Transcription from the Stromelysin Promoter Is Induced by Interleukin-1 and Repressed by Dexamethasone*

(Received for publication, July 16, 1987)

Steven M. Frisch† and H. Earl Ruley§
From the Department of Pediatrics, Children's Hospital, Washington University School of Medicine, St. Louis, Missouri 63110 and the §Center for Cancer Research, E17-520, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The stromelysin gene encodes a potent tissue-degrading proteinase whose activity is important in tissue-remodeling processes such as wound healing, the inflammatory reaction, rheumatoid arthritis, tumor invasion, and possibly embryonic development. In light of the ability of interleukin-1 to amplify, and ability of glucocorticoids to attenuate the inflammatory response, we tested interleukin-1 and dexamethasone for regulatory effects on stromelysin gene expression. We report that interleukin-1 induces the stromelysin gene, and dexamethasone diminishes the level of induction by interleukin-1, epidermal growth factor, phorbol ester, and cAMP elevation (elicited by cholera toxin). Similar responses are conferred upon a chloramphenicol acetyltransferase coding sequence by a 700-base pair stromelysin 5'-flanking fragment, implying transcription regulation by sequence elements in this region.

Extracellular matrix degradation is a key element in such physiologic processes as wound healing (1), inflammation (2), differentiation, and development (3, 4) and in the pathology of rheumatoid arthritis (5) and tumor invasion (6). Degradation of extracellular matrix/connective tissue absolutely requires the cooperation of multiple proteinases. Collagenase alone is capable only of introducing a single cleavage in the collagen triple helix, leaving further collagen degradation and degradation of other matrix components to be dealt with by different proteinases.

The control of genes encoding the relevant proteinases is only beginning to be elucidated. The large number of reports of elevated extracellular proteinase activity in tumor cells (6) suggests that activated oncogenes, possibly by influencing normal growth factor or lymphokine signal transduction pathways, play a regulatory role with regard to these proteinase genes. Recently, we reported the molecular cloning of a cDNA encoding rabbit stromelysin, a phorbol ester-induced, secreted metalloproteinase which has potent, and fairly promiscuous, matrix-degrading activity (7); Matrias et al. (8) reported the cloning of transin, an epidermal growth factor-induced gene of unknown function. We (7) and they (9) subsequently found transin and stromelysin to be very homologous or identical except for species differences. The rat transin gene was found also to be inducible by transformation mediated by polyoma, Rous sarcoma virus, or activated Ha-ras (10), consistent with the SV40 transformation inducibility of human stromelysin observed previously (11). As in the rabbit synovial fibroblast system, the transin gene was inducible by cytochalasin B (10), but the tumor promoter, TPA,‡ induced transin only in the presence of serum, while TPA induced the rabbit stromelysin gene only in the absence of serum (12).

While these second messenger modulators are useful for revealing the signal transduction pathways that have the potential of inducing the stromelysin gene, it is also important to identify the possible physiologic inducers and repressors of this highly tissue-destructive enzyme. Stromelysin-I (II-1) is an attractive candidate, considering its central role in promoting the inflammatory response (13) and, in particular, its stimulation of connective tissue-degrading activity (e.g. Ref. 14) partly attributable to collagenase gene induction (15, 16).

We also chose to examine the effects of the synthetic glucocorticoid dexamethasone on stromelysin gene expression in light of the potent anti-inflammatory (17) and anti-arthritic (18) properties of the glucocorticoids and, in particular, their repression of collagenase gene expression (19). Also, we reasoned that, because fibronectin is readily digested by stromelysin (20), repression of stromelysin synthesis by glucocorticoids would be consistent with (or possibly responsible for) the phenomenon that dexamethasone causes restoration of normal levels of fibronectin accumulation in certain transformed cell lines (21).

Induction of stromelysin gene expression by interleukin-1 and repression by dexamethasone are reported in this paper. DNA elements located in the region −700 to −20 were found to be sufficient to confer both of these regulatory responses upon a heterologous coding sequence, chloramphenicol acetyltransferase, as well as the regulatory responses reported previously (induction by EGF, cAMP elevation, and TPA). Sequence analysis of the stromelysin 5'-flanking region revealed some interesting homologies with the corresponding regions of other, similarly regulated genes.

MATERIALS AND METHODS

Cell Culture—Rabbit synovial fibroblasts were prepared and cultured as described previously (12). Agents tested for induction were EGF (Collaborative Research, receptor grade), human recombinant II-1β-R007 (Cistron Technology, Pine Brook, NJ; provided by Dr. C. P. A. Stein) and polyoma virus. Tumor transin was a gift from Dr. D. E. Powers. The abbreviations used are: TPA, 12-O-tetradecanoylphorbol-13-acetate; II-1, interleukin-1; EGF, epidermal growth factor; SDS, sodium dodecyl sulfate; IBMX, isobutylmethylxanthine; bp, base pair; kbp, kilobase pair; PIPES, 1,4-piperazinediethanesulfonic acid; protein kinase C, Ca2+/phospholipid-dependent enzyme.

*This work was supported by Grant CA40602 (to H. E. R.) and Core Grant 14051 (to H. E. R.) and the §Center for Cancer Research, E17-520, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

‡Present address and to whom correspondence should be addressed: Dept. of Medicine, Dermatology Division, Washington University School of Medicine, St. Louis, MO 63110.

1 The abbreviations used are: TPA, 12-O-tetradecanoylphorbol-13-acetate; II-1, interleukin-1; EGF, epidermal growth factor; SDS, sodium dodecyl sulfate; IBMX, isobutylmethylxanthine; bp, base pair; kbp, kilobase pair; PIPES, 1,4-piperazinediethanesulfonic acid; protein kinase C, Ca2+/phospholipid-dependent enzyme.
A. Dinarello, Boston, MA), cholesta toxin, IBMX, TPA, and dexamethasone (Sigma). Standard doses were 50 ng/ml EGF, II-1 as indicated in the text, 1 µg/ml cholesta toxin, 15 nM IBMX, 50 ng/ml TPA, 2 µM dexamethasone.

Filter Blot Hybridization—Total RNAs were prepared, Northern blotted, and hybridized as described previously (22); oligonucleotide primed restriction fragments were isolated from low-melt agarose gels and labeled by the method of Feinberg and Vogelstein (23). A rabbit genomic DNA library in λ Charon 4A was screened by standard methods (24). Southern blots were performed using the alkaline transfer method (25).

Other Nucleic Acid Methods—DNA sequencing was performed by double stranded plasmid sequencing (26) of exonuclease 111-mung bean nuclease deletion mutants (27).

The stromelysin promoter chloramphenicol acetyltransferase plasmids pSLCATMP10 and pSLCATMP16 (having ~700 bp and ~6.1 kbp of 5′-flanking region, respectively) were constructed by digesting a bluescript plasmid containing a 14.5-kbp EcoRI-EcoRI insert of stromelysin DNA with SacI and either ScaI (MP10) or SmaI (MP16). Digests were blotted, and hybridized as described previously (22); oligonucleotide methasone (Sigma). Standard doses were 50 ng/ml EGF, 11-1 as a bluescript plasmid containing a 14.3-kbp EcoRI-EcoRI insert of stromelysin DNA with SacI and either ScaI (MP10) or SmaI (MP16). Indicated in the text, 1 pg/ml cholera toxin, 15 pSV2CAT in which the SV40 promoter/enhancer has been replaced by a pUC13 polylinker; provided by M. Gilman, M.I.T).

Ribonuclease protection analysis was performed by transcribing an XhoI-cut exonIII deletion mutant in blue scribe (Stratagene) containing ~650 bp of stromelysin sequence, from +115 to +500 (relative to the presumed start site deduced by primer extension) with T7 RNA polymerase under conditions recommended by the supplier (Stratagene). Bluescript plasmid linearized with various restriction enzymes and transcribed with T7 RNA polymerase served as RNA molecular weight markers. T3 transcripts were hybridized with 10 µg of total TPA-induced rabbit synovial cell RNA under the primer annealing conditions above, except at 52 °C, diluted 10-fold, and digested with 40 µg/ml RNase A and 2 µg/ml RNase T1 for 45 min at 30 °C followed by proteinase K treatment and phenol extraction; the protected RNA products were then analyzed on a 6% sequencing gel.

Chloramphenicol acetyltransferase assays were performed by calculating phosphatase transfecting 10 µg of plasmid DNA per 100-mm dish of subconfluent rabbit synovial fibroblasts for 6 h, using the method of Spandidos and Wilkie (29), washing with serum free medium, and incubating for 16 h in serum free medium prior to the addition of stromelysin gene regulators. After 24 h of induction, cells were harvested and protein extracts prepared by lysis in 0.25 M Tris, pH 7.5, 0.5% Triton X-100; extracts were assayed as described by Gorman (30).

Protein concentrations were checked by using the Bio-Rad protein assay reagent. β-Galactosidase assays were performed on extracts from cells that had been co-transfected with RSVβgal as described by Gorman (30) except that SDS 0.2% was added at the end of the reaction period to prevent precipitation of Triton X-100 caused by the addition of sodium carbonate.

RESULTS

Regulatory Behavior of the Endogenous Stromelysin Gene—Total RNA was isolated from rabbit synovial fibroblasts and analyzed on Northern blots using a stromelysin cDNA as probe. II-1 elicited a dose-dependent (Fig. 1a) induction (maximum 63-fold) of stromelysin mRNA levels in rabbit synovial fibroblasts, with a substantial effect observed even at 4 ng/ml (100 pM). By contrast, the inflammatory mediator tumor necrosis factor, whose biologic activities are often similar to those of II-1, had no effect on stromelysin mRNA levels even at 2 µg/ml (data not shown).

The stromelysin gene was induced by several other factors as well. TPA elicited a large (120-fold) induction of stromelysin mRNA levels (Fig. 1a), consistent with our previous study (7) showing that stromelysin mRNA constitutes 1–2% of the total mRNA of TPA-treated rabbit synovial fibroblasts. EGF had a weaker effect, inducing stromelysin mRNA levels by 5-fold (Fig. 1a). These results were in contrast to the rat fibroblast transin system, in which EGF was more effective than TPA (10).
Treatment of rabbit synovial fibroblasts with cholera toxin in the presence of IBMX, which elicits a large accumulation of cAMP (31), induced the stromelysin gene to about the same extent as EGF, implicating cAMP-dependent protein kinase activation as one possible mode of signal transduction for the gene.

Because glucocorticoids have been shown to suppress extra-cellular matrix degradation in cell culture systems (9) and in vivo (17), and because the collagenase gene is one target of glucocorticoid repression (9), we examined the effect of dexamethasone on stromelysin gene expression. We found that dexamethasone diminished stromelysin induction by all inducing agents tested (by 5-fold for TPA, 5-fold for EGF, 7.5-fold for IL-1, 14-fold for cholera toxin; Fig. 1a); in fact, even basal stromelysin expression was repressed. To exclude the possibility that dexamethasone elicited a nonspecific repression of transcription, RNA from control and dexamethasone-treated cells was translated in vitro, and the translation products were analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 1b). Dexamethasone neither had an effect on the amount of total translation product synthesized in vitro nor on the synthesis of the 43-kDa translation product corresponding to actin (compare lanes 1 to 2, 3 to 4, 5 to 6, and 7 to 8). Also, the dexamethasone effect was not due to a general inhibition of protein synthesis, as determined by an [35S]methionine pulse-labeling experiment (data not shown).

Mapping of the Transcription Start Site—Stromelysin genomic clones were isolated by screening a rabbit genomic library using a 1.2-kbp stromelysin cDNA clone (7), and one of the five unique genomic clones was further characterized by hybridization with probes generated from pAct3, a Bluescript plasmid containing an 1822-bp stromelysin cDNA insert (from a λgt11 clone provided by Dr. E. Fini, M.I.T.).

The position of the transcriptional start site of the stromelysin gene within a 1.1-kbp fragment (which hybridized with a probe representing 146 nucleotides of the extreme 5'-end of pAct3) was determined by primer extension analysis using a 433-nucleotide penultimate cDNA fragment as primer, and total RNA from TPA-induced rabbit synovial fibroblasts as template for reverse transcription. The major primer extension product was ~590 nucleotides (Fig. 2a, determined from a shorter exposure), indicating that the start site was approximately 11 nucleotides upstream of the 5'-end of the cDNA. Alignment of the cDNA with the genomic fragment was accomplished by sequencing the latter and comparing it with the cDNA sequence, yielding the approximate start position indicated in Fig. 3. Verification of the approximate start site was provided by ribonuclease protection hybridization, using a T3 polymerase-generated RNA transcript representing +115 to ~500 (with respect to the presumed start site). When hybridized with total RNA from TPA-induced rabbit synovial fibroblasts, and digested with ribonucleases, the major product (Fig. 2b) was about 110 nucleotides, closely agreeing with the primer extension result and with the location of a "TATA box" (in this case TATAAAATT) at 20–20 nucleotides upstream of the indicated start site (Fig. 3).

Construction and Transfection of Stromelysin Promoter-Chloramphenicol Acetyltransferase Fusions—DNA fragments spanning from the transcription start site (Sac I at position −10) to various distances upstream were fused upstream of a chloramphenicol acetyltransferase coding sequence as described under "Materials and Methods." When constructs containing either a 700-bp fragment (Fig. 4a) or a 6.1-kbp fragment (data not shown) were transfected into rabbit synovial fibroblasts, the basal expression level was very low, but could be induced by about 20-fold by treatment with TPA, suggesting that a phorbol ester-inducible sequence element resides within the 700-bp fragment. (As a control, TPA was found to induce RSVCAT by only ~2-fold; data not shown.) IL-1 induction of chloramphenicol acetyltransferase activity was also conferred by the 700-bp SL fragment, although magnitude of the induction (8-fold) was smaller than that observed in Northern blot analysis. EGF and cholera toxin activations of the indicated start site (Fig. 3).

---

2 E. Fini, unpublished data.
Transcription Induced by II-1 and Repressed by Dexamethasone

FIG. 4. a, induction of chloramphenicol acetyltransferase activity in rabbit synovial cells transfected with pSLCATMP10. Symbols are as in Fig. 1, b, effect of dexamethasone on chloramphenicol acetyltransferase activity in rabbit synovial cells transfected with pSLCATMP10 or control plasmids. Symbols: SV = pSV2CAT (SV40 enhancer); RSV = RSVCAT (Rous sarcoma virus long terminal repeat enhancer); other symbols are pSLCATMP10 transfecteds treated as in Fig. 1.

comavirus long terminal repeat in RSV-CAT or driven by the SV40 enhancer in pSV2CAT were diminished only slightly by dexamethasone treatment (Fig. 4b).) Total β-galactosidase activity present in the same protein extracts used for the chloramphenicol acetyltransferase assays (endogenous β-galactosidase plus β-galactosidase from the plasmid RSVβgal which was cotransfected with the stromelysin plasmids) was unaffected as well as any of these treatments (data not shown).

DISCUSSION

Induction of stromelysin gene expression by II-1 demonstrated above is consistent with the role of II-1 as an inflammatory mediator. For example, II-1 induces the synthesis of collagenase and prostaglandins in human synovial cells (31). Because of the extreme substrate specificity of collagenase for a single cleavage site in collagen, collagenase synthesis is probably necessary but insufficient for the massive cartilage degradation observed in rheumatoid arthritic synovia (where II-1 is found in elevated concentration compared to normal synovia (32)) or for the cartilage degradation resulting from experimental II-1 treatment of synovia (14). In at least three systems, rabbit chondrocytes (34, 35), endothelial cells (36), and synovia (14), secretion of a proteoglycan-degrading (or in vitro caseinolytic) activity results from II-1 treatment. (One problem in the interpretation of these reports has been that assays of enzyme activity, in vivo or in vitro, can be heavily influenced by the levels of metalloproteinase inhibitors, as in Ref. 37, for example, where large amounts of endothelial cell collagenase are masked by tissue inhibitor of metalloproteinases.) Our present observation that the stromelysin gene is induced by II-1 identifies the stromelysin gene product, at least in the synovial cell system, as an important component of the tissue-degradative process. We have also demonstrated that II-1 inducibility can be conferred upon a heterologous promoter as a result of the tissue-degradative process. We have also demonstrated that II-1 inducibility can be conferred upon a heterologous promoter as a result of the tissue-degradative process. We have also demonstrated that II-1 inducibility can be conferred upon a heterologous promoter as a result of the tissue-degradative process. We have also demonstrated that II-1 inducibility can be conferred upon a heterologous promoter as a result of the tissue-degradative process. We have also demonstrated that II-1 inducibility can be conferred upon a heterologous promoter as a result of the tissue-degradative process. We have also demonstrated that II-1 inducibility can be conferred upon a heterologous promoter as a result of the tissue-degradative process. We have also demonstrated that II-1 inducibility can be conferred upon a heterologous promoter as a result of the tissue-degradative process. We have also demonstrated that II-1 inducibility can be conferred upon a heterologous promoter as a result of the tissue-degradative process. We have also demonstrated that II-1 inducibility can be conferred upon a heterologous promoter as a result of the tissue-degradative process.
copy number of the transfected gene. For example, in Ref. 46, the TPA induction of SV40 enhancer is observed only at low DNA input per plate. Alternatively, additional II-1 inducibility elements may reside in regions other than the 700-bp fragment.

Partial protection by glucocorticoids against inflammation-induced tissue degradation (17) may be partly attributed to repression of collagenase synthesis, as has been shown for synovial fibroblasts (18) and macrophages (19). The repression of stromelysin synthesis presently reported implicates an additional mechanism of protection.

Both an increase in matrix-degrading protease activity (6) and a decrease in fibronectin accumulation (38) have been observed in a wide variety of tumor cells. Treatment of several transfected cell lines having both of these properties leads to restoration of nearly normal fibronectin levels (e.g., Ref. 21). If the level of fibronectin in transformants is abnormally low because of increased degradation by proteases such as stromelysin, then the glucocorticoid repression of stromelysin synthesis may partly explain restoration of normal fibronectin levels.

Our observation that dexamethasone repressed stromelysin expression under the influence of a variety of inducers (TPA, EGF, IL-1, cholera toxin) suggests two possible mechanisms: (i) the blockage is in a late step of signal transduction (which would hypothetically be a common signal generated by all of the above inducers, since the early responses to these inducers are diverse); or (ii) the glucocorticoid receptor-dexamethasone complex binds to the stromelysin 5′-flanking region so as to repress transcription. Thus far, we have insufficient evidence to distinguish between these possibilities. Although there is no precedent in the literature, to our knowledge, for direct inhibitory effect of glucocorticoid receptor on transcription, there is no precedent in the literature, to our knowledge, for direct inhibitory effect of glucocorticoid receptor on transcription, which could be involved in stable activation of stromelysin gene expression.

Acknowledgments—We thank Dr. F. E. Fini (M.I.T.) for exchanging her longer cDNA clone for our shorter one and Dr. David Perlmuter for helpful discussions.

REFERENCES

1. Gross, J. L., Moscatelli, D. M., and Rifkin, D. B. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2623-2627
2. Murphy, G., and Reynolds, J. J. (1985) Bioessays 5, 55-60
3. Ermolin, S., and Kayalar, B. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9203-9207
4. Delollz, R., and Spierer, P. (1986) Nature 323, 688-692
5. Ermolin, S., and Kayalar, B. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9203-9207
6. Gross, J. L., Moscatelli, D. M., and Rifkin, D. B. (1983) J. Invest. Dermatol. 70, 835-846
7. Liotta, L., Rio, C. N., and Barsky, S. H. (1983) Lab. Invest. 49, 636-847
8. Frisch, S. M., Clark, E. J., and Werb, Z. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 2900-2904
9. Matrisian, L. M., Glueckshaus, N., Gesnel, M.-C., and Breathnach, R. (1986) EMBO J. 5, 1343-1351
10. Breathnach, R., Matrisian, L., Gesnel, M.-C., Staub, A., and Leroy, P. (1987) Nucleic Acids Res. 15, 1139-1151
11. Matrisian, L., Lero, P., Rubenke, G., Gesnel, M.-C., and Breathnach, R. (1986) Mol. Cell. Biol. 6, 1679-1686
12. Frisch, S. M., Herron, G. S., and Werb, Z. (1986) Fed. Proc. 44, 91
13. Angell, J., Frisch, S. M., and Werb, Z. (1984) J. Cell Biol. 96, 1652-1671
14. Lomard, J. T., Kimmel, P. L., Gubler, U., Stern, A. S., and Chizhovic, R. (1984) Cold Spring Harbor Symp. Quant. Biol. 51, 631-639
15. Peterson, E. R., Higgs, G. A., and Aaronson, B. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9749-9753
16. Dayer, J. M., Banz, J., Che, L., and Kame, S. M. (1979) J. Clin. Invest. 64, 1386-1392
17. Polisithwate, A., E. A. (1983) J. Exp. Med. 157, 801-808
18. Coleman, R., R. S., Sterling, K. M., and Schull, S. (1986) in Regulation of Matrix Accumulation (Mecham, R., ed.) pp. 119-176, Academic Press, Orlando
19. McGuire, M. B., Murphy, G., Reynolds, J. J., and Russell, G. R. (1981) Clin. Sci. 61, 703-710
20. Werb, Z., Foeley, R., and Munck, A. (1978) J. Immunol. 121, 115-121
21. Chin, J. R., Murphy, G., and Werb, Z. (1986) J. Biol. Chem. 260, 12367-12376
22. Armaia, M. C. S., and Armaia, H. A. (1983) J. Cell Biol. 97, 459-465
23. Frisch, S. M., Davidson, J. M., and Werb, Z. (1986) Mol. Cell. Biol. 5, 253-260
24. Feinberg, A. V., and Vogelstein, B. (1984) Anal. Biochem. 136, 266-272
25. Maniatis, T., Hardington, R. C., Lacy, E., Lazer, J., O'Connell, C., Quin, D., Sim, G. K., and Efstratiadis, A. (1975) Cell 15, 687-701
26. Reed, K. C., and Mains, D. A. (1986) Nucleic Acids Res. 13, 7207-7229
27. Chen, E. H., and Seelburg, P. H. (1986) DNA (NY) 4, 165-170
28. Laronde, R., and Will, K. M. (1984) in Transcription and Translation, a Practical Approach (Hames, B. D., and Higgins, S. J., eds.) pp. 49-70, IRL Press, Oxford
29. Spandidos, D., and Wilkie, N. M. (1984) in Transcription and Translation, a Practical Approach (Hames, B. D., and Higgins, S. J., eds.) pp. 5-48, IRL Press, Oxford
30. Gorman, C. (1986) in DNA Cloning II (Glover, D. M., ed.) pp. 143-165, IRL Press, Oxford
31. Winn, W., A., Lee, B. B., Olahaw, N., Ears, H. S., and Pledger, W. J. (1985) J. Cell Physiol. 111, 201-206
32. Dayer, J. M., deRochaMonteaux, B., Bunnus, B., Demouch, S., and Dinarello, C. A. (1986) J. Clin. Invest. 78, 1107-1131
33. Dawai, A. A., March, L. D., Nelson, D. S., and Brooks, C. M. (1987) J. Rheumatol. 14, 65-59
34. Schenly, J., Payne, T., and Dinarello, C. A., (1987) J. Immunol. 138, 499-503
35. Murphy, G., Hembry, R. M., and Reynolds, J. J. (1986) Colliders Relat. Phys. 6, 351-364
36. Harvey, W., Sandy, J., and Muroo, P. (1986) Br. J. Rheumatol. 25, 107-113
37. Herron, G. S., Banda, M. J., Clark, E. J., Gavrilov, J., and Werb, Z. (1986) J. Biol. Chem. 261, 2514-2520
38. Yamasaki, A., and Oder, K. (1987) Nature 225, 179-184
39. Yamamoto, K. R. (1985) Annu. Rev. Genet. 19, 200-252
40. Angel, P., Imagnawa, M., Chua, G., Stein, B., Imba, R., Rahnsdorf, H., Jonet, C., Hebib, L., and Karin, M. (1987) Cell 49, 729-739
41. Comb, M., Birleh, N. C., Seasholtz, A., Herbert, E., and Goodman, H. (1986) Nature 322, 648-654
42. Downward, J., Waterfield, M. D., and Parker, P. J. (1985) J. Biol. Chem. 260, 14616-14624
43. Yoshimura, T., Stedel, D., Bovier, M., Letkowitz, R. J., and Caron, M. G. (1987) Nature 327, 67-70
44. Abraham, R. T., Ho, S., Barna, T. J., and Mckean, D. E. (1987) J. Biol. Chem. 262, 2719-2728
45. Langdon, R., Burchall, N., Kupper, T., and McGuire, J. (1987) J. Invest. Dermatol. 88, 529-532
46. Imba, R. J., and Karin, M. (1986) Nature 323, 555-558