Mol. Cell. Biol. 12, 302–308
28. Cai, H., Ehrhart, P., Szeeberényi, J., Diaz-Meco, M. T., Moscat, J., and Cooper, G. M. (1992) Mol. Cell. Biol. 12, 5329–5335
29. Diaz-Meco, M. T., Quiñones, S., Munícuo, M. M., Sanz, L., Bernal, D., Cabrero, E., Saus, J., and Moscat, J. (1991) J. Biol. Chem. 266, 22597–22602
30. Dominguez, I., Diaz-Meco, M. T., Munícuo, M. M., Berra, E., García de Herreros, A., Cornet, M. E., Sanz, L., and Moscat, J. (1992) Mol. Cell. Biol. 12, 3776–3783
31. Dominguez, I., Sanz, L., Arenzana-Seisdedos, F., Diaz-Meco, M. T., Virelizier, J. L., and Moscat, J. (1993) Mol. Cell. Biol. 13, 1290–1295
32. Diaz-Meco, M. T., Berra, E., Munícuo, M. M., Sanz, L., Lezano, J., Dominguez, I., Diaz-Gelpe, V., Lain de Lera, M. T., Alcañiz, J., Arenzana, F., Payá, C. V., Virelizier, J. L., and Moscat, J. (1993) Mol. Cell. Biol. 13, 4770–4775
33. Berra, E., Diaz-Meco, M. T., Dominguez, I., Munícuo, M. M., Sanz, L., Lezano, J., Chapkin, R. S., and Moscat, J. (1993) Cell 74, 555–562
34. Crews, C. M., and Erikson, R. L. (1993) Cell 74, 215–217
35. Kolch, W., Heidecker, G., Lloyd, P., and Rapp, U. R. (1991) Nature 348, 426–428
36. Owen, R. D., and Ostrowski, M. C. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3866–3870
37. Edmondson, D. G., and Olson, E. N. (1990) J. Biol. Chem. 265, 755–758
38. Boise, L. H., Petryniak, B., Mas, X., June, C. H., Wang, C.-Y., Lindsten, T., Bravo, R., Kovalsky, K., Leiden, J. M., and Thompson, C. B. (1995) Mol. Cell. Biol. 15, 1911–1919