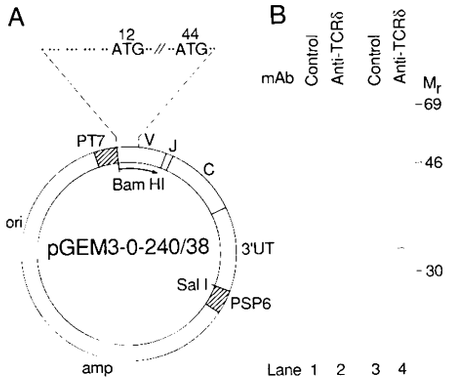

Fig. 4. Recognition of in vitro translated product of cDNA clone IDP2 O-240/38 by anti-TCR δ1. (**A**) pGEM3-O-240/38 construct used for in vitro transcription and translation. IDP2 O-240/38 cDNA clone (1.3 kb) begins within codon 7 of the composite group O sequence and includes the remaining coding region and most of the 3′ untranslated region (*16*). This insert was cleaved as a single Eco RI–Eco RI fragment from λgt10 arms by partial Eco RI digestion to prevent cleavage of the internal Eco RI site. This fragment was subcloned into the Bluescript+ vector (Stratagene). The clone was cleaved as a single Bam HI–Sal I fragment (ends are from the Bluescript vector polylinker) and was then directionally cloned into pGEM-3 (Promega Biotec) downstream from the T7 promoter to generate pGEM3-O-240/38. (**B**) Samples were immunoprecipitated with control mAb P3 (lanes 1 and 3) or with anti-TCR δ1 (lanes 2 and 4). Translations contained no RNA (lanes 1 and 2) or pGEM3-0-240/38–derived RNA template (lanes 3 and 4). pGEM3-0-240/38 plasmid was linearized with Sal I, and capped transcripts were synthesized with the use of T7 polymerase (*11*, *22*, *23*). Integrity and size of the transcripts were checked on a portion of the reaction mixture to which ^{32}P-labeled adenosine triphosphate had been added. A single RNA species of 1.3 kb was obtained. In vitro translation in the presence of [^{35}S]methionine was performed in a rabbit reticulocyte lysate (*24*). After in vitro translation, the samples were boiled in 1% SDS with 2 m*M* dithiothreitol followed by the addition of 10 volumes of 2% TX-100 in tris-buffered saline, *p*H 8.0. The samples were then immunoprecipitated with the above antibodies and analyzed by SDS-PAGE followed by fluorography (*25*).

minor species noted (Fig. 4, lane 4).

Taken together, the correlation between predicted and determined extent of glycosylation and peptide size, the selective expression and rearrangement in TCR γδ cells, and the direct serological recognition of the polypeptide encoded by IDP2 0-240/38, are compelling evidence that this candidate cDNA represents the gene encoding the IDP2 TCR δ subunit. The constant region of this cDNA clone is 79% identical at the nucleotide level to the recently described murine Cx gene (*17*), which may therefore correspond to the murine TCR δ equivalent. Cloning of both TCR γ and δ make it possible to more fully elucidate the expression, rearrangement, and diversity of the receptor. Moreover, mAbs against TCR δ that bind to the surface should facilitate functional studies and move us closer to understanding the role these cells play in the immune system.

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The Adenovirus Major Late Transcription Factor Activates the Rat γ-Fibrinogen Promoter

LEWIS A. CHODOSH, RICHARD W. CARTHEW, JOHN G. MORGAN, GERALD R. CRABTREE, PHILLIP A. SHARP

The major late transcription factor (MLTF) is a 46-kilodalton polypeptide that specifically binds to and activates transcription from the major late promoter of adenovirus. The presence of this promoter-specific transcription factor in uninfected HeLa cell extracts suggests that MLTF is also involved in the transcription of cellular genes. This report demonstrates that MLTF specifically stimulates transcription of the rat γ-fibrinogen gene through a high-affinity binding site. Stimulation of transcription by MLTF was not dependent on the exact position of the MLTF binding site with respect either to the transcription initiation site or to adjacent promoter elements. These results suggest that one of the cellular functions of MLTF is to control γ-fibrinogen gene expression.

A WEALTH OF BIOCHEMICAL AND GENETIC evidence in eukaryotic cells suggests that the regulation of transcription initiation by RNA polymerase II is mediated by the interaction of multiple sequence-specific DNA-binding proteins with the promoter elements of these genes. One of these sequence-specific DNA-binding proteins, the major late transcription factor (MLTF or USF), was originally identified by its ability to selectively bind to and stimulate transcription from the adenovirus major late promoter (MLP) (*1*–*4*). Since this promoter-specific factor was found in extracts of uninfected HeLa cells, it was presumed that MLTF would also selectively stimulate transcription of cellular genes containing an appropriately positioned MLTF-binding site. We report here that MLTF selectively stimulates transcription from the rat γ-fibrinogen (γ-FBG) promoter.

Fibrinogen, an essential blood coagulation factor, is a 340-kD protein composed of two sets of three polypeptides (α, β, and γ), which are produced in the liver. These polypeptides are encoded by three separate genes whose transcription is coordinately regulated (*5*). Fibrinogen genes are also coordinately regulated along with a number of other genes in the mammalian liver during the acute phase response to infection, inflammation, and tissue injury. Recently, the promoter elements of the fibrinogen genes have been genetically identified in

L. A. Chodosh, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, and Harvard Medical School, Boston, MA 02115.
R. W. Carthew and P. A. Sharp, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
J. G. Morgan and G. R. Crabtree, Stanford University School of Medicine, Stanford, CA 94305.

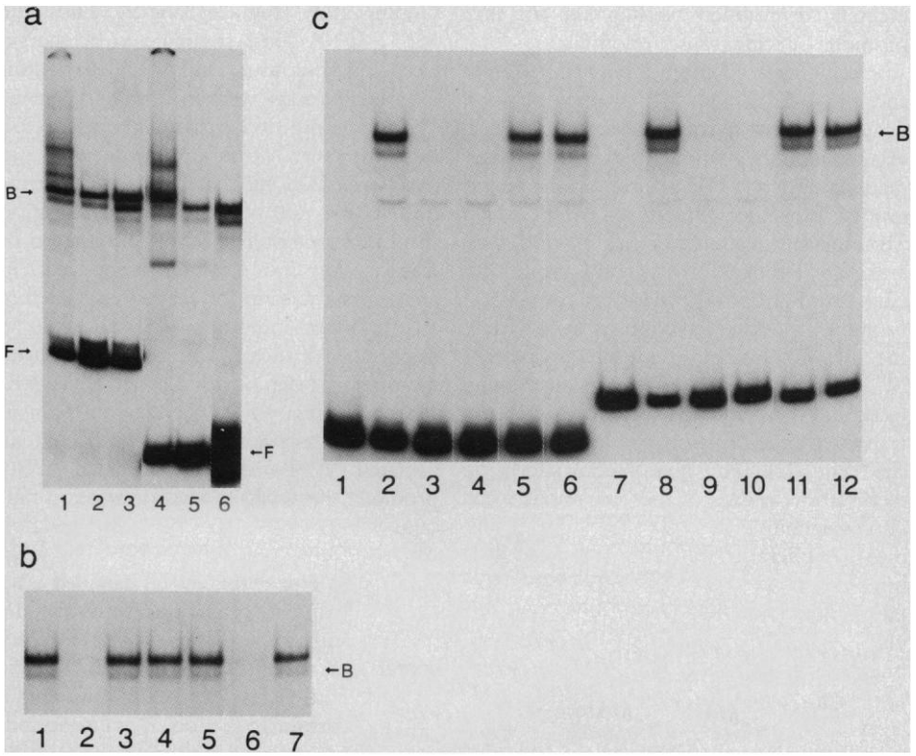

Fig. 1. Specific binding of MLTF to the γ-FBG promoter and the major late promoter. Binding reactions and native gel electrophoresis were as described (*1*), except that γ-FBG reactions were done in the absence of $MgCl_2$. (**a**) A portion (1.1 ng) of a 256-bp end-labeled ML probe (*4*) (lanes 1 to 3) or 1.0 ng of an 80-bp end-labeled γ-FBG probe (lanes 4 to 6) containing sequences from −95 to −39 relative to the transcription initiation site was added to 4 μg of poly(dI-dC) · (dI-dC) and either 10 μg (lane 1) or 20 μg (lane 4) of HeLa whole-cell extract, 6 μg (lane 2) or 9 μg (lane 5) of partially purified MLTF fraction AA (*4*), or 0.4 ng (lane 3) or 0.8 ng (lane 6) of affinity-purified MLTF fraction M4 (*4*). (**b**) MLTF fraction AA (9 μg) was added to a mixture of 4 μg of poly(dI-dC) · (dI-dC), 1.0 ng of γ-FBG probe and either no DNA (lane 1), or a 40-fold molar excess of an unlabeled DNA fragment containing sequences from −95 to −39 of the γ-FBG promoter (lane 2), −100 to −43 of the MOPC 41 V_K promoter (*19*) (lane 3), −138 to +5 of the H-$2K^b$ promoter (*20*) (lane 4), −110 to +19 of the human α1-globin promoter (*6*) (lane 5), −174 to +33 of the adenovirus 2 MLP (*4*) (lane 6), or −329 to +246 of the adenovirus 2 EIV promoter (*1*) (lane 7). (**c**) MLTF fraction AA (9 μg) (lanes 2 to 6 and 8 to 12) was added to a mixture of 4 μg of poly(dI-dC) · (dI-dC) and either 1.1 ng of an 80-bp γ-FBG probe (lanes 1 to 6) or 1.1 ng of a 110-bp MLP probe (lanes 7 to 12) and no further addition (lanes 2 and 8) or a 40-fold molar excess of an unlabeled DNA fragment containing sequences from −174 to +33 of the MLP (lanes 3 and 9), −95 to +38 of the γ-FBG promoter (lanes 4 and 10), −51 to +33 of the MLP (lanes 5 and 11), or −66 to +38 of the γ-FBG promoter (lanes 6 and 12). No protein was added to lanes 1 and 7.

vivo (*6*). Inspection of deletion mutants of the γ-FBG promoter revealed that an upstream element involved in transcriptional activation of this gene contained a 12-bp sequence homologous to the MLTF binding site in the adenovirus MLP. This suggested that MLTF might mediate transcription from the γ-FBG promoter. Accordingly, we tested the ability of MLTF to bind to this promoter by means of the gel electrophoresis DNA-binding assay (*1, 7, 8*).

Incubation of 100,000-fold affinity-purified MLTF with a radiolabeled γ-FBG–promoter DNA fragment resulted in the formation of a discrete protein-DNA complex with a mobility similar to that previously shown for the MLTF-MLP complex (Fig. 1a). This indicated that a protein present in a highly purified preparation of MLTF binds with high affinity to both the γ-FBG promoter and the adenovirus MLP. A partially purified chromatographic fraction (AA) that contains MLTF, but not other proteins that specifically bind to these promoters, formed a complex identical in mobility to that observed with purified MLTF. Incubation of HeLa whole-cell extract with MLP or γ-FBG–promoter DNA gave rise to this same complex and several others that result from the specific binding of distinct proteins to each of these promoters [Fig. 1a; (*9*)].

To further establish the specificity of the binding activity present in purified preparations of MLTF, competition binding studies were performed in which radiolabeled γ-FBG–promoter DNA was mixed with an excess of various unlabeled competitor DNA fragments (Fig. 1b). Only the γ-FBG and adenovirus MLP fragments were able to effectively compete for formation of the putative MLTF–γ-FBG probe complex, indicating that formation of this complex requires a DNA sequence common to both promoters. By titration of the amount of competitor DNA necessary to suppress complex formation, it was estimated that in the absence of $MgCl_2$ the relative affinity of MLTF for the γ-FBG promoter was approximately equal to its affinity for the MLP (*9*). Surprisingly, addition of 5 m*M* $MgCl_2$ to the binding reaction increased the relative affinity of MLTF for the MLP fivefold, while decreasing its affinity for the γ-FBG promoter fourfold (*9*). To establish that the common DNA sequence required for complex formation was in fact the MLTF–binding site homology, cross competitions were performed in which formation of the putative MLTF-DNA complex was competed by a 40-fold molar excess of either wild-type or deleted promoter fragments (Fig. 1c). The 12-bp MLTF binding site homology is located between −63 and −52 and −85 and −74 relative to the transcription initiation sites in the MLP and γ-FBG promoters, respectively (*10*). Both wild-type γ-FBG and wild-type MLP competitor DNA fragments were able to abolish formation of the MLTF-promoter complex on either the γ-FBG or MLP probes. The γ-FBG or MLP mutants in which the MLTF-binding site had been deleted failed to compete for complex formation.

To further delineate the MLTF-binding site in the γ-FBG promoter, competition studies were performed with unlabeled DNA fragments derived from a series of deletion mutants of the promoter (Fig. 2a). The 5′ deletion mutants retaining the 12-bp MLTF binding site homology (Δ−135, Δ−95, Δ−87) competed efficiently for MLTF binding; 5′ deletion mutants lacking this site (Δ−66, Δ−52) were unable to compete for MLTF binding. Similarly, internal deletion mutants that retain the MLTF binding site homology and are lacking sequences from −54 to −62 or −54 to −65 competed efficiently for MLTF binding. Removal of sequences from −54 to −74 resulted in only partial competition, indicating that a portion of the MLTF binding site had been altered. Larger internal deletions extending through the MLTF homology from −54 to −80 or −54 to −100 resulted in the complete elimination of competition for MLTF binding. Thus, the 12-bp MLTF homology within the γ-FBG promoter closely corresponds to the experi-

mentally determined binding site for this promoter-specific transcription factor.

Specific sites of contact between MLTF and the γ-FBG and ML promoters were determined by a methylation-interference assay to identify guanine residues that, when methylated at the N-7 position in the major groove, prevented binding of MLTF (*11*). This methylation-interference pattern was strikingly similar for the two promoters (Fig. 2b). For both the MLP and the γ-FBG promoter, all observed contacts were within the MLTF homology. Each promoter-MLTF complex formed six guanine contacts, and these contacts were identical with respect to their position within the binding site (Fig. 2c). The quantitative effects of methylation at each of these positions were also essentially identical. Methylation of the most peripherally located base in the MLTF binding site (γ-FBG, −74; MLP, −52) partially interfered with MLTF binding to either promoter (Fig. 2c). Deletion of this guanine residue from the γ-FBG promoter also partially impaired MLTF binding as assayed by the gel electrophoresis DNA binding assay (Fig. 2a, lane 9). Thus, the MLTF-DNA contacts predicted by the gel electrophoresis assay and the methylation-interference assay were in close agreement.

The binding of a particular transcription factor to a site within a promoter does not necessarily indicate that the factor contributes to the rate of transcription initiation from the promoter in vivo. Stimulation of transcription upon binding of the factor in an in vitro transcription reaction is therefore an important indication of the functional importance of such an interaction. S1 nuclease mapping demonstrated that RNA synthesized from a γ-FBG DNA template in an in vitro transcription reaction was accurately initiated (*9*). To identify particular γ-FBG promoter elements, a series of deletion mutants was transcribed in an in vitro reaction containing HeLa whole-cell extract (Fig. 3a). A wild-type template was included as an internal control. Deletion through the MLTF binding site from −87 to −66 resulted in a threefold reduction in specific transcription. Deletion to −52 resulted in a further fourfold reduction in transcription, which suggested the presence of an additional transcriptional element between −66 and −52 bp. Internal deletions that removed this additional element (ID−74/−54, ID−65/−54, ID−62/−54) had little effect on transcription, whereas internal deletions that removed this additional element as well as the MLTF binding site (ID−80/−54, ID−100/−54) resulted in a fivefold decrease in transcription. This suggests that the MLTF binding site can substitute for the putative transcriptional element located between −66 and −52. Moreover, three of these internal deletion mutants bring the MLTF binding site 2, 5, or 14 bp closer to the transcription initiation site, yet appeared to have no effect either on the overall transcriptional activity of the promoter or on the ability of the promoter to be stimulated by MLTF (see Fig. 3, a and c). Because these deletions both translocate and rotate the MLTF binding site relative to the TATA element, these results suggest that stereospecific protein-protein contacts between MLTF and the TATA box–binding factor may be less crucial for this particular

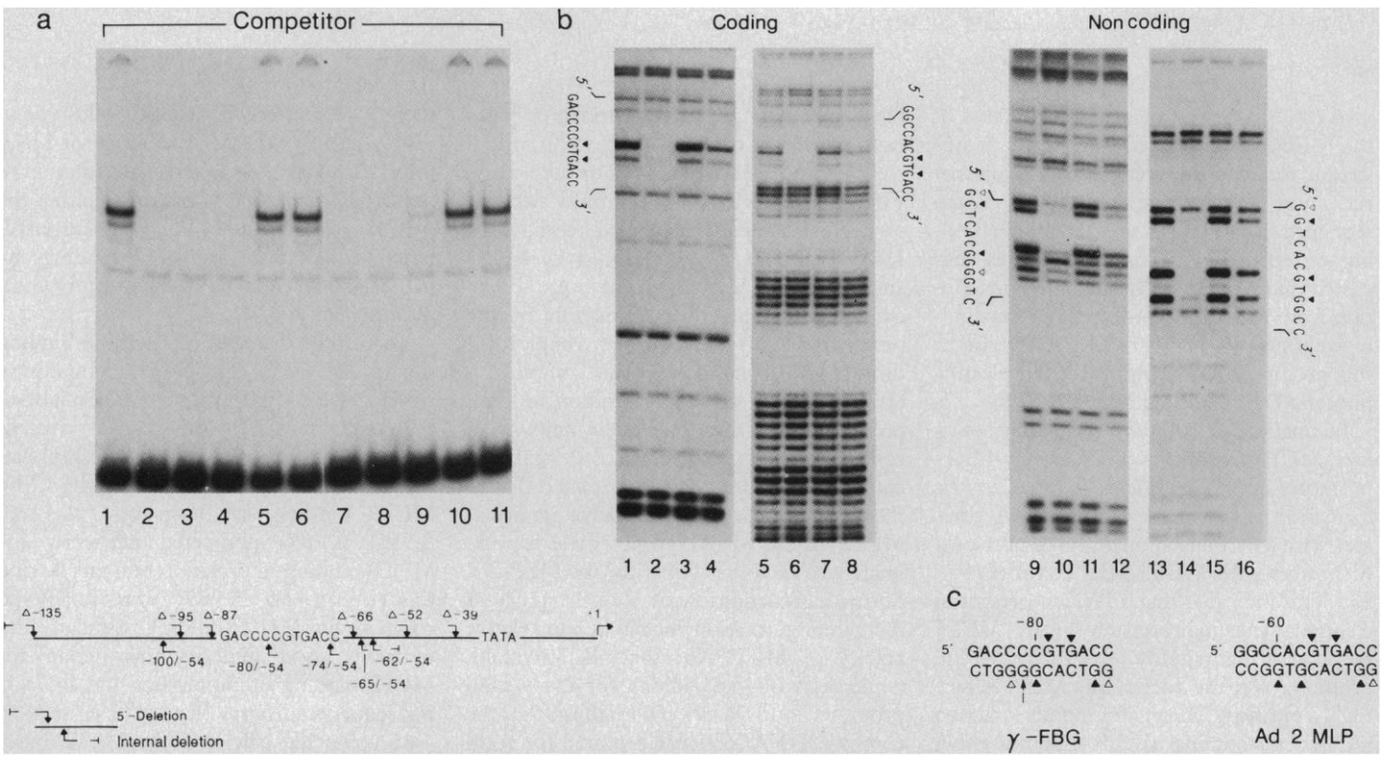

Fig. 2. Localization of MLTF binding to the γ-FBG promoter. (**a**) MLTF fraction AA (9 μg) was added to a mixture of 4 μg of poly(dI-dC) · (dI-dC), 1.0 ng of γ-FBG probe, and either no DNA (lane 1) or a 40-fold molar excess of an unlabeled DNA fragment derived from either a γ-FBG 5′ deletion mutant that contained sequences from −135 to +38 (lane 2), −95 to +38 (lane 3), −87 to +38 (lane 4), −66 to +38 (lane 5), −52 to +38 (lane 6), or from an internal deletion mutant that lacked sequences from −62 to −54 (lane 7), −65 to −54 (lane 8), −74 to −54 (lane 9), −80 to −54 (lane 10) or −100 to −54 (lane 11). Reaction conditions and electrophoresis were as described above. (**b**) Methylation interference was performed as described (*20*). γ-FBG or MLP probes (3′ end-labeled) were partially methylated with dimethylsulfate, purified, and used as substrate in a binding reaction with 20 μg poly(dI-dC) · (dI-dC) and 150 μg of MLTF fraction AA. Bound (lanes 2, 6, 10, and 14) and free (lanes 1, 3, 5, 7, 9, 11, 13, and 15) probes were separated by native gel electrophoresis, eluted, purified, and cleaved with piperidine, and an equal number of counts were electrophoresed on denaturing polyacrylamide gels along with a portion of original methylated DNA, which was piperidine-cleaved to generate a G ladder (lanes 4, 8, 12, and 16). The MLTF recognition sequence is shown for each strand of each promoter. Residues at which methylation interferes with MLTF binding are indicated with triangles. (**c**) Summary of effects of partial methylation on MLTF binding to the γ-FBG and ML promoters. Filled triangles indicate a greater degree of interference than hollow triangles. The arrow indicates a residue at which methylation increases MLTF binding.

promoter than for the MLP (*2*). These in vitro results were qualitatively similar to those obtained in vivo upon transfection of a hepatoma cell line (*6*).

To show directly that MLTF stimulates transcription from the γ-FBG promoter, a partially purified in vitro transcription system was used that did not contain MLTF activity (Fig. 3b). In contrast to the results obtained with whole-cell extract, the partially purified reconstituted transcription system transcribed γ-FBG templates containing or lacking the MLTF binding site with approximately equal efficiency. Addition to this system of increasing amounts of a crude chromatographic fraction containing MLTF resulted in a fivefold stimulation of transcription from the wild-type γ-FBG promoter (Δ−95) relative to the γ-FBG deletion mutant lacking the MLTF binding site (Δ−39). Similarly, the addition of MLTF stimulated transcription of the wild-type MLP sevenfold relative to the mutant γ-FBG promoter. If this transcription activation indeed resulted from the sequence-specific interaction of MLTF with the promoter, then a correlation should be observed between MLTF binding and MLTF-mediated transcription stimulation for each of the γ-FBG deletion mutants (Fig. 3c). Addition of an amount of 500-fold purified MLTF sufficient to stimulate transcription from the wild-type MLP 20-fold, resulted in a 4- to 5-fold stimulation of transcription from γ-FBG templates containing the MLTF binding site (Δ−135, Δ−87). Deletion through the MLTF-binding site (Δ−66, Δ−52) completely abolished the stimulation of transcription by MLTF. Internal deletions that retained the MLTF binding site (ID−62/−54, ID−65/−54) had no effect on the degree of MLTF stimulation, whereas further deletion through the MLTF binding site (ID−80/−54, ID−100/−54) eliminated the ability of MLTF to stimulate transcription from these templates. Internal deletion of sequences from −54 to −74 only partially diminished stimulation of transcription by MLTF, in accord with the partial effect that this mutation has on the binding of MLTF (Fig. 3c, lanes 15 and 16; Fig. 2a, lane 9; Fig. 2c). Thus, in all cases, the ability of MLTF to stimulate transcription from various mutants of the γ-FBG promoter can be predicted from the relative affinity with which MLTF binds to each of these mutants.

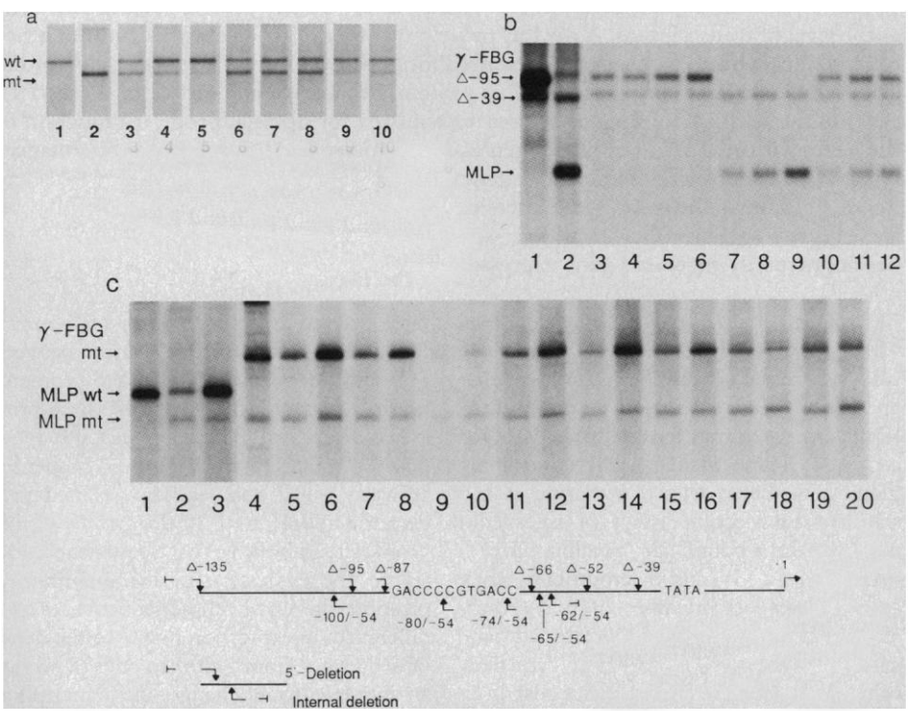

Fig. 3. Analysis of MLTF stimulation of γ-FBG transcription. (**a**) In vitro runoff transcription of γ-FBG deletion mutants was performed and processed as previously described (*1*). Transcriptions (20 μl) contained HeLa whole-cell extract (9 μl), poly(dI-dC) · (dI-dC) at 20 μg/ml, Fsp I–linearized Δ−87 at 10 μg/ml (lanes 1 and 3 to 10), and the following Xmn I–linearized mutants (mt) at 10 μg/ml: Δ−87 (lanes 2 and 3), Δ−66 (lane 4), Δ−52 (lane 5), ID−62/−54 (lane 6), ID−65/−54 (lane 7), ID−74/−54 (lane 8), ID−80/−54 (lane 9), or ID−100/−54 (lane 10). γ-FBG mutants were constructed as described previously (*6*). (**b**) In vitro runoff transcriptions were carried out either with whole-cell extract as above (lanes 1 and 2) or with a partially purified reconstituted transcription system lacking MLTF (lanes 3 to 12) as previously described (*1*). Reconstituted system reactions were supplemented with no further addition (lanes 3, 7, and 10), 1 μl of tenfold concentrated MLTF fraction AA (AA$_c$) (lanes 4, 8, and 11), 2 μl of fraction AA$_c$ (lane 5), or 3 μl of AA$_c$ (lanes 6, 9, and 12). All runoff transcript reactions contained Xmn I–linearized Δ−39 at 5 μg/ml (lanes 1 to 12), and were supplemented with Sca I–linearized Δ−95 at 5 μg/ml (lanes 1, 3 to 6, and 10 to 12) and/or the MLP template Sma I–linearized pBalE at 5 μg/ml (lanes 2 and 7 to 12). γ-FBG mutants Δ−95, Δ−39, and Δ−24 were constructed by cloning the Bst XI/Bst EII, Eco RI/Bst EII, and Hae III/Bst EII fragment, respectively, of rat γ-FBG into pUC18 by means of standard cloning procedures. (**c**) In vitro runoff transcriptions were performed with the HeLa whole-cell extract as above (lanes 1 and 4) or with the reconstituted transcription system as above (lanes 2, 3, and 5 to 20). Lanes 3, 6, 8, 10, 12, 14, 16, 18, and 20 were supplemented with 2 μl of 500-fold purified MLTF fraction M3 (*4*). Templates used were the wild-type MLP construct, pFLBH at 5 μg/ml (*21*), linearized with Tth I (lanes 1 to 3); the 5′ deletion mutant MLP construct, which lacks the MLTF-binding site—pXB806—linearized with Pvu II and at 5 μg/ml (*1*) (lanes 1 to 20), with or without each of the following Xmn I–linearized γ-FBG mutants at 5 μg/ml: Δ−135 (lanes 4 to 6), Δ−87 (lanes 7 and 8), Δ−66 (lanes 9 and 10), ID−62/−54 (lanes 11 and 12), ID−65/−54 (lanes 13 and 14), ID−74/−54 (lanes 15 and 16), ID−80/−54 (lanes 17 and 18), or ID−100/−54 (lanes 19 and 20).

The results of this study emphasize the close correlation between binding and transcription activities of promoter-specific transcription factors. They also support and extend the general observation that promoter-specific transcription factors, identified on the basis of their involvement in viral gene transcription, are also involved in transcription of cellular genes (*12–14*). Some differences, however, were noted between MLTF transcription stimulation of the MLP and of the γ-FBG promoter. Stimulation of the MLP by MLTF was several times greater than that of the γ-FBG promoter. This may have resulted from the higher affinity with which MLTF binds to the MLP compared to the γ-FBG promoter. Alternatively, this lower degree of MLTF responsiveness may reflect the more distal location of the MLTF binding site in the γ-FBG promoter, or may result from the effect of additional proteins that bind specifically to the γ-FBG promoter in the region between the MLTF and the TATA box homologies (*6, 9*).

Consistent with its proposed role in the transcription of the liver-specific rat γ-FBG gene, MLTF has been found in rat liver as well as in bovine and mouse liver (*6, 9*). However, MLTF has also been found in several other cell types and tissues including B cells, fibroblasts, erythroleukemia cells, embryonal carcinomal cells and in bovine brain and is active in these cell types since they support lytic adenovirus replication (*15*). It is therefore unlikely that MLTF plays a direct role in determining the tissue-specific expression of the γ-FBG gene. Consistent with this hypothesis, the β- and γ-

FBG genes contain MLTF binding sites, whereas the α-FBG gene does not. The observation that MLTF is present in cell types in which the γ-FBG gene is not expressed suggests that in these cell types either MLTF is unable to productively interact with the γ-FBG promoter in vivo or a dominant transcriptional element is able to repress transcription of this gene. Evidence exists that tissue-specific transcriptional extinguishers play just such a role in the transcription of liver-specific genes (*16, 17*).

The fact that induction of γ-FBG gene transcription, either by glucocorticoids or as part of the acute phase response, does not appear to require the presence of an MLTF binding site is analogous to mouse metallothionein I gene expression in which MLTF appears to contribute to the constitutive level of transcription without affecting heavy metal induction (*18*). Thus, MLTF appears to function in these inducible promoters as a constitutive transcription factor. Virtually no environmentally or developmentally regulated promoter has been described that consists solely of a binding site for a regulated transcription factor. Rather, each of these regulated promoters appears to be also bound by at least one constitutive transcription factor, which suggests that promoters evolve by combining regulatory elements with constitutive elements to establish an appropriate pattern and level of expression.

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Interaction of a Liver-Specific Nuclear Factor with the Fibrinogen and α_1-Antitrypsin Promoters

GILLES COURTOIS, JOHN G. MORGAN, LINDA A. CAMPBELL, GENEVIEVE FOUREL, GERALD R. CRABTREE

The orderly and sequential activation of genes during development is hypothesized to be related to the selective expression of groups of regulatory proteins acting primarily at the level of transcription. A nuclear protein was found in hepatocytes, but not other cell types, that binds to a sequence required for hepatocyte-specific transcription of the gene for the β chain of fibrinogen. This protein, hepatocyte nuclear factor 1 (HNF1), also interacts with homologous sequences required for optimal promoter function of the genes for the α chain of fibrinogen and α_1-antitrypsin. The promoter or enhancer regions for several viral and cellular genes not expressed in the liver did not compete for this binding. The restricted expression of HNF1 and its selective interaction with the control regions of several liver-specific genes indicate that it is involved in developmentally regulated gene expression in the liver.

REGIONS OF DNA IN OR NEAR PROMOTERS from several genes expressed selectively in the liver confer tissue-specific expression upon an unrelated gene when transfected into hepatocyte cell lines. These tissue-specific control regions have been defined by using either transient transfection assays or transgenic mice for the albumin (*1*), prealbumin (*2*), α_1-antitrypsin (*3*), and α-fetoprotein genes (*4*). At least one member of this group, albumin, is transcribed with greater efficiency in nuclear extracts from liver cells than other cell types (*5*). This implies that liver cells contain soluble factors capable of specifically activating the transcription of genes normally expressed selectively in the liver. Whereas progress has been made in the definition of these nuclear factors in B lymphocytes (*6*) and the endocrine pancreas (*7*), they have been elusive in the liver despite its obvious biochemical advantages.

In transfection experiments we examined 5′ deletion mutations of the promoter for the β chain of fibrinogen for their ability to be expressed in several cell lines derived from different tissues (Table 1). Transfection efficiency was controlled by cotransfecting the indicated plasmid with a plasmid in which transcription of the firefly luciferase gene is directed by the SV40 early region promoter (*8*). In the two hepatocyte cell lines derived from rat (Faza) and human (Hep G2), deletion of sequences between 500 and 78 bp upstream of the promoter resulted in a reduction to 1/40 the expression of the linked indicator gene CAT. Finer deletion mutations that removed sequences between −117 and −78 bp resulted in a decrease to 1/15 to 1/20 the activity of this promoter, indicating that sequences essential to the expression of the β-fibrinogen promoter lie within these sequences. These differences in expression due to the deletion of sequences from −500 to −78 were not attributable to a change in transcription initiation, since each of the constructs led to the production of properly initiated transcripts when RNA from transfected cells was examined by ribonuclease mapping.

A different pattern of expression was seen when the same mutations were examined after transfection into the nonhepatocyte lines KB, L cells, and Jurkat T lymphocytes (Table 1). After normalization for transfection efficiency with the pSV2-luciferase internal control, expression was 1/40 of or less that in the hepatocyte cell lines, and there was little or no detectable change upon deletion of sequences from −117 to −78.

We prepared an internal deletion mutation replacing sequences between −109 and −78 with an 8-bp Sph I linker that bears little similarity to the original sequence. This deletion reduced to 1/18 the expression of the β-fibrinogen promoter, which indicated that a sequence essential to the expression of

Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.