Cloning of Murine Tissue Factor and Regulation of Gene Expression by Transforming Growth Factor Type β1*

(Received for publication, August 20, 1990)

Gouri Ranganathan†, Stanley P. Blatt‡, Malayannan Subramaniam, David N. Fass§, Nita J. Maihle, and Michael J. Getz||

From the Department of Biochemistry and Molecular Biology and the §Section of Hematology Research, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

We have cloned a serum- and cycloheximide-inducible mRNA from AKR-2B murine fibroblasts which encodes a protein with significant sequence similarity to human tissue factor, a cellular initiator of the blood coagulation cascade. Information derived from this clone was used to establish the presence of a virtually identical sequence in mouse brain. Most importantly, cDNA-directed expression in a quail fibroblast cell line produced high levels of tissue factor procoagulant activity, confirming the identity of this protein as murine tissue factor. Additional studies demonstrate that transforming growth factor type β1 stimulates tissue factor gene transcription and is a potent inducer of tissue factor procoagulant activity in fibroblasts. Other tested mitogens such as platelet-derived growth factor, epidermal growth factor, and insulin were weak inducers. These results may reflect a role for transforming growth factor β1 in the maintenance of hemostasis or, alternatively, a role for tissue factor in cellular functions unrelated to blood coagulation.

Tissue factor (TF) is a transmembrane glycoprotein which serves as a cell surface receptor for plasma coagulation factor VII/VIIa, a serine protease. Together they form an active bimolecular complex responsible for initiating the protease cascade leading to blood coagulation (1). TF is known to be expressed in a variety of tissues including brain, lung, and placenta (2, 3) and can be induced in certain cell types by a number of different mitogenic and/or inflammatory stimuli (4-9). While the physiological significance of inducible TF activity is not known, the inappropriate expression of such activity may play a role in a variety of thrombogenic diseases, collectively known as disseminated intravascular coagulation, and in atherosclerosis (reviewed in Ref. 10). Thus, an understanding of the mechanisms which regulate TF expression may shed light on the role of TF in both normal cell physiology and in disease. Recently, we reported the isolation of several cDNA clones which corresponded to mRNAs specifically induced in AKR-2B mouse fibroblasts stimulated with EGF in the presence of cycloheximide, an inhibitor of protein synthesis (11). We now report that one of these, previously designated c70, encodes murine TF and demonstrate that TF gene expression is specifically regulated by TGF-β1, a pleiotropic growth factor known to mediate a variety of functions ranging from normal embryonic development (12) to wound healing and tissue repair (13).

MATERIALS AND METHODS

Cell Culture—AKR-2B mouse embryo-derived fibroblasts were maintained as described previously (14). For experimental purposes, cells were plated in 100-mm dishes and grown to near confluence in McCoy's 5a medium supplemented with 5% fetal calf serum (FCS). Cells were made quiescent by serum deprivation for 48 h in serum-free medium MCDB 402, then stimulated with fresh medium to which growth factor, 20% FCS, or 20% FCS + cycloheximide was added as specified in each experiment. QT6 quail fibroblast cells (15) were maintained in Dulbecco's modified Eagle's medium supplemented with 4.5 g of glucose/liter, 4% fetal bovine serum, and 1% chick serum.

Construction of cDNA Library—The construction and replica screening of a λgt11 library leading to the original c70 isolate was described previously (11). Since this library yielded no c70 clones longer than 622 base pairs, AKR-2B cells, cultured as above, were stimulated with 20% FCS + 10 μg/ml cycloheximide in MCDB 402 for 3 h. Total cellular RNA was extracted using the guanidinium isothiocyanate method as described by Maniatis et al. (16). Poly(A)*RNA was separated using oligo(dT)-cellulose chromatography as described (17) and was shipped to Clontech Laboratories, Inc. for the construction of a new λgt11 cDNA library.

Sequenceing of AKR-2B-βc70 Clone—The partial c70 cDNA clone, described previously (11), was subcloned and sequenced using a dyeoxy double-stranded DNA sequencing system (Promega Biotech). Oligonucleotide primers corresponding to determined regions of specific c70 and λgt11 vector sequences were used to amplify (polymerase chain reaction) longer c70-specific clones from the newly constructed cDNA library (described above) using the method of Friedman et al. (18). The oligonucleotide primers used contained linked T7 and SP6 phage promoter sequences. The polymersase chain reaction-amplified DNA was transcribed and sequenced as described by Sarkar and Sommer (19).

Detection and Sequencing of a c70-related mRNA in Mouse Brain—Total RNA was isolated from C3H/HeJ congenic mouse brain tissue by the guanidinium isothiocyanate method (16). RNA amplification with transcript sequencing (RAWTS) was performed as described by Sarkar and Sommer (19, 20). Briefly, oligo(dT)-primed single-stranded DNA synthesis was performed using reverse transcriptase. The specific oligonucleotide primers used corresponded to regions 5' and 3' of the previously determined, presumptive c70 protein-coding sequence. Primer A (5' TGAAGCCCCCGAGACC 3') located on the sense strand 34 bases upstream of the predicted ATG translation initiation codon was synthesized with a linked T7 phage promoter. Primer B (5' GAAGGCAGCCTCTCT 3') was located on the anti-sense strand 121 bases downstream of the TAG-stop codon.
Northern Blot Analysis—Northern blot analysis of total RNA isolated from AKR-2B cells stimulated with FCS, with or without cycloheximide or actinomycin D as specified, was performed by the electrophoresis of RNA in 1% agarose gels containing formaldehyde and formamide, followed by hybridization to a nick-translated c70 probe as previously described (14). 

Nuclear Run-off Transcription Assay—Nuclei were isolated from AKR-2B cells stimulated with TGF-β1 (10 ng/ml) or FCS (20%) in MCDB 402 for the specified time period, and in vitro transcription assays were performed exactly as previously described (21).

Construction of c70 and Human Tissue Factor Expression Vectors—The complete predicted coding sequence of c70 along with some 5' and 3' untranslated sequence was amplified using the c70 primers described above. For cloning purposes, the primers were synthesized with linked XhoI (5') and BamHI (3') recognition sequences and a short clamp sequence. The approximately 1-kilobase pair DNA gel band that was amplified from the cDNA library was gel-purified, cloned into pGem4, and sequenced. The c70 insert was then excised using appropriate restriction enzymes and was subcloned into the BamHI site of pSSB, a eukaryotic expression vector previously described by Mahle et al. (22). The XhoI site of the insert was converted into a blunt end using Klenow fragment, and a BamHI linker was attached. Clones containing the c70 insert in the correct transcriptional orientation were selected by restriction enzyme mapping.

The human tissue factor clone (HTF8) described by Scarpati et al. (23) was used as the source of an 1100-base pair insert containing the complete coding sequence of human tissue factor. The insert was excised using ScaI, the ends were filled using Klenow fragment, and the insert was cloned into the pSSB vector at the BamHI site which was also repaired using Klenow fragment. All pSSB constructs were propagated in the Escherichia coli strain DH5.

DNA Transfection and Preparation of Cell Extracts—QT6 cells were seeded on 100-mm plates and grown to 50% confluency (24 to 48 h). Cells were washed twice with serum-free medium 24 h after transfection and incubated for 10 min at 37°C in 5% glycerol in phosphate-buffered saline (PBS). This buffer was aspirated off and the cells were harvested in 3 ml of sterile water. The cell lysates were stored frozen (−20°C) until they were assayed for tissue factor activity.

Measurement of Tissue Factor Activity—Tissue factor activity was measured in rat plasma or in factor VII-deficient human plasma to suppress alternative factor X activation. Typically, 50 μl of cell extract was mixed with 100 μl of human factor VII-deficient plasma and 100 μl of 15% inosithin, a soybean phospholipid preparation in imidazole-buffered saline (25, 26). Clotting time was measured at 37°C on an uncoated clotting plate using a 1:1 mixture of serum-stimulated AKR-2B cells typically yielding clot times between 37 and 45 s. All measurements were corrected for total protein in each sample, and activities were quantitated by reference to standard curves generated with commercial rabbit brain thromboplastin. Samples with maximum activity were assayed using factor VII-deficient human plasma as substrate to ensure that the activity being measured was characteristic of tissue factor.

RESULTS

Nucleotide and Predicted Protein Sequence of c70 and a Homologous Sequence Expressed in Mouse Brain—A combination of conventional and polymerase chain reaction-amplified cDNA sequencing techniques was used to establish the c70 nucleotide sequence shown in Fig. 1. A search of the GenBank nucleic acid sequence data base revealed that the illustrated sequence was 68.4% identical with a portion of human TF mRNA (nucleotides 43–1329) as recently reported by several groups (23, 27–29). Translation of the c70 sequence indicated the presence of a single open reading frame encoding the illustrated 291-amino acid protein.

Because of the unexpected similarity between the c70 sequence and that of human TF, it was important to determine whether c70 was expressed in a normal adult mouse tissue known to elaborate TF activity. To achieve single nucleotide resolution, we employed the recently developed RAWTS technique (18, 20) to obtain sequence information directly from total RNA isolated from mouse brain. Two independent amplification and sequencing reactions yielded an identical sequence which differed from that of c70 mRNA in three positions as noted in Fig. 1. Two of these, an A at position 311 and a T(U) at position 454 are third position codon changes which do not alter the amino acid sequence of the encoded protein. The third change, a C at position 111 results in the replacement of an isoleucine with a threonine at amino acid residue 26. However, this change occurs within a predicted signal peptide sequence (see Fig. 2) and, therefore, may not be retained in the mature protein.

The c70 Protein Is Homologous to Human Tissue Factor—Fig. 2 shows a comparison between the predicted amino acid sequence of c70 and that of human TF (27). Although localized regions of high sequence homology are evident, for example c70 residues 35–94, the overall percentage of amino acids which are identical (54.4%) or functionally similar (68.8%) is somewhat lower than expected for homologous human and murine proteins. Nevertheless, there are several shared features which suggest functional relatedness.

The amino-terminal 27 residues of c70 and the first 30 of human TF are largely hydrophobic and followed by a predicted (30) signal peptide cleavage site (between A-29 and G-30 for c70 and between A-32 and S-33 for human TF). At the COOH terminus, both sequences exhibit a stretch of 23 consecutive hydrophobic amino acids followed by a short stretch...
of charged, basic amino acids. These features are characteristic of membrane spanning proteins (31) and contribute to procoagulant activity when introduced into heterologous cells.

The c70 Clone Directs the Synthesis of Biologically Active Tissue Factor in Quail Fibroblasts—To test directly the possibility that the c70 protein represents murine TF, we examined the ability of c70 cDNA to direct the synthesis of TF procoagulant activity when introduced into heterologous cells. A portion of the c70 sequence corresponding to nucleotide positions 1–1040 was amplified by polymerase chain reaction and subcloned into the BamHI site of pSSB, a eukaryotic expression vector containing the 5’ LTR of an integrated avian leukosis virus provirus and a portion of a c-erbB cDNA transcribed in the presence of cycloheximide, we analyzed the effect of TF specific activity relative to extracts from untransfected control cells or from cells transfected with the parental pSSB vector lacking an insert. When tested against human plasma, extracts from pSSB-c70-transfected cells were about one-third as active (relative to activity in rat plasma) while extracts from cells transfected with the human TF cDNA retained most, if not all, of their activity. Except for this small difference in specific activity against human plasma, the procoagulant activity of the c70 protein was indistinguishable from that of human TF. Although we cannot formally exclude the possibility that additional TF proteins are expressed in mouse cells, these experiments provide strong evidence that the c70 protein represents at least one form of murine tissue factor.

Induction and Superinduction of c70 mRNA by Serum and Cycloheximide—Previously, we employed nuclear run-off transcription assays to demonstrate serum-inducibility of c70 transcription (11). Since the cDNA library from which c70 was originally selected was derived from cells growth-stimulated in the presence of cycloheximide, we analyzed the effect of cycloheximide on c70 expression.

Northern blot hybridization using a c70 probe (Fig. 3) demonstrated the induction of an approximately 2.1-kilobase transcript upon serum stimulation of quiescent AKR-ZB fibroblasts. This transcript reached maximum levels within about 1 h, then declined with an apparent half-life of 60 min or less. The inclusion of 10 μg/ml cycloheximide during serum stimulation blocked this decline and resulted in a strong superinduction of c70 mRNA by 6 h. When cells were serum-stimulated for 1 h, then additionally treated for 5 h with a
combination of cycloheximide and actinomycin D, the superinduction effect was clearly reduced but not eliminated. Since we have previously shown that the transcriptional response of the c70 gene to serum stimulation is transient, reaching maximal levels at 30 min then rapidly declining (11), the addition of actinomycin D at 1 h after serum stimulation would be expected to have little effect on superinduction of c70 mRNA unless cycloheximide induced a prolonged transcriptional response. To test this possibility, the nuclear run-off transcription experiments shown in Fig. 4 were performed. As previously observed, c70 transcription was activated by 30 min following serum stimulation, then declined to near basal levels by 4 h. However, when cycloheximide was included, the transcriptional response was extended through at least 4 h. Moreover, treatment with cycloheximide alone was found to induce c70 transcription in the absence of serum components. Similar results were obtained for two other serum-inducible genes (β-actin and c-fos) while the transcription of a non-serum-inducible gene (α-tubulin) was unaffected by cycloheximide treatment. Taken together, the data in Figs. 3 and 4 indicate that cycloheximide-mediated superinduction of c70 mRNA is the result of an extended transcriptional response to serum coupled with an increase in mRNA stability.

**Regulation of Tissue Factor Procoagulant Activity by Purified Growth Factors**—Initial studies (not shown) revealed that serum stimulation of AKR-2B cells resulted in a strong induction of TF procoagulant activity in cell extracts by 6 to 10 h, followed by a decline to near basal levels by 30 h. To determine whether the induction of TF activity was an invariant response to the stimulation of cell proliferation or a selective response to one or more individual growth factors, AKR-2B cells were grown to near confluency in medium containing 5% fetal calf serum; these cells were then rendered quiescent by switching to serum-free medium (MCDB 402) for 48 h. Cells were stimulated for 6 h by the addition of fresh medium containing either 20% fetal calf serum or several purified growth factors which have been previously shown to be mitogenic for AKR-2B cells (32, 33). Fig. 5 illustrates that PDGF, EGF, and insulin were weak inducers of TF procoagulant activity when compared to serum. In contrast, TGF-β1 induced 75 to 80% of the activity found in serum-stimulated cells while the combination of TGF-β1 and EGF induced somewhat higher levels. EGF also potentiated the ability of PDGF to induce TF activity but had only a small effect on induction by insulin.

Fig. 6 illustrates a more detailed comparison of the ability of serum and TGF-β1 to induce TF activity in AKR-2B cells.

**Fig. 5.** Induction of tissue factor procoagulant activity by serum and purified peptide growth factors. Quiescent AKR-2B cells in serum-free medium were stimulated for 6 h with fresh medium containing 20% FCS or the indicated purified growth factors at the following concentrations. TGF-β1, 10 ng/ml; PDGF, 2.5 ng/ml; EGF, 10 ng/ml; insulin, 500 ng/ml. Cell extracts were prepared and assayed for TF activity as described under "Materials and Methods."

**Fig. 6.** Kinetics of induction of tissue factor procoagulant activity following treatment with serum or TGF-β1. Quiescent AKR-2B cells in serum-free medium were stimulated for the indicated times with fresh medium containing either 20% FCS (○) or 10 ng/ml TGF-β1 (●). The bars represent the range of two independent determinations.

**Fig. 7.** Stimulation of c70 gene transcription by TGF-β1. Quiescent AKR-2B cells in serum-free medium were stimulated for the indicated times with fresh medium containing 10 ng/ml TGF-β1. Nuclear run-off transcription assays were performed as described. The probes used were the same as indicated in Fig. 4 except that a γ-actin clone and an additional fibronectin clone representing the 5′ end of the mRNA were included.

Although serum was a more effective inducer at early times following stimulation, TGF-β1 induced high levels of TF activity by 8 to 10 h poststimulation.

**Stimulation of c70 Gene Transcription by TGF-β1**—To de-
termine whether the induction of TF procoagulant activity in TGF-β1 stimulated cells was preceded by an increase in c70 gene transcription, nuclear run-off transcription assays were again performed using nuclei isolated from cells stimulated for various times with purified TGF-β1. The results are shown in Fig. 7. TGF-β1 stimulated an increase in c70 transcription which was detectable within 20 min and which continued for at least 3 h. In contrast to the pattern in serum-stimulated cells (11), no attenuation of c70 transcription was apparent during the course of the experiment. Although the hybridization signal to c70 DNA was weak relative to the other TGF-β1-inducible genes included for comparison (fibronectin, β- and γ-actin, c-fos), no hybridization was observed to the pBR322 or pGem4 plasmid cloning vectors included as controls. We conclude that c70 gene transcription is specifically induced following stimulation with TGF-β1 but at a lower rate than several other TGF-β1-responsive genes. Similar relative rates of transcription are also observed in serum-stimulated cells (Fig. 4 and Ref. 11).

DISCUSSION

The initial experiments which lead to the isolation of the cDNA clone termed c70 were designed to identify genes involved in regulating cell growth and division. Thus, the sequence similarities between c70 and human TF, the cellular initiator of the protease cascade leading to blood coagulation, were surprising. Nevertheless, the demonstration that the c70 clone directs the synthesis of biologically active TF when introduced into quail fibroblasts provides convincing evidence that the c70 protein represents murine TF and not the product of related gene. This conclusion is reinforced by the demonstration that the c70 protein is not unique to AKR-2B cells but is also expressed in mouse brain, a tissue known to elaborate high levels of TF procoagulant activity. Interestingly, the brain mRNA sequence, which differs from that of c70 in three positions, is identical with the nucleotide sequence of a growth factor-responsive mRNA recently identified by Hartzell et al. (34) in BALB/c 3T3 cells. Although the protein encoded by this mRNA was not directly determined to possess TF procoagulant activity, other experiments indicated that the protein was extensively glycosylated and present on the surface of BALB/c 3T3 cells, as would be expected of TF. Taken together, these data suggest that the c70 sequence and the brain/3T3 sequence may represent allelic variants of the mouse TF gene which encode identical mature proteins.

To our knowledge, the induction of TF gene expression and procoagulant activity is a novel and previously unrecognized response of fibroblast-type cells to TGF-β1. Although the activation of TF gene transcription is also a primary response to serum stimulation, the lack of a close correspondence between the mitogenicity of a given growth factor and its ability to induce TF (Fig. 5) suggests that TF induction is not obligatory to cell division per se. A more likely possibility is that TF induction is a specific response to TGF-β1 which is not directly coupled to mitogenesis. This could reflect a role for TGF-β1 in the regulation of hemostasis, or alternatively, a role for TF which is not directly related to blood coagulation. In this regard, it is interesting to note that TF appears to be the only protein in the coagulation pathway for which a congenital deficiency has not been reported (35). If indeed none exists, then TF may have additional properties which are essential to normal embryonic development. Since these would seem unlikely to include the binding of coagulation factor VII, TF may be a multifunctional receptor with specificity for more than one ligand. The nature of such ligands is strictly speculative but could include serine proteases other than factor VII, perhaps including proteases involved in mediating cell adhesion and/or cell migration (reviewed in Ref. 36). This would be consistent with the several known biological functions of TGF-β1 such as tissue morphogenesis (12), wound healing (13), cell migration (37), and modulation of proteolytic enzyme activity (38). The derivation of cell lines in which regulated expression of TF is perturbed should allow elucidation of possible alternative functions.

Acknowledgments—The technical assistance of Michelle Sanders in the transfection studies, Chris French in tissue culture, and Marylou Stewart in the coagulation assays is gratefully acknowledged. We are indebted to Dr. J. Evan Sadler for a gift of the human tissue factor cDNA clone HT8.

REFERENCES

1. Furie, B., and Furie, B. C. (1986) Cell 53, 506-518
2. Williams, W. J. (1964) J. Biol. Chem. 239, 933-942
3. Williams, W. J. (1965) J. Biol. Chem. 241, 1840-1846
4. Nawroth, P. P., Stern, D. M., Kusiel, W., and Bach, R. (1985) Thromb. Res. 40, 677-691
5. Nawroth, P. P., Handley, D. A., Esmon, C. T., and Stern, D. M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3640-3644
6. Nawroth, P. P., and Stern, D. M. (1986) J. Exp. Med. 163, 740-745
7. Maynard, J. R., Dreyer, B. E., Stermerman, M. B., and Pitlick, F. A. (1977) Blood 50, 387-396
8. Maynard, J. R., Fintet, D. A., Pitlick, F. A., and Nemerson, Y. (1978) Lab. Invest. 35, 542-549
9. Gregory, S. A., Morrissey, J. H., and Edgington, T. S. (1989) Mol. Cell. Biol. 9, 2752-2758
10. Carson, S. D. (1984) Proc. Clin. Pathol. 9, 1-14
11. Blatti, S. P., Foster, D. N., Ranganathan, G., Moses, H. L., and Getz, M. J. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1119-1123
12. Heine, U. I., Munoz, E. F., Flanders, K. C., Ellsworth, L. R., Lam, H.-Y. P., Thompson, N. L., Roberts, A. B., and Sporn, M. B. (1987) J. Cell Biol. 105, 2861-2876
13. Montesano, R., and Orci, L. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4894-4897
14. Hodgen, C. P., Elder, P. K., Ono, T., Foster, D., and Getz, M. J. (1983) Mol. Cell. Biol. 3, 2221-2231
15. Moscovici, C., Moscovici, M. G., Jimenez, H., Lai, M. M., Hayman, M. J., and Vogt, P. K. (1977) Cell 11, 95-103
16. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Getz, M. J., Elder, P. K., Benz, E. W., Jr., Stephens, R. E., and Moses, H. L. (1976) Cell 7, 255-265
18. Friedman, K. D., Rosen, N. L., Newman, P. J., and Montgomery, R. K. (1986) Nucleic Acids Res. 14, 8713
19. Sarkar, G., and Sommer, S. S. (1988) Nucleic Acids Res. 16, 5197
20. Sarkar, G., and Sommer, S. S. (1989) Science 244, 331-334
21. Ranganathan, G., and Getz, M. J. (1990) J. Biol. Chem. 265, 3001-3004
22. Mayhle, N. J., Raines, M. A., Flickinger, T. W., and Kung, H.-J. (1988) Mol. Cell. Biol. 8, 4869-4876
23. Scarpati, E. M., Wen, D., Broze, G. J., Miletech, J. P., Flander- meyer, R. R., Siegel, N. R., and Sadler, J. E. (1987) Biochemistry 26, 5234-5238
24. Graham, F. L., and Van der Eb, A. J. (1973) Virology 52, 456-467
25. Bowie, G. J. W., Thompson, J. H., Jr, Didisheim, P., and Owen, C. A., Jr. (1971) in Mayo Clinic Laboratory Manual of Hemostasis (Bowie, E. J., ed) pp. 105-111, W. B. Saunders Co., Philadelphia
26. Bowie, G. J. W., Thompson, J. H., Jr, Didisheim, P., and Owen, C. A., Jr. (1971) in Mayo Clinic Laboratory Manual of Hemostasis (Bowie, E. J., ed) p. 165, W. B. Saunders Co., Philadelphia
27. Morrissey, J. H., Fakhrai, H., and Edgington, T. S. (1987) Cell 50, 129-135
28. Spicer, E. K., Horton, R., Bloem, L., Bach, R., Williams, K. R., Guha, A., Kraus, J., Lin, T.-C., Nemerson, Y., and Konigsberg, W. H. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 5148-5152
29. Fisher, K. L., Gorman, C. M., Vehar, G. A., O'Brien, D. P., and

Murine Tissue Factor
Murine Tissue Factor

Lawn, R. M. (1987) *Thromb. Res.* **48**, 89–99
30. von Heijne, G. (1986) *Nucleic Acids Res.* **14**, 4683–4690
31. Hopp, T. P., and Woods, K. R. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78**, 3824–3828
32. Shipley, G. D., Tucker, R. F., and Moses, H. L. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82**, 4147–4151
33. Shipley, G. D., Childs, C. B., Volkenant, M. E., and Moses, H. L. (1984) *Cancer Res.* **44**, 710–716
34. Hartzell, S., Ryder, K., Lanahan, A., Lau, L., and Nathans, D. (1989) *Mol. Cell. Biol.* **9**, 2567–2573
35. Carson, S. D., Henry, W. M., and Shows, T. B. (1985) *Science* **229**, 991–993
36. Matusian, L. M., and Hogan, B. L. M. (1990) in *Growth Factors and Development* (Nilsen-Hamilton, M., ed) pp. 219–250, Academic Press, Orlando, FL
37. Postlethwaite, A. E., Keski-Oja, J., Moses, H. L., and Kang, A. H. (1987) *J. Exp. Med.* **165**, 251–256
38. Laiho, M., Saksela, O., Andreasen, P. A., and Keski-Oja, J. (1986) *J. Cell Biol.* **103**, 2403–2410