Regulation of γ-Fibrinogen Chain Expression by Heterogeneous Nuclear Ribonucleoprotein A1*

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Earlier studies showed that HepG2 cells stably transfected with any one fibrinogen chain cDNA enhanced the expression of the other two fibrinogen chains. In this report, a regulatory element “TGCTCTC” in the γ-fibrinogen promoter region, −322 to −316, is identified, which is involved in increased expression of γ chain in HepG2 cells that are transfected with Bβ fibrinogen cDNA. By electrophoretic mobility shift assay, three DNA-protein complexes were found to form with the regulatory element. The amount of the protein complexes that bind with the regulatory element was much reduced in HepG2 cells transfected with Bβ cDNA. By DNA-affinity chromatography, mass spectrometry, and supershift assay, human heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) was identified as a component of the complexes. Overexpression of hnRNP A1 suppressed basal γ-fibrinogen transcription. These results indicate that the basal expression of γ-fibrinogen is regulated by a constitutive transcriptional repressor protein, hnRNP A1, and the decreased binding activity of hnRNP A1 leads to the overexpression of γ chain in HepG2 cells that overexpress the Bβ chain.

Fibrinogen (340 kDa) is a dimer with each half-molecule composed of three different polypeptide chains as follows: Aα, 67 kDa; Bβ, 56 kDa; and γ, 47 kDa. Two of the chains, Bβ and γ, are glycoproteins with N-linked sugars (1, 2). Each chain is encoded by a distinct gene, and these genes are clustered in a region of ~50 kb located on chromosome 4q23-q32 (3–6). The three chains of fibrinogen are mainly synthesized in liver hepatocellular parenchymal cells, and the nascent chains are then processed, glycosylated, and assembled in the endoplasmic reticulum in a stepwise manner and eventually secreted into the circulating plasma (7–10).

Fibrinogen is an acute phase protein, and its biosynthesis may increase 2–10-fold during the acute phase reaction (11). Interleukin 6 (IL-6)1 and glucocorticoids are two key factors that are involved in the increased expression and synthesis of fibrinogen in the acute phase response (12–14). A transcription factor, STAT-3, can be activated by IL-6 that acts on the Jak-like thymidine kinase gene transcriptional activity by binding to its DNA element binding complex.

STAT-3 have been identified in the promoter region of all three fibrinogen genes (15, 16). Thus at least two phenomena control expression of the fibrinogen genes as follows: liver-specific constitutive regulation and modulation during the acute phase response. At the basal level, there appears to be different levels of gene regulation. For example, there is an excess of Aα and γ and smaller amounts of Bβ chains in HepG2 cells, a human hepatocellular carcinoma cell line, partly due to reduced synthesis of Bβ chain, suggesting that Bβ is rate-limiting for the assembly and secretion of mature fibrinogen. The different amounts of intracellular fibrinogen chains, however, can also be due to a combination of different rates of expression and intracellular degradation (7, 8, 17, 20, 21). In HepG2 cells, overexpression of any one fibrinogen gene, elicited by transfection, leads to the concurrent up-regulation of the other two genes, suggesting coordinate gene expression (22–24). The up-regulation of fibrinogen genes is because of the increased RNA biosynthesis (22). However, the process that coordinates the basal expression of the three fibrinogen chains is not understood. In this study, a regulatory element involved in this coordinated expression is identified in the γ gene promoter region, and heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) is shown to be a component of this regulatory element binding complex.

There are over 20 heterogeneous nuclear ribonucleoproteins (hnRNPs), designated A–U, in human cells (25). These proteins contribute to the complex around nascent pre-mRNA and thus are able to modulate RNA processing (26, 27). hnRNP A1 is the best characterized protein from this family. It has a role in pre-mRNA processing and mRNA transport and participates in telomeric length maintenance (28–32). Recently, an additional role for hnRNP A1 in RNA biogenesis has been reported because it could be a regulator of gene expression through direct DNA binding or interaction with other proteins (33–37). For example, hnRNP A1 has been described to suppress human thyroidine kinase gene transcriptional activity by binding to its promoter (34). hnRNP A1 can also modulate apoE promoter activity by interacting with the ~219T allelic form (37). The interaction of hnRNP A1 with hormone-response elements of vitamin D receptor can cause vitamin D resistance (35, 36). In this work, human hnRNP A1 is identified as a constitutive transcriptional repressor protein that regulates fibrinogen γ chain gene transcription.

EXPERIMENTAL PROCEDURES

Materials—The rabbit polyclonal antibodies to human hnRNP A/B and hnRNP C1/C2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Goat polyclonal antibodies to human hnRNP A3 and hnRNP A0 were also purchased from Santa Cruz Biotechnology. Polyclonal rabbit antibody to AUF1 (hnRNP D) was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY), and a mouse monoclonal antibody to hnRNP A2B1 was from Abcam, Inc. (Cambridge, MA). The mouse monoclonal antibody to hnRNP A1 was a generous gift from Dr. G. Dreyfuss (University of Pennsylvania).

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and Bβ plasmid. After transfection, Neo- and Bβ plasmid expression vector pGL3 upstream of the luciferase (Luc) gene. All constructs were transfected into Neo- and Bβ-HepG2 cells. Luciferase expression was measured 48 h after transfection. Relative luciferase activity is determined as a ratio of luciferase activities, in relative light units, to activities of β-galactosidase. Values are the mean of four independent experiments for each construct, and the error bars indicate the standard deviation.

**Cell Culture**—HepG2 cells were maintained in Eagle’s minimal essential medium containing 10% fetal calf serum and penicillin/streptomycin. The stable cell lines Bβ-HepG2 cells that were transfected with Bβ cDNA expression vector and overexpress Bβ chains and the control cells, Neo-HepG2, transfected with control vector were maintained in DEME medium with 0.6 mg/ml geneticin as described previously (22–24).

**Plasmid Constructs and Mutagenesis**—A series of DNA fragments containing different lengths of γ promoter regions was obtained from the genomic DNA of HepG2 cells by PCR and was cloned into the polylinker region of a luciferase reporter gene vector pGL3 (Promega). Site-directed mutagenesis in the γ promoter region in the pGL3 vector was performed according to the protocol supplied by the manufacturer (Stratagene). The expression vectors for human hnRNP A1 were generous gifts from Dr. John S. Adams (Burns and Allen Research Institute, CA) and Dr. Amy S. Lee (University of Southern California).

**Transient Transfection and Luciferase Activity Assay**—Aliquots (20 × 10⁴ cells) of Neo- and Bβ-HepG2 cells were plated in 24-well plates and cultured as described above to 80% confluence. After 24 h of culture, cells were transfected with 0.4 µg of various γ luciferase plasmids and site-directed mutant constructs using Lipofectamine Plus (Invitrogen). To determine transfection efficiency, 0.1 µg of Rous sarcoma virus β-galactosidase plasmid was cotransfected with each test plasmid. After transfection, Neo- and Bβ-HepG2 cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum for 48 h and then lysed using 1× reporter lysis buffer (Promega) and processed for luciferase assay (Promega). For transfections analyzing the effect of hnRNP A1, normal HepG2 cells were cotransfected with reporter plasmids, β-galactosidase plasmid, and hnRNP A1 expression plasmid or the control plasmid. After incubation for 48 h with the appropriate medium, the cells were harvested, and extracts were assayed for luciferase and β-galactosidase activities.

**Electrophoretic Mobility Shift Assay (EMSA)**—Nuclear extracts from normal HepG2 cells or Neo- and Bβ-HepG2 cells were prepared using NE-PER nuclear and cytoplasmic extraction reagents (Pierce) according to the manufacturer's protocol. Protein concentration was quantified by spectrophotometry using the Bio-Rad protein assay. Double-stranded oligonucleotide probes were synthesized as complementary single strands (Invitrogen) and annealed at 92 °C for 10 min, followed by slow cooling to room temperature. Sequences of the various oligonucleotides used were as follows: γ-fibrinogen wild-type probe (WT), 5'-AGACTAGGTTGTGCTAGCTGG-3', γ-fibrinogen WTP1, 5'-TCTGTTTCTCTTCCAGGCAG-3', mutant probe 1 (MP1), 5'-TCAGCCATGACGGCATCCTACGT-3'. Probes were prepared by end labeling the double-stranded oligonucleotides with [32P]ATP using T4 polynucleotide kinase, followed by G-50 column purification. The nuclear extracts were preincubated for 10 min at room temperature with 2 µg of poly(dI-dC) in the binding buffer (20 mM Tris-HCl, pH 8.0, 60 mM KCl, 1 mM EDTA, 12% glycerol, 1.5 mM dithiothreitol, and 1 µg/ml bovine serum albumin). The labeled probe was then added to each reaction for a 20-min incubation at room temperature, and the DNA-protein complexes that formed were analyzed on a 6% polyacrylamide gel. For competition assays, unlabeled probes were used at 30× excess to radiolabeled probes. For supershift assays, the nuclear extracts were preincubated overnight with antibody at 4 °C prior to performing the EMSA procedures.

**DNA Affinity Chromatography**—A DNA affinity resin was prepared as described by Kadonaga and Tjian (19). The high pressure liquid chromatography-purified 28-mer oligonucleotide containing three copies of the identified binding site (5'TGGTTTGCTCCTGCTCTGCTGCTGCAGC-3') was coupled to CNBr-activated Sepharose CL4B. Affinity chromatography was performed by combining the nuclear extract from HepG2 cells with competitor DNA poly(dI-dC), pelleting the insoluble protein-DNA.
complexes by centrifugation, and loading the resulting soluble material onto the DNA affinity column (2 ml) equilibrated with buffer B (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 12% glycerol, 1.5 mM dithiothreitol) containing 60 mM KCl. Then the affinity column was washed with 20 column volumes of the same buffer and eluted stepwise with buffer B containing 0.2, 0.3, 0.4, 0.6, 0.8, and 1 M of KCl. The eluted fractions were subjected to EMSA and SDS-PAGE analysis. Furthermore, the SDS-PAGE protein bands of interest were excised following Coomassie Blue staining. Protein fingerprinting was performed by tryptic digestion and matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) by the Protein Core Facility of Columbia University.

**Southwestern Blotting Assay—** Affinity-purified proteins were loaded onto 10% SDS-PAGE. The separated proteins were electroblotted to a nitrocellulose membrane. The membrane was processed for binding assay with labeled probe as described by Wilkinson et al. (38).

**Western Blotting—** Nuclear extracts from Neo- and Bβ-HepG2 cells, separated by SDS-PAGE, were electroblotted onto nitrocellulose membranes. The nonspecific binding sites of the membranes were blocked using 5% non-fat milk, followed by addition of the mouse monoclonal antibody to hnRNP A1 or CREB1 (Santa Cruz Biotechnology) or rabbit polyclonal antibody to AUF1. The amount of primary antibody bound to the proteins was detected using an Immun-Star chemiluminescence kit.

**Metabolic Labeling and Immunoprecipitation—** Neo- and Bβ-HepG2 cells were labeled with L-[35S]methionine for 1 h. The incubation media were collected, and secreted fibrinogen was isolated, as described previously (24) from the incubation media by immunoprecipitation. In pulse-chase experiments, normal HepG2 cells transfected with Bβ fibrinogen cDNA (Bβ-HepG2) were metabolically labeled for 1 h with L-[35S]methionine and the amount of radioactive fibrinogen secreted into the incubation media was measured, the Bβ-HepG2 cells synthesized and secreted more fibrinogen than the control Neo-HepG2 cells. These cell systems (Neo- and Bβ-HepG2 cells) were used to identify the regulatory elements in the promoter region involved in increased expression of intact fibrinogen promoter was used and when deletions were compared in both control HepG2 cells (Neo-HepG2) and in Bβ-HepG2 cells (Fig. 1B). The luciferase activities were measured and compared in both control HepG2 cells (Neo-HepG2) and in Bβ-HepG2 cells (Fig. 1B). The luciferase activities were higher in the Bβ-HepG2 cells as compared with the control cells when intact fibrinogen promoter was used and when deletions were made up to −357. However, when the γ chain promoter was deleted from −357 to −307, the Bβ-HepG2 cells had the same luciferase activity as the control cells. These results indicate
that the −357 to −307 region of γ-fibrinogen promoter contains important elements that are involved in its overexpression in Bβ-HepG2 cells.

**Identification of the Regulatory Elements That Are Involved in γ-Fibrinogen Overexpression in Bβ-HepG2 Cells**—To determine whether any binding factors may reside in the −357 to −307 region of γ-fibrinogen promoter, two γ-fibrinogen wild-type probes (WTPI and WTPII) covering the γ promoter region −357 to −309 were used for gel shift assays (Fig. 2). Nuclear proteins were isolated from Neo- and Bβ-HepG2 cells. By using WTPI probe (−357 to −328), a nuclear protein was bound to the labeled probe. The nuclear protein complex from Neo-HepG2 cell had a similar intensity to that from Bβ-HepG2 cells. However, at least three prominent DNA-protein complexes (complexes I, II, and III) were observed with WTP II probe (−328 to −309). Most interestingly, the intensity of the three nuclear complexes was much reduced when the nuclear proteins were from Bβ-HepG2 cells rather than from control Neo-HepG2 cells. This result suggests that the γ promoter region −328 to −309 may contain a regulatory element involved in its overexpression and that the nuclear factors bound to the element may be transcription inhibitors.

To characterize the binding elements, two mutant forms of the WTP II probe were constructed by altering selected nucleotides. Mutant probe (MP) I changes 2 bases located in the middle part of WTP II probe, and MPII changes 3 bases around the middle part of WTP II probe. The two mutant oligonucleotides were used as competitors in EMSA employing nuclear proteins obtained from normal HepG2 cells. Results are shown in Fig. 3. Three protein complexes were detected with the wild-type probe (WTP II) (Fig. 3, lane 2), and unlabeled WTP II probe almost abolished the formation of the protein-DNA complexes. However, 30-fold excess of unlabeled MPI and MPII could not abolish the formation of the protein-DNA complexes (Fig. 3, lanes 3 and 4). These results demonstrate that there is a protein-binding site around the region of the CTCTC sequence in the WTP II probe.

**Functional Analysis of the Forming Site of the Three Nuclear Protein Complexes**—To prove further that the γ-fibrinogen promoter region from −320 to −316 (“CTCTC” sequence) contains a regulatory element, luciferase reporter assays were performed with γ-fibrinogen promoter constructs containing internal deletions. The pGL3-γ357 vector was used for site-directed mutagenesis. Based on the EMSA results described above, we deleted a 5-bp “TCTCA” (from −319 to −315) fragment from the pGL3-γ357 vector. The mutagenized construct was then transfected into Neo- and Bβ-HepG2 cells. Luciferase reporter assays with the mutagenized construct showed that deletion of this region significantly reduced the luciferase activity in Bβ-HepG2 cells to near control levels (Fig. 4A). This result indicates that the region within the γ-fibrinogen promoter region from −320 to −315 not only binds with nuclear proteins but also contains a functional regulatory site involved in overexpression of γ-fibrinogen in Bβ-HepG2 cells.
accurately the regulatory element involved in overexpression of γ-fibrinogen in Bβ-HepG2 cells, a series of pGL3-γ357 vectors were constructed containing single or double base deletions within the “CTCTCA” sequence. The sites altered are shown in Fig. 4B. These vectors were then transfected into Neo- and Bβ-HepG2 cells, and luciferase assays were performed. Single or double base deletions within the box “TGCTCTC” (−322 to −316) reduced luciferase activities in Bβ-HepG2 cells to the level in Neo-HepG2 cells (Fig. 4B). However, wild-type construct and two mutations outside the box (Fig. 4B, constructs 5 and 6) had no effect. Therefore, this box TGCTCTC in the γ-fibrinogen promoter region from −322 to −316 may be a regulatory site involved in the overexpression of γ-fibrinogen in Bβ-HepG2 cells.

**Affinity Purification of the Protein Generating One of the DNA-Protein Complexes**—The experiments described above suggested that the TGCTCTC box may be involved in the overexpression of γ-fibrinogen gene and that several protein complexes are bound to this sequence. To identify the proteins, nuclear extracts from HepG2 cells were subjected to DNA affinity chromatography. The DNA affinity resin was prepared by coupling a 28-mer oligonucleotide containing three copies of TGCTCTC to CNBr-activated Sepharose CL4B. The eluted fractions from the affinity column were subjected to gel mobilization and analyzed by SDS-PAGE and Western blotting.

**Fig. 5. Characterization of isolated proteins binding to the TGCTCTC element.** A, DNA affinity column containing three repeat TGCTCTC sequences was used to isolate the binding proteins (see “Experimental Procedures”). The eluted fractions were assayed by EMSA, and the arrow denotes the bound labeled probes present in fractions 8 and 9 of the 0.6 M KCl eluate. The nuclear extract not subjected to fractionation is shown as a control. B, the DNA affinity fractions were subjected to SDS-PAGE analysis and stained with Coomassie Blue. Three major protein bands in fraction 8 are marked with arrowheads. C, Southwestern blot analysis was performed on the eluted fractions using radiolabeled 3X TGCTCTC probe. A 34-kDa protein band in fraction 8 was identified and is marked with an arrowhead. D, the isolated nuclear protein fractions were separated by SDS-PAGE and transferred to nitrocellulose membranes, and Western blot was performed with a mouse monoclonal antibody to hnRNP A1. E, nuclear extracts from Neo- and Bβ-HepG2 cells were separated by SDS-PAGE, and Western blots were performed with anti-hnRNP A1, anti-CREB1, and anti-AUF1 antibodies.
shift assays with a series of antibodies were performed. The probe used in the supershift assay is wild-type probe II (WTP II). A, the antibodies used are nonimmune serum (NIS), lane 1; anti-hnRNP A/B, lane 2; and anti-hnRNP C1/C2, lane 3. B, nonimmune serum, lane 1; anti-hnRNP A0, lane 2; A3, lane 3; A1, lane 4; A2/B1, lane 5; and anti-AUF1, lane 6. The DNA-protein complexes are marked as I–III.

The DNA-protein complexes are marked as I–III. The results are shown in Fig. 6. In Fig. 6A, lane 1, three complexes were competed away by addition of this antibody, and in addition a supershifted band was observed (Fig. 6A, lane 2). However anti-hnRNP C1/C2 was not able to inhibit the formation of the three complexes (Fig. 6A, lane 3). Because the hnRNP A/B antibody is a rabbit polyclonal antibody and reacts with several kinds of hnRNP (A0, A1, A2, A3, B1, and D etc.), the result suggests that all three complexes may be derived from hnRNPs. Next, further supershift assays were performed with a series of antibodies. As shown in Fig. 6B, the addition of a mouse monoclonal antibody to human hnRNP A1 reduced the binding of complexes I and III with WTP II probe (Fig. 6B, lane 4). Except for anti-hnRNP A1 that decreased the amounts of complexes I and III, other antibodies tested (Fig. 6B, lanes 2, 3, 5, and 6) had no effect, suggesting that complex II may be derived from other hnRNPs.

Effect of hnRNP A1 on the Transcriptional Activity of γ-Fibrinogen Promoter—To confirm the functional link between fibrinogen expression and hnRNP A1-mediated transcription, further reporter studies were carried out using HepG2 cells cotransfected with a γ-luciferase reporter vector together with expression constructs for hnRNP A1 or empty vector alone (control). The results are shown in Fig. 7A. By using the wild-type luciferase reporter vector pGL-γ357, overexpression of hnRNP A1 was able to suppress basal γ-fibrinogen transcription. However, the suppression effect was abolished when either the mutant vector in which the hnRNP A1 binding sequence TGCTCTC (−322 to −316) was deleted from the pGL-γ357 or the shortened γ promoter-luciferase reporter vectors (pGL-γ307 and pGL-γ280) were used. These results also explain the previous EMSA finding in which the intensity of the nuclear protein complex binding with WTP II probe was much reduced in the Bβ-fibrinogen cells, indicating that it is the decreased binding of repressor protein hnRNP A1 with γ promoter leads to the overexpression of γ-fibrinogen in Bβ-HepG2 cells.

The effect of overexpression of hnRNP A1 on the synthesis of fibrinogen was also determined. HepG2 cells were transfected with either hnRNP A1 expression vector or an empty vector as a control. After 48 h the transfected cells were pulse-labeled with 1-[35S]methionine for 8 min, and the amounts of radioactive fibrinogen and hnRNP A1 were determined. As expected, cells transfected with hnRNP A1 cDNA synthesized more hnRNP A1 but less γ-fibrinogen than control cells. Most interestingly, the synthesis of the other two fibrinogen chains, Aα and Bβ, was also reduced in the HepG2 cells transfected with hnRNP A1 (Fig. 7B), indicating that hnRNP A1 may also be involved in regulating, by suppression, the expression of the Aα and Bβ chains. In addition, the overexpression of fibrinogen in Bβ-HepG2 cells might be due to decreased amounts and activity of hnRNP A1 in the cell nucleus.

DISCUSSION

Fibrinogen expression is regulated at the following two levels: the basal or constitutive level and during the acute phase response when it is stimulated by IL-6. When fibrinogen is expressed at basal levels, in HepG2 cells, there is steady state pool of surplus Aα and γ chains, present as Aα-γ and free γ chains (7, 8). Our previous studies indicate that overexpression of the basal rate of fibrinogen expression can be accomplished in HepG2 cells by transfection with vectors containing individual fibrinogen chain cDNAs (22–24). In this situation, in which a single fibrinogen chain is overexpressed, the cells compensate by up-regulating the expression of the other two chains. Thus the expression of all three fibrinogen genes is tightly linked,
but the mechanism by which this occurs is not yet understood. A comparison of the promoter regions of the three fibrinogen genes does not show striking general homology, which suggests that the genes may be independently expressed. However, many cis-acting elements in the three fibrinogen genes have been identified in common (15, 39–43). In addition to these identified regulatory elements, a number of other as yet unidentified factors may be involved in regulating transcription of the three genes.

Epidemiological studies support a direct correlation between certain polymorphisms in the promoter region of the B chain and elevated circulating fibrinogen levels (44–46). An elevated level of plasma fibrinogen is also correlated with cardiovascular disease. The trans-activation protein complexes that bind to the B chain promoter containing the −455/G and −854/G polymorphism has been described (47, 48). It has also been shown that −455/G and −854/G polymorphisms show increased Bβ fibrinogen chain expression (47, 48) and that these relatively common polymorphic forms are associated with increased levels of plasma fibrinogen in healthy middle-aged men (48). These naturally occurring in vivo situations may be compared with the HepG2 system used in this study that overexpresses the Bβ chain.

The current study identifies a regulatory sequence TGCTCTC in the γ-fibrinogen promoter that is involved in overexpression of the γ chain in HepG2 cells transfected with Bβ cDNA (Bβ-HepG2). Three protein complexes (I–III) particularly bind with the TGCTCTC box, and a nuclear protein, hnRNP A1, was identified as a component of complexes I and III. Furthermore, the EMSA results showed that the amount of nuclear complexes bound with TGCTCTC box was much reduced in Bβ-HepG2 cells, and in addition, overexpression of hnRNP A1 suppressed basal γ-fibrinogen transcription. Taken together these findings indicate that hnRNP A1 is a constitutive transcriptional repressor protein that regulates the basal expression of γ-fibrinogen. Overexpression of hnRNP A1 in normal HepG2 cells also leads to decreased synthesis of Aα and Bβ chains, suggesting that this factor may be a repressor not only for γ-fibrinogen but also affects the expression of the other two fibrinogen chains (Fig. 7B). Several similar elements with TGCTCTC sequence are also found in both Aα and Bβ gene promoters, indicating that hnRNP A1 may also regulate Aα and Bβ basal expression.

hnRNP A1 is an abundantly expressed protein, which is better known as a participant in splicing, mRNA transport, and telomere biogenesis. In addition, it has also been shown recently that hnRNP A1 can act as either a transcriptional activator or a repressor. Although the sequence specificity for

![Diagram](image)
hnRNP A1 binding with double-stranded DNA, is not very clear, recent studies have identified a specific 36-bp target sequence (18). The 36-bp sequence is as follows: 5′-GGCTTGG-TCTTGAACCTCTGTCA(A/G)GCTCA(A/G)GTGA(T/C)TCC-3′.

Although the sequence does not contain the reported “ATTT” motif present in the human thymidine kinase gene promoter, which has been shown to bind hnRNP A1 and corresponds to its recognition sequence in RNA (34), the 36-bp sequence includes two adjacent sites, “AGCTCA” and “AGGTGA,” which are underlined and are similar to a recently reported hnRNP A1 binding sequence “AGCTCA.” The AGCTCA site can bind hnRNP A1 and leads to vitamin D resistance (36). Most interestingly, within the hnRNP A1 binding sequence TGCTTCTC, which is present in the γ-fibrinogen promoter region, there are two sequences similar to the underlined site in the 36-bp sequence. One sequence “TGCTCT” (−322 to −317) is similar to the AGCTCA site, and another one has the same sequence as “CTCAG” (−318 to −314 in the γ promoter region). This result suggests that the underlined site (A/G)GCTCA(A/G)GTGA in the 36-bp sequence may participate in hnRNP A1 binding.

Most interestingly, in this report three protein complexes are found to bind to the TGCTTCTC box in the γ-fibrinogen promoter region. EMSAs with a monoclonal antibody to hnRNP A1 indicate that complexes I and III contain hnRNP A1, whereas complex II, which is affected by a polyclonal antibody to several hnRNPs but not by the monoclonal antibody to hnRNP A1, may contain an hnRNP family member. hnRNPs are known to have a modular structure, and some of the glycine-rich domains may mediate protein-protein interactions (26, 27). Thus, they have the opportunity to form homodimers or trimers and to facilitate or interfere with the oligomerization of other hnRNPs or cellular factors. For example, it has been reported that hnRNP A1 can bind to its site together with other hnRNPs. Both p37AUF (hnRN D) and hnRNP A1 can bind the ATTT site in human thymidine kinase gene promoter. On the other hand, hnRNP K cannot inhibit the ability of both p37AUF and hnRNP A1 to bind to the thymidine kinase gene promoter by protein-protein interaction with the two hnRNPs (34).

Although this study identifies hnRNP A1 as a repressor factor involved in the expression of γ-fibrinogen in Bβ-HepG2 cells, which overexpress Bβ chains, the mechanism by which overexpression of any one fibrinogen chain up-regulates the expression of the other two chains is not yet fully understood. In this study, it is shown that there are less amounts of nuclear hnRNP A1 in Bβ-HepG2 than that in control cells. Thus, the decreased amounts of repressor hnRNP A1 leads to less binding on the γ-fibrinogen promoter and to up-regulation of the γ-fibrinogen gene. However, we do not yet know the sensing mechanism that either the increased levels of Bβ fibrinogen RNA or of its protein chain triggers the events leading to a decrease of nuclear hnRNP A1.

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