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Induction of fibronectin gene transcription and mRNA is a primary response to growth-factor stimulation of AKR-2B cells

(extracellular matrix/cytoskeleton/differential gene expression)

STANLEY P. BLATTI*, DOUGLAS N. FOSTER†, GOURI RANGANATHAN*, HAROLD L. MOSES‡, AND MICHAEL J. GETZ*§

*Department of Biochemistry and Molecular Biology, Mayo Clinic, Mayo Foundation, Rochester, MN 55905; †Department of Poultry Science, The Ohio State University/Ohio State Agricultural Research and Development Center, Wooster, OH 44691; and ‡Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

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ABSTRACT **A cDNA library, prepared from poly(A)$^+$ RNA isolated from quiescent AKR-2B cells 4 hr after stimulation with epidermal growth factor in the presence of cycloheximide, was screened to identify RNA transcripts whose abundance is specifically increased as a primary response to growth stimulation. Approximately 40% of the inducible clones detected by this procedure corresponded to either cytoskeletal β- or γ-actin genes. One nonactin clone, designated c99, was found to be derived from an 8.5-kilobase RNA whose abundance began to increase as early as 30 min after stimulation. DNA sequencing established the identity of this RNA as fibronectin. Several additional mitogens were then tested and found to efficiently induce fibronectin mRNA. These included fetal calf serum, platelet-derived growth factor, and transforming growth factor type β. For at least one inducer, fetal calf serum, the increase in mRNA was preceded by an increase in fibronectin gene transcription. This increase was rapid, reaching maximal levels within 10 min, and was accompanied by near-coordinate increases in both c-*fos* and β-actin transcription. These results indicate that fibronectin is a member of a class of "early-response" genes, typified by c-*fos* and including β-actin, whose rapid expression may be important in mediating cellular responses to peptide growth factors.**

Peptide growth factors govern a wide range of cellular activity including embryonic development and differentiation, maintenance of cell-type homeostasis, and wound healing. Genetic and biochemical evidence indicates that cellular responses to growth factors are mediated, in part, through the regulation of specific gene expression. Thus, one approach to understanding how growth factors influence cell behavior is to clone and identify responsive genes. Estimates derived from such endeavors are inexact (1) but suggest that growth factors exert their influence through the action of perhaps 50–100 genes.

In studies of cell growth control, particular attention has been focused on genes expressed soon after growth-factor stimulation of quiescent cells in culture. Such genes presumably encode proteins important to growth stimulation and have been shown to include the c-*fos* and c-*myc* protooncogenes (2, 3). Interestingly, enhanced expression of the cytoskeletal actin genes is also a prominent and rapid response to mitogenic stimuli in several cell types (4–7). This contrasts with the traditional view of the cytoskeletal actins as passive structural proteins but is broadly consistent with other data suggesting an important role for actin microfilaments in mediating cellular responses to growth factors (8) and in neoplastic transformation (9).

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Although the exact role of actin in cell growth control is not clear, the degree of anchorage of cells to the extracellular matrix has long been known to influence both cell division and cell differentiation (reviewed in ref. 10). Among the major mediators of cell adhesion are transmembrane glycoproteins that link actin microfilaments on the cytoplasmic side of the membrane with extracellular matrix proteins such as fibronectin (FN) (11–14). Furthermore, these interactions are complex and appear to facilitate conformational changes associated with cell division (15, 16).

In the present study, we sought to identify additional genetic responses to peptide growth factors by using, as a model system, epidermal growth factor (EGF) stimulation of quiescent AKR-2B mouse embryo fibroblasts. As expected, cytoskeletal actin mRNA transcripts were abundantly represented in a group of cDNA clones derived from inducible mRNAs. Several nonactin clones were also isolated, and one of these was subsequently identified as murine FN (MuFN).¶ Most interestingly, stimulation with a variety of mitogens produced near coordinate increases in the levels of MuFN and β-actin mRNAs. These results suggest that the cellular effects of a variety of peptide growth factors are mediated, in part, through the regulation of specific genes encoding components of the cytoskeleton and the extracellular matrix.

MATERIALS AND METHODS

Cell Culture and RNA Preparation. Mouse embryo-derived AKR-2B cells were cultured as described (17). Quiescent cultures were obtained by growing cells to near confluency in McCoy's 5a medium supplemented with 5% (vol/vol) fetal calf serum (FCS), then switching to serum-free medium MCDB 402 for 48 hr. Stimulated cells were obtained by the addition of fresh medium containing the indicated concentrations of purified growth factors or fresh 20% (vol/vol) FCS in the presence or absence of cycloheximide (1 or 10 μg/ml) as indicated. Growth factors used were mouse EGF, human platelet-derived growth factor (PDGF) (R. D. Systems, Minneapolis, MN), human transforming growth factor type β1 (TGF-β1) (Collaborative Research, Waltham, MA), and bovine insulin (Sigma). Phorbol 12-myristate 13-acetate (PMA) was a gift from G. Woloschak. Total cellular RNA was prepared by the guanidinium isothiocyanate procedure described by Maniatis *et al.* (18).

Abbreviations: FN, fibronectin; EGF, epidermal growth factor; MuFN, murine FN; FCS, fetal calf serum; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor type β; PMA, phorbol 12-myristate 13-acetate.

§To whom reprint requests should be addressed.

¶This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03646).

Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography as described (19).

Construction and Replica Screening of cDNA Library. Roller bottle cultures of AKR-2B cells were grown to near confluency in McCoy's 5a medium containing 10% (vol/vol) FCS, then placed in serum-free MCDB 402 for 48 hr. Quiescent cells were treated with EGF at 10 ng/ml plus cycloheximide at 1 μg/ml for 4 hr and then harvested. Twenty-five micrograms of poly(A)$^+$ mRNA from these cells was used for single-strand cDNA synthesis by 2–3 units of reverse transcriptase per μg of mRNA. The Klenow fragment of DNA polymerase I was used to synthesize the second strand of cDNA, and the hairpin loop was removed by S1-nuclease treatment. Synthetic *Eco*RI linkers were added, and the double-stranded cDNA was ligated to purified phage λgt11 DNA (20). The recombinant molecules were packaged *in vitro* and grown in *Escherichia coli* Y1088 to generate the cDNA library. This library contained 8×10^6 individual members of which 38% were recombinant. Approximately 40,000 plaques were initially screened by differential hybridization to replica filter lifts (21) by using cDNA probes made from poly(A)$^+$ RNA isolated from quiescent cells or quiescent cells that were treated for 4 hr with EGF (10 ng/ml) and cycloheximide (1 μg/ml). Those clones that showed differential expression were picked and plaque-purified by two additional rounds of differential screening.

Blot Analysis of RNA. Electrophoresis of RNA in 1% agarose gels containing formaldehyde and formamide, transfer of RNA to nitrocellulose, and hybridization to cloned probes, ^{32}P-labeled by nick-translation, were performed as described (17).

DNA Sequencing. DNA sequencing was performed by the GemSeq double-stranded DNA sequencing system (Promega Biotec, Madison, WI) according to protocols supplied by the manufacturer. Synthetic oligonucleotides corresponding to determined sequences were synthesized on an Applied Biosystems (Foster City, CA) model 380A DNA synthesizer and utilized as internal primers to extend and complete the sequence. The sequences of both DNA strands were determined.

Run-Off Transcription. Nuclear run-off transcription assays were performed as described (5).

RESULTS

Replica Screening of a cDNA Library to Identify EGF-Inducible Sequences. Mouse embryo-derived AKR-2B cells display an anchorage- and density-dependent pattern of growth (22) and normally initiate DNA synthesis 12–14 hr after EGF stimulation (23). To identify EGF-inducible sequences, a λgt11 cDNA library was constructed with poly(A)$^+$ RNA from quiescent cells stimulated for 4 hr with EGF in the presence of cycloheximide. Differential screening (21) of 40,000 clones led to the isolation of 28, which corresponded to inducible mRNAs.

Since our laboratory had shown (5, 17, 24) that transcripts of both mouse *VL30* genes, a class of retrovirus-like mobile genetic elements, and β- and γ-actin genes are induced to high levels by EGF stimulation, the 28 positive clones were tested for their ability to hybridize to cloned probes specific for either VL30 or actin sequences. Of these, 12 were identified as either β- or γ-actin-specific clones, 11 were found to be *VL30*-specific clones, while 5 (c70, c94, c95, c98, and c99) represented unrelated sequences not belonging to either the actin or the *VL30* groups.

Differential Effects of EGF and Cycloheximide on Expression of Selected Clones. To test the relative effects of EGF and cycloheximide on inducible RNA sequences, poly(A)$^+$ RNA from cells stimulated with EGF, cycloheximide, or a combination was analyzed by RNA gel blot hybridization with various selected clones as probes. In these experiments, insulin was included in the stimulation protocol for reasons outlined in a following section. The results obtained with clone c99 are shown in Fig. 1. The c99 probe hybridized to a major transcript with an apparent chain length of 8.5 kilobases (kb) and to two minor transcripts of 4 and 2 kb, respectively. These minor transcripts could represent either varients or degradation products of the 8.5-kb RNA or functionally unrelated RNAs that share limited sequence identity. The abundance of the 8.5-kb RNA was increased by 30 min after stimulation, and cycloheximide had little if any effect on this induction.

Nucleotide Sequence of c99 Reveals Extensive Similarity with FN. A 900-base-pair (bp) insert from clone c99 was subcloned into the plasmid pGem4 and sequenced by the dideoxy chain-termination technique (25). A search of the GenBank data base¶ for sequences similar to the resulting 905-bp sequence revealed that c99 was >90% identical to the 3′ end of rat FN mRNA (26) as shown in Fig. 2. This similarity included an open reading frame corresponding to the carboxyl-terminal 103 amino acids of rat FN, a TAA termination codon, and 583 bp of 3′-untranslated sequence.

Fig. 3 illustrates a comparison between the deduced amino acid sequence encoded by the open reading frame of c99 and the carboxyl-terminal amino acid sequence of rat (26) and human (27) FN. This sequence was found to be 98% similar to rat FN and 93% similar to human FN. Interestingly, both the murine and rat sequences contain an insertion of 3 nucleotides relative to the human sequence resulting in an additional amino acid in the carboxyl-terminal region.

Differential Effects of Other Mitogens on FN mRNA Induction. To determine whether the induction of MuFN mRNA occurs in response to a broad spectrum of mitogens, we tested the ability of several mitogens to stimulate MuFN mRNA accumulation. These included medium supplemented with fresh 20% (vol/vol) FCS and serum-free medium containing EGF, insulin, PDGF, TGF-β, or the phorbol ester tumor promoter PMA. Insulin alone is not mitogenic for AKR-2B cells but has been shown to greatly potentiate the mitogenic effect of EGF in serum-free medium (23).

Cells were grown to near confluency in medium containing 5% (vol/vol) FCS then switched to serum-free medium MCDB 402. To ensure complete removal of residual serum-factors, cells were washed twice with serum-free medium and then incubated in serum-free medium for 2 days. They were then stimulated for 6 hr by the addition of fresh medium

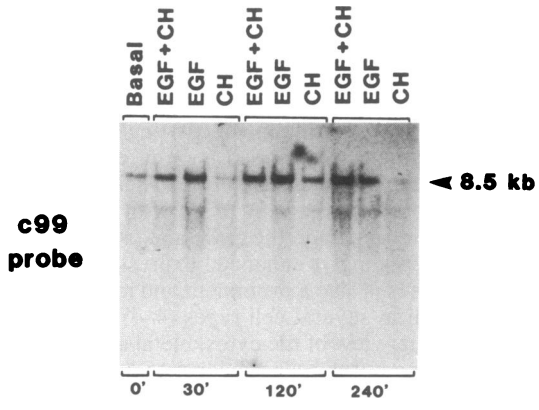

FIG. 1. Kinetics of c99 mRNA accumulation in response to EGF, cycloheximide, or both. Quiescent cells were treated for the indicated times with EGF (10 ng/ml), cycloheximide (CH, 1 μg/ml), or both in the presence of insulin (500 ng/ml). Total RNA was prepared and analyzed by RNA gel blot hybridization with c99 as probe.

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                                                                450
FIBRONECTIN TGGAAAGGGAGAATTCAAATGCGATCCCCATGAAGCAACGTGTTATGACGACGGGAAGAC
C99                 GAATTCAAGTGTGATCCCCATGAAGCAACGTGTTATGACGATGGGAAGAC
                                                                    50
                                                  500
FIBRONECTIN CTACCACGTAGGAGAACAGTGGCAGAAAGAGTATCTCGGAGCCATTTGCTCCTGCACGTG
C99         CTACCATGTAGGAGAACAGTGGCAGAAAGAAATATCTCGGAGCCATTTGTTCCTGCACGTG
                                                                100
                                              550
FIBRONECTIN TTTCGGGGGCCAGCGGGGCTGGCGCTGTGACAACTGCCGCAGACCTGGGGCTGCTGAACC
C99         TTTCGGAGGCCAGCGGGGCTGGCGCTGTGACAACTGCCGTAGACCTGGGGCTGCTGAACC
                                    600                         150
FIBRONECTIN CAGTCCCGATGGTACCACTGGCCACACCTACAACCAGTATACACAGAGATACCATCAGAG
C99         CAGTCCCGATGGCACCACCGGCCACAACTACAACCAGTATACACAGAGATACAATCAGAG
                              650                   200           700
FIBRONECTIN AACGAACACTAATGTAAATTGCCCAATTGAATGCTTCATGCCGTTGGACGTGCAGGCTGA
C99         AACAAACACTAACGTAAATTGCCCCATTGAGTGCTTCATGCCGTTAGATGTGCAAGCTGA
                                    250                         750
FIBRONECTIN CAGAGATGATTCCAGAGAGTAATCTTTCCATCCAGCCCAAGCCAACAAGTGTCTCTCTAC
C99         CAGAGACGATTCTCGAGAGTAATCTTTCCA----GCCCCACCCTACAAGTGTCTCTCTAC
                        300                                 800
FIBRONECTIN CAAGGTCAATCCACACCCCAGTGATGTTAGCAGACCCTCCATTTCTGAGTGGTCATTTCA
C99         CAAGGTCAATCCACACCCCAGTGATGTTAGCAGACCCTCCATCTTTGAGTGGTCCTTTCA
                  350                                       400
                                                850
FIBRONECTIN CCCTTAAGCCTTCTGCTCTGGAGTCAAGTTCTCAGCTTCAGCTCAACTTACAGCTTCTCC
C99         CCCTTAAGCCTTTTGCTCTGGAGCCTAGTTCTCAGCTTCAGCACAATTTACAGCTTCTCC
                                          900                   450
FIBRONECTIN AAGCATCGCCCCGCGGGATGTTTTGAGACTTCCCTCTTAAATGGTGACAGTTGGTG-CCC
C99         AAGCATCGCCCCGTGGGATGTTT-GAGACTTCTCTCCTCAATGGTGACAGTTGGTCACCC
                        950                         500             1000
FIBRONECTIN TGTTCTGCTTCAGGGTATTCAGTACTGCTCAGTATTATTGTCTAAGAGAATCAAAAGTTC
C99         TGTTCTGCTTCAGGGT-TTCAGTACTGCTCAGTG---TTGTTTAAGAGAATCAAAAGTTC
                                    550                         1050
FIBRONECTIN TTGTGATTTGGTCTGGGATCAA-AGGGAAACACAGGTAGCCAACCACGATGCAATGAATT
C99         TTATGGTTTGGTCTGGGATCAATAGGGAAACACAGGTAGCCAACTAGGAGGAAATGTACT
                              600                   1100
FIBRONECTIN GAATGGTAGTACCCAAGAGCGGGAGCAGGAAGTTAAACCAGACAGTTCTGCTTTCTTTTG
C99         GAATGCTAGTACCCAAGACCTGAGCAAGGAAAGTCACCCAGACACCTCTGCTTTCTTTTG
                        650                         1150            700
FIBRONECTIN CCGTCTGATCTGCAGCACTGTCAGGAGGCCTGTCCTGTGGCTGTGTC-CAAACACCCC--
C99         CCATCTGACCTGCAGCACTGTCAGGACATGG--CCTGTGGCTGTGTGTCAAACACCCCTC
                                          1200                  750
FIBRONECTIN --ACAGGACTCACT--GTCCCAACAATCCTAATTGCCTAGAAATATCTTTCTCTTACCTG
C99         CCACAGAACTCACTTTGTCCCAACAATTCAGATTGCCTAGAAATACCTTTCTCTTACCTG
                                    1250                        800
FIBRONECTIN TT----ATTTATCAATTTTTCCCAGTATTTTTATACGGAAAAAATTGTATTGAAGACACT
C99         TTTGTTATTTATCAATTTTTCCCAGTATTTTTATACGGAAAAAATTGTATTGAAGACACT
                              1300                  850
FIBRONECTIN TTGTATGCAGTTGATAAG
C99         TTGTATGCAGTTGATAAGAGGAATTC
                                    900
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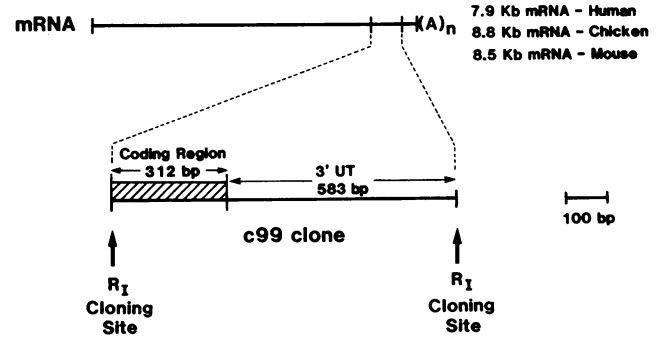

FIG. 2. (*Upper*) Similarity of the c99 cDNA sequence to 1 kb of known sequence from 3' end of a rat FN cDNA clone. *Eco*RI (R_I) cloning sites (underlined) and translation termination codon TAA (box) are shown. Dots indicate identical nucleotides. (*Lower*) Schematic representation of FN mRNA and its relationship to c99 sequence. UT, untranslated.

containing the factors or combinations of factors indicated in Fig. 4. Total RNA was prepared and analyzed by RNA gel blot hybridization with c99 (MuFN) or an isotype-specific β-actin cDNA clone (28) as probe.

The data displayed in Fig. 4 demonstrate that the patterns of FN and actin mRNA accumulation in response to the tested factors are quite similar but complex. Both 20% (vol/vol) FCS and PDGF were strong inducers. PMA was a weak but significant inducer while TGF-β produced an intermediate response. Two strong synergistic effects were

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RA-FIBRONECTIN  RMSCTCLGNGKGEFKCDPHEATCYDDGKTYHVGEQWQKEYLGAICSCTCFGGQRGWRCDNCRRP
C99                       EFKCDPHEATCYDDGKTYHVGEQWQKEYLGAICSCTCFGGQRGWRCDNCRRP
HU-FIBRONECTIN  MMSCTCLGNGKGEFKCDPHEATCYDDGKTYHVGEQWQKEYLGAICSCTCFGGQRGWRCDNCRRP

RA-FIBRONECTIN  GAAEPSPDGTTGHTYNQYTQRYHQRTNTNVNCPIECFMPLDVQADRDDSRE COOH
C99             GAAEPSPDGTTGHNYNQYTQRYNQRTNTNVNCPIECFMPLDVQADRDDSRE COOH
HU-FIBRONECTIN  GG-EPSPEGTTGQSYNQYSQRYHQRTNTNVNCPIECFMPLDVQADREDSRE COOH
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FIG. 3. Derived amino acid sequence for c99 and its identity to the carboxyl-terminal amino acid sequence of rat (RA) and human (HU) FN. The single-letter amino acid code is used.

also noted. First, whereas neither EGF nor insulin alone gave a significant response, the combination did, suggesting that the synergistic effect of these factors on DNA synthesis (23) is mimicked at the level of specific mRNA induction. Second, EGF alone greatly potentiated the TGF-β induction but had no measurable effect on the ability of PDGF to induce mRNA accumulation. In fact, the combination of TGF-β and EGF produced a response equal to, if not greater than, fresh 20% (vol/vol) FCS. Additional experiments (data not shown) demonstrated that the inclusion of cycloheximide at 10 μg/ml did not inhibit the ability of any factor tested to induce MuFN mRNA.

Coordinate Induction of FN, β-Actin, and c-*fos* Gene Transcription in Serum-Stimulated Cells. Having determined that a variety of mitogenic stimuli were capable of inducing FN mRNA, it was of interest to examine the effect of such stimuli on FN gene transcription. AKR-2B cells, rendered quiescent as before, were stimulated with fresh 20% (vol/vol) FCS for periods from 10 min to 3 hr. Nuclei were then isolated and used in nuclear run-off transcription assays as described (5). To provide a comparison with genes whose transcription is known to be serum-inducible, we examined c-*fos* and β-actin; increased transcription of these genes is among the earliest catalogued responses to growth factor stimulation (4, 5).

FN gene transcription was strongly stimulated within 10 min of serum addition (Fig. 5). As expected, transcription of c-*fos* and β-actin was stimulated with similar kinetics. All three responses were transient, declining to near basal levels by 60 min. The transcription of a fourth tested gene, α-tubulin, appeared to be only minimally altered during the course of the experiment demonstrating that the strong induction and subsequent attenuation of c-*fos*, β-actin, and

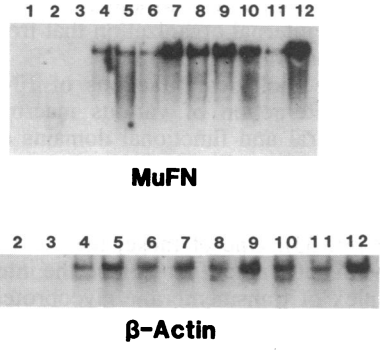

FIG. 4. Stimulation of MuFN and β-actin mRNA by various growth factors and mitogens. Quiescent AKR-2B cells in serum-free medium were stimulated for 6 hr with various factors and combinations. Total RNA was isolated and analyzed by RNA gel blot hybridization by using as probe c99 (*Upper*) or a β-actin 3'-untranslated sequence clone (*Lower*). Each lane contained 4 μg of RNA. Lanes: 1, fresh medium alone; 2, insulin (500 ng/ml); 3, EGF (10 ng/ml); 4, EGF (10 ng/ml) and insulin (500 ng/ml); 5, TGF-β (10 ng/ml); 6, TGF-β (10 ng/ml) and insulin (500 ng/ml); 7, PDGF (2.5 ng/ml); 8, PDGF (2.5 ng/ml) and insulin (500 ng/ml); 9, 20% (vol/vol) FCS; 10, PDGF (2.5 ng/ml) and EGF (10 ng/ml); 11, PMA (100 ng/ml); 12, TGF-β (10 ng/ml) and EGF (10 ng/ml).

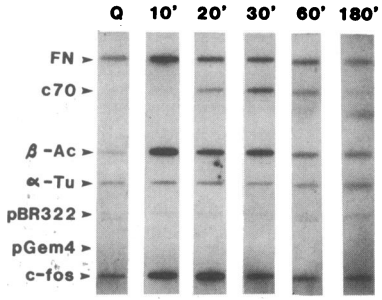

FIG. 5. Effect of serum stimulation on specific gene transcription in AKR-2B cells. Quiescent AKR-2B cells in serum-free medium were stimulated for the indicated times (in min) by the addition of fresh medium containing 20% (vol/vol) FCS. Nuclei were isolated and used for nuclear run-off transcription as described (5). ^{32}P-labeled transcripts were then hybridized to the indicated cloned probes or plasmid cloning vehicles immobilized on nitrocellulose in the form of slots. Lane Q, quiescent AKR-2B cells.

FN gene transcription is a specific response to serum stimulation.

Finally, we also examined the transcriptional response of the gene corresponding to the EGF-inducible clone c70. As shown in Fig. 5, c70 transcription was stimulated in response to 20% (vol/vol) FCS. However, the kinetics of induction lagged behind FN, reaching maximal levels at 30 min and then declining. In related experiments (data not shown), c70 was found to be derived from a 2.1-kb RNA that was maximally induced between 30 and 60 min after serum stimulation, but DNA sequencing failed to establish its identity with any catalogued sequence.¶ However, it may correspond to one of several similarly regulated transcripts of comparable size identified by Lau and Nathans (29).

DISCUSSION

FN, a high molecular weight glycoprotein, is a major component of the extracellular matrix and an important determinant of cell adhesion both *in vivo* and *in vitro* (reviewed in ref. 30). A variety of functions depend upon its properties including the maintenance of normal morphology, the attachment of cells to substrata, wound healing, and embryological development and differentiation. In addition, the loss of cell-surface FN is believed to account for the diminished ability of transformed cells to adhere to normal substrates and the loss of internal organization that frequently accompanies cell transformation (31, 32).

The multiple biological functions of FN are mediated through the interaction of various macromolecules with various structural and functional domains of the FN subunits. For example, other components of the extracellular matrix such as collagen and fibrin are linked to the cell surface through interactions with specific FN domains (reviewed in ref. 33). One of these, the cell-binding domain, interacts with actin microfilaments in the interior of the cell by a complex of transmembrane glycoproteins termed the FN receptor (11). This receptor and possibly other transmembrane complexes (34) comprise the physical linkage through which FN provides a focal point for the anchorage of actin microfilaments and serves as an organizer of cell configuration.

Despite the central role of FN and other extracellular matrix proteins in cellular and developmental regulation, studies of the controlling influence of peptide growth factors on FN expression are few and relatively narrow in scope (35–37). Ignotz and Massague (36) demonstrated that stimulation of a variety of fibroblastic and epithelial cell lines with TGF-β results in the increased synthesis of FN and collagen and proposed that cellular actions of TGF-β may be mediated by changes in the extracellular matrix. Our results support and expand this hypothesis by demonstrating that the activation of several growth factor receptors directly stimulate FN mRNA in AKR-2B cells. In addition, our data suggest that various combinations of growth factors can have strong synergistic effects and that these effects are also manifest upon a major cytoskeletal protein mRNA, β-actin. Finally, our results indicate that the increase in FN mRNA in serum-stimulated cells results, at least in part, from the direct stimulation of FN gene transcription. Most significantly, this increase occurs within minutes of serum addition and is virtually indistinguishable from a similar increase in the transcription of β-actin and the c-*fos* protooncogene. Lau and Nathans (29) identified several additional genes in serum-stimulated BALB/c 3T3 cells that are transcriptionally activated with similar kinetics. Thus, FN and β-actin may be members of a diverse class of rapidly activated genes, typified by c-*fos*, that comprise the initial genetic response of fibroblast-like cells to peptide growth factors.

An apparent paradox in our data concerns the synergistic effect of EGF and insulin on MuFN and β-actin mRNA. The data displayed in Fig. 4 suggest that in serum-free conditions both of these factors are required for induction. In other experiments (data not shown) purified insulin-like growth factor I (somatomedin C) has been found to similarly potentiate EGF suggesting that the insulin effect is mediated through the insulin-like growth factor I receptor rather than the insulin receptor. However, neither insulin nor insulin-like growth factor I were added to the culture medium of the original AKR-2B cells used to produce our cDNA library. One explanation for the induction of MuFN mRNA under these conditions might be that these cells were grown in roller bottles with medium containing a high concentration of FCS and were not washed prior to their transfer to serum-free medium. Thus, residual serum factors may have been sufficient to potentiate the ability of EGF to induce MuFN mRNA. Our laboratory has shown (5) that β-actin mRNA can be induced by EGF alone when added to medium containing a low concentration (0.5%) of FCS.

With the exception of insulin, all factors that we tested have been shown to be mitogenic for AKR-2B cells grown in monolayer culture (23, 38). In the "competence–progression" model for cell-cycle control (39, 40), EGF and insulin (or insulin-like growth factors) are generally viewed as progression factors, whereas PDGF is considered to be a competence factor. TGF-β is a "pleiotropic" agent that can either stimulate or inhibit cell growth, depending on cell type (41, 42). In AKR-2B cells, TGF-β stimulates monolayer growth in a manner consistent with that of a potent competence factor (38, 43). PMA, while not a peptide growth factor, is believed to exert its mitogenic effect by directly activating protein kinase C (44). The virtually identical pattern of induction of FN and β-actin mRNA in response to this diverse group of mitogens (Fig. 4) coupled with the coordinate activation of gene transcription after serum stimulation (Fig. 5) suggests that coexpression of FN and cytoskeletal actin genes may be an important early event during the transition of cells from a resting to a proliferating state. Moreover, these data raise the issue of whether common factors, perhaps working at the transcriptional level, may be the molecular basis for the observed patterns of coordinate expression. In this regard, Dean *et al.* (45) reported the nucleotide sequence of the promoter and associated 5′ region of the human FN gene. An inspection of their data reveals the sequence 5′ GATGACCGCAAAGGAAACC 3′, located 224 bp upstream from the promoter. This sequence is related to several members of a conserved family of specific protein-binding sequences that have been shown to be required for growth factor-dependent expression. These include sequences associated with the human c-*fos* gene

(46), the rat prolactin gene (47), and a *Xenopus laevis* cytoskeletal actin gene (48). Our laboratory has cloned a serum-inducible mouse β-actin gene promoter and identified another related sequence in 5′-flanking DNA (49). It will be important to determine whether the FN and β-actin sequences are required for serum-dependent expression and, if so, whether a common trans-acting factor is involved.

Irrespective, the stimulatory effects of growth factors upon genes that encode components of the extracellular matrix and the cytoskeleton may facilitate an understanding of how various growth factors affect cell anchorage and cell morphology (23, 38). These effects, in turn, appear to play an important role in regulating cell division and cell differentiation (10). This is particularly true of TGF-β, which induces striking morphological changes in a variety of cell types (23, 36, 38). The elucidation of the postreceptor pathway(s) by which TGF-β and other growth factors regulate FN and actin gene expression may provide valuable insight into the initial stages of this process.

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