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.

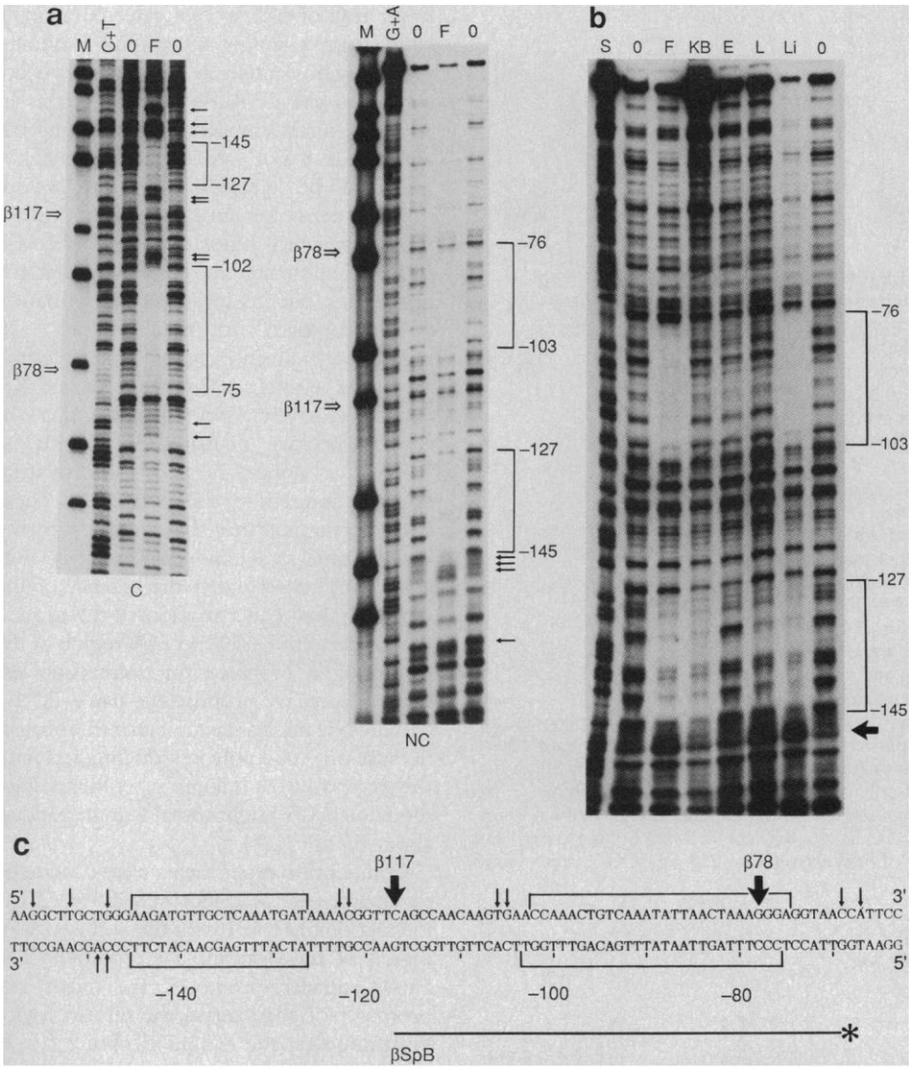

Fig. 1. Deoxyribonuclease I footprinting of the rat β-fibrinogen promoter. (**a**) Analysis of the coding (C) and noncoding (NC) strands. Probe C extends from −291 to +1 and probe NC extends from −222 to +1. The Faza nuclear extract was prepared essentially as described (*7*). The nuclei were extracted with 0.3*M* $(NH_4)_2SO_4$, and the fraction that contained the nuclear proteins precipitated with 0.2 g of $(NH_4)_2SO_4$ per milliliter was used for the footprint. The DNase I footprint assay was carried out as described (*7, 9*) with poly(dI-dC) as nonspecific competitor. M, molecular weight markers (Msp I digest of pBR322); C + T and G + A, Maxam and Gilbert sequencing reactions; 0, digestion of the probes after incubation with 25 μg of bovine serum albumin (BSA) as control; F, digestion of the probe after incubation with 25 μg of nuclear extract from the Faza cell line. The brackets and numbers indicate, respectively, the extent and positions of the footprinted regions, the small arrows indicate the hypersensitive sites, and the empty arrows represent the localization of the 5′ deletion mutants β117 and β78. (**b**) Footprint analysis of the β-fibrinogen noncoding strand with various nuclear extracts. S, G + A Maxam and Gilbert sequencing reaction; 0, digestion of the probe after incubation with 25 μg of BSA as control; F, KB, E, L, and Li, digestion of the probe after incubation with 25 μg of nuclear proteins from Faza, KB, EL4 (mouse T cell), and L cell lines and rat liver, respectively. (**c**) Diagrammatic summary of the protected sequences of the β promoter. The thick bar represents βSpB, the probe used in the mobility shift assay (see Fig. 3).

the β-fibrinogen promoter in hepatocytes lies between −117 and −78.

One interpretation of the cis-active sequences detected in promoters is that they represent binding sites for specific nuclear proteins. To search for such sites in the β-fibrinogen promoter, we used the footprinting technique (*9*) based on the ability of a bound protein to protect a sequence from digestion by deoxyribonuclease I (DNase I). We first analyzed the 200-bp region upstream of the β-fibrinogen cap site. Two different probes labeled either on the coding or the noncoding strand of the β-fibrinogen promoter were incubated with a nuclear extract prepared from the Faza cell line. After treatment with DNase I, the proteins were phenol-extracted, and the digested probes were analyzed on a sequencing gel (Fig. 1a). Two protected regions were detected on both strands extending from −145 to −127 and −102 to −75 on the coding strand and from −147 to −127 and −103 to −76 on the noncoding strand (Fig. 1, a and c). The proximal footprint was localized almost completely between the 5′ deletion mutants β117 and β78, which delimit a sequence important for transcriptional activity of the β-fibrinogen promoter as described above.

When we used nuclear extracts from other cell lines or tissues (Fig. 1b) we observed the distal footprint in every extract, although its intensity was variable. In contrast, the proximal footprint was detected with extracts from the Faza cell line and from the rat liver, which synthesize fibrinogen, but not with extracts prepared from KB, L, or EL4 cells, which do not synthesize fibrinogen. These results suggest that the −102 to −75 footprint could represent the interaction of a liver-specific nuclear protein with the β-fibrinogen promoter.

The footprinting analysis does not allow the accurate determination of differences in the concentration of DNA-binding proteins since the protected region must be almost fully occupied for a convincing footprint to be visualized. To detect low levels of proteins that bind to the −102 to −75 region of the β-fibrinogen promoter, we used the mobility shift assay (*10, 11*). A 49-bp probe, βSpB, which contains the proximal footprint (Fig. 1c), was prepared and incubated with various amounts of Faza nuclear extract (Fig. 2b). As expected, the probe formed a complex, confirming that the −102 to −75 region binds one or more nuclear proteins. Furthermore, the analysis of nine different cell lines or tissues indicated that this complex is exclusively formed with nuclear extracts derived from hepatocytes. Extracts from the Faza cell line, the rat liver, and the human Hep G2 cell line gave a similar signal after incubation with βSpB (Fig. 2a). In contrast, no complex was observed with either the human Jurkat or KB cell lines or the mouse L, C_2C_{12}, EL4, or TEPC cell lines even when we used a fourfold greater amount of these extracts (Fig. 2a). These differences were not related to species differences since extracts from rat, mouse, and human hepatocytes bind to βSpB with similar affinity. On the basis of these results we estimate the concentration of the protein to be at least 100-fold greater in hepatic than nonhepatic cells.

To be certain that the nonhepatic extracts were not inactivated or degraded during the purification, we tested their quality using the mobility shift assay and a labeled probe containing the human α-globin promoter. After incubation of this DNA fragment with the different nonhepatic extracts (Fig. 2a,

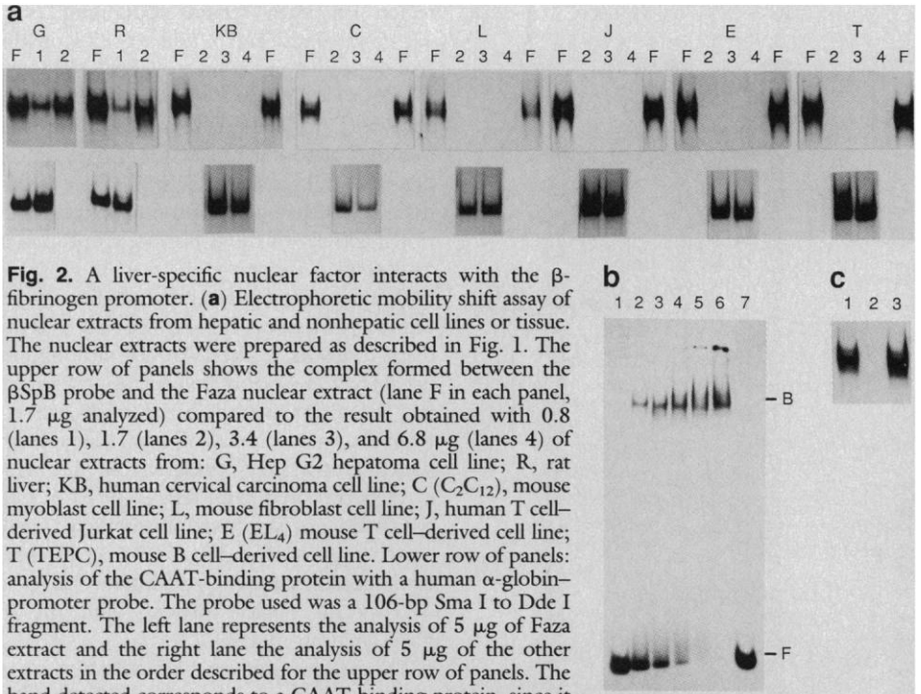

Fig. 2. A liver-specific nuclear factor interacts with the β-fibrinogen promoter. (**a**) Electrophoretic mobility shift assay of nuclear extracts from hepatic and nonhepatic cell lines or tissue. The nuclear extracts were prepared as described in Fig. 1. The upper row of panels shows the complex formed between the βSpB probe and the Faza nuclear extract (lane F in each panel, 1.7 μg analyzed) compared to the result obtained with 0.8 (lanes 1), 1.7 (lanes 2), 3.4 (lanes 3), and 6.8 μg (lanes 4) of nuclear extracts from: G, Hep G2 hepatoma cell line; R, rat liver; KB, human cervical carcinoma cell line; C (C_2C_{12}), mouse myoblast cell line; L, mouse fibroblast cell line; J, human T cell–derived Jurkat cell line; E (EL_4) mouse T cell–derived cell line; T (TEPC), mouse B cell–derived cell line. Lower row of panels: analysis of the CAAT-binding protein with a human α-globin–promoter probe. The probe used was a 106-bp Sma I to Dde I fragment. The left lane represents the analysis of 5 μg of Faza extract and the right lane the analysis of 5 μg of the other extracts in the order described for the upper row of panels. The band detected corresponds to a CAAT-binding protein, since it is in competition with the α-globin wild-type promoter but not with an α-globin promoter containing a mutated CAAT box (data not shown). (**b**) Electrophoretic mobility shift assay with a Faza nuclear extract and the probe βSpB (see Fig. 1). The assay was carried out essentially as described by Singh *et al.* (*11*), with 0.2 ng (5000 cpm) of βSpB and 3 μg of poly(dI-dC) in 15 μl of reaction volume. F, free probe; B, complex formed between the nuclear proteins and the probe; nuclear proteins in micrograms: lane 1, 0; lane 2, 1.7; lane 3, 3.5; lane 4, 7.0; lane 5, 14; lane 6, 35; lane 7, 0. (**c**) Mixing experiment. The probe βSpB was incubated with: lane 1, 1.25 μg of Faza extract; lane 2, 5 μg of KB extract; lane 3, 1.25 μg of Faza extract + 5 μg of KB extract.

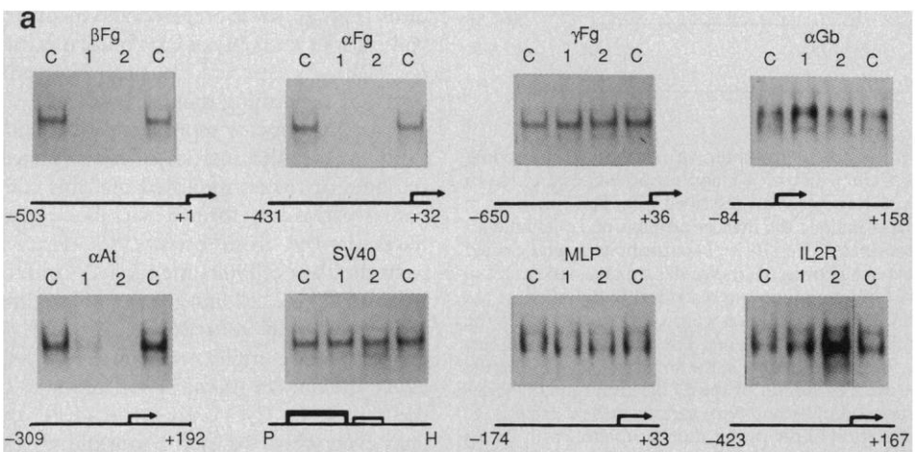

Fig. 3. (**a**) Competition for the binding of HNF1 to the β-fibrinogen promoter with various viral or cellular promoters. βFg, rat β-fibrinogen promoter; αFg, rat α-fibrinogen promoter; γFg, rat γ-fibrinogen promoter; αGb, human α-globin promoter; αAt, human α_1-antitrypsin promoter; SV40, Hind III (H) to Pvu II (P) enhancer-promoter region of SV40; MLP, major late promoter of adenovirus; IL2R, human IL-2 receptor-promoter. For each experiment the competitor used is described under the autoradiogram. The mobility shift assay was carried out as described in Fig. 3, with a Faza extract. The competitor and the probe were mixed before addition of the nuclear extract. The arrows indicate the cap sites; the large empty box and the small empty box in the SV40 fragment represent the 72-bp and the 21-bp repeats, respectively. C, control; lane 1, 20-fold molar excess of competitor; lane 2, 100-fold molar excess. (**b**) Sequence similarities between rat α-fibrinogen (*25*), rat β-fibrinogen (*25*), and human α_1-antitrypsin (*17*) promoters. The arrow indicates a sequence shared by the three promoters.

lower row of panels), we detected a single band, corresponding to a CAAT-binding protein, whose intensity and integrity were similar to the CAAT band from Faza. In addition, intact complexes representing the adenovirus major late transcription factor (*12*) could be detected in every extract with a probe derived from the rat γ-fibrinogen promoter, which binds this factor (*13*). Finally, to eliminate the possibility that the nonhepatic extracts inhibited the formation of the complex with the −102 to −75 region of β fibrinogen, we carried out a mixing experiment using the Faza and KB extracts (Fig. 2c). No difference was observed when we incubated βSpB with the Faza extract alone (lane 1) or with a mixture of Faza and KB extracts (lane 3). These controls suggest that the results from the footprinting assay and the mobility shift assay truly reflect either the absence or the extremely low concentration of the protein that binds to the −102 to −75 region of the β-fibrinogen promoter in nonhepatic cell lines. Hence, we propose the name HNF1 (hepatocyte nuclear factor 1) for this protein because only one polypeptide interacts with this region [cross-linking experiments with ultraviolet (UV) light reveal a single band of about 85 kD (*14*)].

Competition experiments were conducted to analyze the sequence specificity of the interaction of HNF1 with the −102 to −75 region of the β-fibrinogen promoter. The SV40 enhancer-promoter, the major late promoter of adenovirus, the human α-globin promoter, and the interleukin-2 (IL-2) receptor-promoter were unable to compete for the formation of the complex with βSpB (Fig. 3a). Using DNA fragments derived from the three fibrinogen genes, we observed, as expected, competition with the β promoter but did not detect any competition with the γ promoter, which interacts with the transcription factors Sp1 (*15*), CAAT-binding protein (*16*), and major late transcription factor (MLTF) (*12, 13*). In contrast, competition was observed when we used sequences extending from −431 to +32 of the α-fibrinogen promoter. Similar competition was observed with a shorter fragment extending from −88 to +32, suggesting that a homologous sequence present in α fibrinogen between −51 and −46 (Fig. 3b) is a binding site for the same factor as β fibrinogen.

Since a similar sequence is present in the α_1-antitrypsin promoter (*17*), between −69 and −58 (Fig. 3b), we carried out a competition experiment and found that a fragment derived from this promoter efficiently competed for binding of the liver-specific factor to β fibrinogen (Fig. 3a). This led us to speculate that a sequence related to AT-

TAAC was the binding site for HNF1 and that this protein was interacting not only with the β-fibrinogen promoter but also with the α-fibrinogen and α_1-antitrypsin promoters. The sequence of the γ-fibrinogen promoter (*18*) does not contain the ATTAAC sequence, a finding that is consistent with the lack of competition for HNF1 by the γ-fibrinogen promoter.

To test the validity of our hypothesis, we performed DNase I protection experiments with the α-fibrinogen and α_1-antitrypsin promoters (Fig. 4). With the α-fibrinogen promoter, two footprinted regions were detected, one of them covering the putative consensus site for HNF1. With α_1-antitrypsin a footprint was also observed in the expected region. Moreover, a synthetic 28-bp fragment corresponding to the proximal footprinted region of β fibrinogen competed for proteins that protect the ATTAAC region in β fibrinogen and also in α fibrinogen and α_1-antitrypsin, which suggests that HNF1 binds to these three promoters.

To confirm that the β-fibrinogen, α-fibrinogen, and α_1-antitrypsin promoters interact with HNF1, we investigated the complexes formed with these promoters using a mobility shift assay. Two probes derived from the α-fibrinogen and the α_1-antitrypsin promoters were isolated and incubated with three different nuclear extracts derived from Faza, KB, and Jurkat cell lines. As shown in Fig. 5, the Faza cell nuclear extract formed a complex with the α-fibrinogen and α_1-antitrypsin probes whose characteristics are very similar to the complex detected with the β-fibrinogen probe. These characteristics can be summarized as follows: first, the complexes observed with β fibrinogen, α fibrinogen, or α_1-antitrypsin migrate at the same position (compare lanes 1 and 3 in Fig. 5, a and b); second, they are tissue-specific since we did not detect them in a KB or a Jurkat extract (Fig. 5a, lanes 4 and 5, and Fig. 5b, lanes 4 to 7); third, the β synthetic 28-bp fragment, the α fibrinogen, and the α_1-antitrypsin promoters did compete for the binding, but the major late promoter of adenovirus did not compete (Fig. 5a, lanes 7 to 10, and Fig. 5b, lanes 10 to 13).

The ATTAAC sequence of the α_1-antitrypsin promoter is within a region essential for the tissue-specific expression of the α_1-antitrypsin gene. Recently the −345 to −30 region of this promoter was shown to confer hepatocyte-specificity of CAT expression (*19*). The ATTAAC sequence between positions −69 and −58 is thus within the sequences necessary for optimum expression of α_1-antitrypsin in hepatocytes.

Moreover, we have investigated the role of the ATTAAC sequence of the α-fibrinogen promoter by examining the behavior of 5′ deletion mutations of this region after transfection into hepatocytes. Although several regions within the α-fibrinogen promoter appear to be important, deletion of sequences between −88 and −33 resulted in a reduction to 1/10 the activity of this promoter in the Faza rat hepatocyte cell line.

Table 1. The β-fibrinogen promoter is only active in hepatoma cell lines. Deletions at the 5′ end of β-fibrinogen promoter were generated from a fusion of the −4500 to +12 sequence of the β-fibrinogen promoter to the JYM-CAT plasmid (*21*) with Bal 31 nuclease (*22*). An internal deletion, βID −109/−78, was also prepared by removing nucleotides between −109 and −78 with Bal 31 and a Sph I linker. Cells (2×10^6) were cotransfected with 20 μg of each construct and 1 μg of pSV2-luciferase (*8*), by means of the calcium phosphate procedure (*23*). After 18 hours of exposure to the precipitate, the cells were shocked by exposure to glycerol for 1 to 2 minutes and incubated for 16 hours at 37°C. CAT and luciferase amounts were determined according to published procedures (*8, 24*). Each percentage conversion has been corrected for transfection efficiency, with pSV2-luciferase being used as internal control. Values represent the percentage of CAT conversion compared to the conversion obtained with a pSV2-CAT plasmid transfected in parallel. Each value represents the mean of at least two experiments. The results did not vary by more than 20% of the indicated value upon repeat experiments.

Cell line	CAT conversion by plasmids					
	β4500	β503	β117	β78	βID −109/−78	SV2
Faza	20	16.2	5.4	<0.5	1.1	100
Hep G2	20	16.7	5.8	<0.5	—	100
KB	<0.5	<0.5	<0.5	<0.5	—	100
L	<0.5	<0.5	<0.5	<0.5	—	100
Jurkat	<0.5	<0.5	<0.5	<0.5	—	100

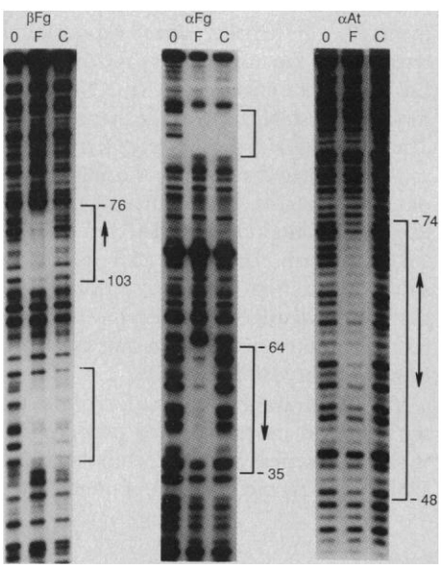

Fig. 4. Footprint analysis of α-fibrinogen and α_1-antitrypsin promoters. βFg, noncoding-strand analysis of rat β-fibrinogen promoter; αFg, coding strand analysis of rat α-fibrinogen promoter; αAt, coding strand analysis of human α_1-antitrypsin promoter. 0, digestion of the probe after incubation with 25 μg of BSA as control; F, digestion of the probe after incubation with 25 μg of Faza nuclear extract; C, same as F, except that a 100-fold molar excess of a synthetic 28-bp fragment corresponding to the −103 to −76 region of β promoter was added to the probe before the nuclear extract. The arrow represents the sequence homology between the three promoters (see Fig. 3b).

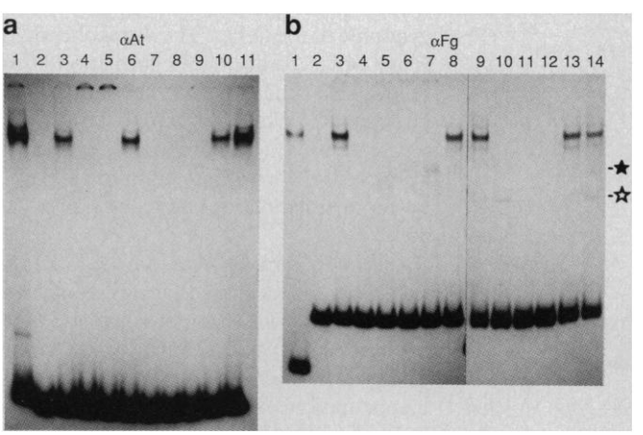

Fig. 5. α-Fibrinogen, β-fibrinogen, and α_1-antitrypsin promoters interact with HNF1. (**a**) Electrophoretic mobility shift assay with an α_1-antitrypsin promoter fragment. The probe used extends from −75 to −30 (*23*). Lane 1, probe βSpB + Faza extract (1.25 μg); lane 2, α_1-antitrypsin probe alone; lanes 3, 6, and 11, α_1-antitrypsin probe + Faza (2.5 μg); lane 4, + KB (10 μg); lane 5, + Jurkat (10 μg); lane 7, competition with 100-fold molar excess of α-fibrinogen promoter (see Fig. 3a); lane 8, competition with β-fibrinogen promoter; lane 9, competition with α_1-antitrypsin promoter; lane 10, competition with adenovirus major late promoter. (**b**) Electrophoretic mobility shift assay with an α-fibrinogen promoter fragment. The probe used extends from −70 to +32 (*22*). Lane 1, probe βSpB + Faza extract (1.25 μg); lane 2, probe α fibrinogen alone; lanes 3, 8, 9, and 14, probe α fibrinogen + Faza (2.5 μg); lane 4, + KB (2.5 μg); lane 5, + KB (5 μg); lane 6, + Jurkat (2.5 μg); lane 7, + Jurkat (5 μg); lane 10, competition with 100-fold molar excess of α-fibrinogen promoter; lane 11, competition with β-fibrinogen promoter; lane 12, competition with α_1-antitrypsin promoter; lane 13, competition with adenovirus major late promoter. Stars indicate interactions with the −32 to +32 portion of the probe.

Thus the α-fibrinogen ATTAAC sequence is also within a region essential for optimum function of this promoter.

One copy of the ATTAAC sequence is present in the rat, mouse, and human albumin promoters in the opposite orientation at position −54, and deletion of this sequence results in a reduction in tissue-specific in vitro transcription (*5*). This region is protected from DNase I digestion by a protein in liver cells (*20*). These results suggest that HNF1 also interacts with the albumin promoter.

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Dorsal, an Embryonic Polarity Gene in *Drosophila*, Is Homologous to the Vertebrate Proto-oncogene, c-*rel*

RUTH STEWARD

The *Drosophila* gene, *dorsal*, is a maternal effect locus that is essential for the establishment of dorsal-ventral polarity in the developing embryo. The *dorsal* protein was predicted from the complementary DNA sequence; it is almost 50 percent identical, over an extensive region, to the protein encoded by the avian oncogene v-*rel*, its cellular homolog, c-*rel*, and a human c-*rel* fragment. The oncogene v-*rel* is highly oncogenic in avian lymphoid, spleen, and bone marrow cells.

THE FINAL IDENTITY OF EACH CELL along the dorsal-ventral axis in the *Drosophila* embryo is dependent on the interaction of maternal effect and zygotic gene products during oogenesis and early embryogenesis. The maternal effect gene products control the expression of zygotic genes that are essential for the elaboration of the asymmetric pattern along the dorsal-ventral axis. Exhaustive mutant screens have led to the identification of 12 such genes (*1*). Females homozygous for loss or reduction of function mutations in 11 of these genes (the "dorsal group") produce embryos missing the ventral pattern elements and body parts; all of the embryonic cells assume a fate normally assigned to cells at a more dorsal position. Genetic experiments suggest that the products of these 11 maternal effect genes participate in a complex morphogenetic pathway resulting in dorsal-ventral polarity in the embryo (*2*).

The *dorsal* locus was the first "dorsal group" gene to be identified (*3*). Females that are homozygous mutant for *dorsal* produce embryos that fail to establish normal dorsal-ventral polarity, irrespective of the genotype of the father. The *dorsal* phenotype is first observed during the formation of the cellular blastoderm, 2.5 to 3 hours after fertilization. While *dorsal* mutations cause severe perturbation along the dorsal-ventral axis of the developing embryo, anterior-posterior polarity appears to be normal (*4, 5*).

Although *dorsal* is a maternal effect locus and must be expressed during oogenesis, two observations suggest that the gene product is active or required early in embryogenesis. First, temperature-shift experiments indicate that the *dorsal* protein is active during a short period in early embryogenesis (between 1.25 and 2.5 hours post-fertilization, before cellular blastoderm formation) (*6*). Second, *dorsal* embryos can be partially rescued by injection of cytoplasm from wild-type, cleavage-stage embryos (*7*). Injection experiments also suggest that the distribution of the *dorsal* rescuing activity changes during early embryogenesis from a uniform distribution on both the ventral and dorsal sides of the embryo immediately after the egg is fertilized to an enrichment on the ventral side by the syncytial blastoderm stage (*8*). It seems likely that other genes in the dorsal group also participate in this asymmetric distribution of the *dorsal* rescuing activity.

The *dorsal* gene has been cloned and characterized (*9*). The *dorsal* transcription unit is about 14 kb in length and encodes a polyadenylated [poly(A)$^+$] RNA of about 2.8 kb (Fig. 1). We found that the gene is only transcribed in adult females and not in males, or at other stages during development (*9*). In females, *dorsal* expression is restricted to the nurse cells of the ovary, and the *dorsal* messenger RNA (mRNA) accumulates in a stable form in the maturing egg. The *dorsal* mRNA persists after fertilization, can be detected throughout the first 2 hours of embryogenesis, and then turns over rapidly and can no longer be detected by cellular blastoderm formation (2.5 hours). In situ hybridization to tissue sections shows that the *dorsal* mRNA is uniformly distributed throughout the ooplasm and cytoplasm of early embryos (*5, 10*).

To extend the characterization of the *dorsal* gene and its product (or products) we isolated a series of *dorsal* complementary DNA (cDNA) recombinants from *Drosophi-*

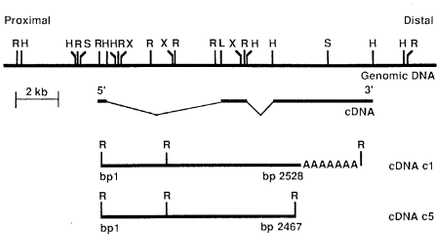

Fig. 1. Restriction map of the *dorsal* region, RNA coding region, and cDNAs. (R) Eco RI; (H) Hind III; (L) Sal I; (S) Sst I; (X) Xho I. The cDNAs were isolated from a cDNA library constructed from 0- to 2-hour poly(A)$^+$ embryonic RNA (*12*).

Department of Biology, Princeton University, Princeton, NJ 08544.