Sp1 and Sp3 Transcription Factors Mediate Malondialdehyde-induced Collagen α1(I) Gene Expression in Cultured Hepatic Stellate Cells

Malondialdehyde, the end product of lipid peroxidation, has been shown to stimulate collagen α1(I) (Col1a1) gene expression. However, mechanisms of this effect are unclear. The purpose of this study was to clarify these mechanisms. Rat hepatic stellate cells were cultured in the presence of 200 μM malondialdehyde, and the effects on collagen gene expression and the binding of nuclear proteins to the col1a1 promoter were analyzed. Malondialdehyde treatment induced an increase in the cellular levels of col1a1 mRNA that was abrogated by pretreating cells with cycloheximide, p-hydroxymercuribenzoate, pyridoxal 5'-phosphate, and mithramycin. Transient transfections showed that malondialdehyde exerted its effect through regulatory elements located between −220 and −110 bp of the col1a1 promoter. Gel retardation assays demonstrated that malondialdehyde increased the binding of nuclear proteins to two elements located between −161 and −110 bp of the col1a1 promoter. These bindings were supershifted with Sp1 and Sp3 antibodies. Finally, malondialdehyde increased cellular levels of the Sp1 and Sp3 proteins and Sp1 mRNA. Our data indicated that treatment of hepatic stellate cells with malondialdehyde stimulated col1a1 gene expression by inducing the binding of regulatory elements located between −161 and −110 bp of the col1a1 promoter.

Lipid peroxidation by-products, particularly reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4HNE), have been incriminated in the pathogenesis of liver fibrosis. Thus, Chojkier et al. (1) first showed that MDA increased significantly the collagen α1(I) (Col1a1) mRNA in cultured human fetal fibroblasts, and Maher et al. (2) found that collagen synthesis doubled in response to MDA in rat kidney fibroblasts. Likewise, Parola et al. (3, 4) showed that 4HNE and other 4-hydroxy-2,3-alkenals, aldehyde end products of lipid peroxidations, were able to stimulate col1a1 gene expression and collagen synthesis in cultured hepatic stellate cells (HSC). Similar results have been reported by Tsukamoto and co-workers (5, 6), who also observed a significant correlation between the liver MDA and 4HNE levels and the hepatic collagen accumulation in a rat model of alcoholic liver disease. Mechanisms by which these reactive aldehydes induce col1a1 gene expression and synthesis are still unclear. However, a number of studies have provided evidence supporting the role of aldehyde-protein adducts in the regulation of col1a1 gene expression. These aldehydes are known to react with sulphydryl or amino groups to form aldehyde-protein adducts (7). These adducts have been found in alcoholic liver disease (8, 9) and other clinical conditions of chronic liver injury associated with active fibrogenesis, as well as in animal models of lipid peroxidation (6, 10–14). Moreover, antioxidant treatment significantly decreased the production of these adducts and prevented the fibrogenesis cascade (10, 13, 15). The purpose of this study was to clarify the mechanisms by which MDA induces col1a1 gene expression, particularly the transcription factors and cis-acting elements involved in the mediation of this effect.

EXPERIMENTAL PROCEDURES

Reagents—Minimum essential Eagle’s medium with Hanks’ balanced salt solution (HMEM), collagenase type VII, p-hydroxymercuribenzoate (pHMB), mithramycin, MDA, pyridoxal 5'-phosphate (P5P), and cycloheximide were from Sigma. Fetal bovine serum (FBS), l-glutamine, nonessential amino acids, and penicillin/streptomycin were purchased from Boehringer Ingelheim and Bio-Whitaker, Verviers, Belgium. Plastic cell culture flasks and dishes were from Nunc (Roskilde, Denmark).agarose and Bradford protein reagent were from Bio-Rad. Nylon filters HybondTM, L-[3H]proline (specific activity, 43 Ci/mmol), [14H]chloramphenicol (specific activity, 56 mCi/mmol), and [α-32P]dCTP (specific activity, 3000 Ci/mmol) were obtained from Amersham Biosciences. Klenow fragment of the Escherichia coli DNA polymerase I, restriction enzymes, and poly(dI- dC) were from Roche Molecular Biochemicals. pBluescript SK+ was obtained from Stratagene, Heidelberg, Germany. Synthetic oligonucleotides were supplied by Amersham Biosciences; silica plates for thin layer chromatography were from Scharlau, Barcelona, Spain; and Sp1, Sp3, NF-1, BTEB, ZAP, AP2, and C/EBPβ antibodies were from Santa Cruz Biotechnology Inc, Santa Cruz, CA. p57/co33 and p5–126/luc reporter plasmids (18) were generously provided by A. Tugores (University of California, San Diego). c-Krox protein and c-Krox antibody was a gift by Dr. Philippe Gale`ra (Laboratoire de Biochimie du Tissu Conjonctif, Faculté de Médecine, Caen, France).

HSC Isolation and Culture—HSC were isolated from adult Sprague-Dawley rat liver by collagenase digestion and purified by fluorescence-activated cell sorting (FACS) as described. HSC were plated in 10-cm dishes at 3 × 105 cells/dish in a minimum essential Eagle’s medium with 10% serum, 2.5% FBS, 2 mM L-glutamine, and 100 units/ml of penicillin/streptomycin. After 12–24 h, the medium was replaced by medium containing 5% FBS.


**Sp Proteins and Malondialdehyde-induced Collagen Expression**

Dawley rats as described originally by Friedman and Roll (19), with the minor modifications introduced by Rippe et al. (20). Cells were cultured at 37 °C in DMEM supplemented with 10% horse serum, 10% FBS, and 2% l-glutamine in a 95% air, 5% CO₂ humidified atmosphere. Growth medium was changed on a daily basis for the 1st week in culture and then every 2 days thereafter. By using this procedure, we obtained an HSC population 95–99% pure. The effect of MDA was examined by addition of this agent to cells cultured in serum-free medium.

**Plasmids**—The p(-3700)ColCAT, p(-905)ColCAT, and p(-220)ColCAT (17) constructs contain the XbaI-XbaI (nucleotides –3700 to +116), the PvuII-XbaI (nucleotides –905 to +116), or the BglII-XbaI (nucleotides –220 to +116) fragment, respectively, and the 5′-untranslated region of the first exon of the col1a1 gene fused to the bacterial chromosomal acetyltransferase (CAT) gene. The vector plasmid pUCCAT, also containing the CAT gene, was obtained by digesting p(-3700)ColCAT (16) with XbaI, removing the XbaI-XbaI fragment, and ligating both ends. The reporter plasmid p(-111)ColCAT was constructed as described elsewhere (21). The reporter plasmid p(-128)Luc was constructed by subcloning the fragment –126 to +58 of the T-cell-specific mal gene promoter into the promoterless plasmid pLuc. This plasmid contains two consensus sites for nuclear factor Sp1 (18). In the reporter plasmid p5SLuc, Sp1 cis-acting elements of the p(-128)Luc had been deleted (18). The mutated plasmids p(-905)FP2/ColCAT and p(-905)FP2/FP3/ColCAT were constructed using the technique of site-directed mutagenesis described by Ho et al. (22). The PCR products were inserted into the p(-905)ColCAT vector after removing the fragment PvuII-XbaI. In the p(-905)FP2/ColCAT construct, the binding site for Sp1 and Sp3 extending from –129 to –110 bp had been converted from GGGGCCCCGGCCG to GGGGAACCTGGCC. In addition to this mutation, in the p(-905)FP2/FP2/ColCAT construct, the sequence between –161 and –135 bp, the binding site for Sp1 and Sp3, had been converted from CCGGCCCTGT to CCGAACCTGCTT. Mobility shift assays confirmed that recombinant Sp1 does not bind oligonucleotides containing these mutated sequences (data not shown). All constructions and the orientation of the inserts were tested by restriction analysis and limited DNA sequencing by using an ABI PRISM 310 Genetic Analyzer (PerkinElmer Life Sciences).

**Northern Blotting**—HSC were incubated as described above in DMEM without FBS in the presence or absence of MDA and processed as described previously (21, 23). Rat complementary DNA for the col1a1 gene promoter into the promoterless plasmid p19Luc. This plasmid contains two consensus sites for nuclear factor Sp1 (18). In the p(-905)ColCAT construct, the sequence between –161 and –135 bp, the binding site for Sp1 and Sp3, had been converted from CCGGCCCTGT to CCGAACCTGCTT. Mobility shift assays confirmed that recombinant Sp1 does not bind oligonucleotides containing these mutated sequences (data not shown). All constructions and the orientation of the inserts were tested by restriction analysis and limited DNA sequencing by using an ABI PRISM 310 Genetic Analyzer (PerkinElmer Life Sciences).

**Preparation of Nuclear Extracts and Gel Retardation Assays**—Nuclear proteins from HSC untreated and treated with 200 μM MDA were extracted according to the method of Dignam et al. (24). The pellet was resuspended in 50 μl of Dignam C buffer, and protein concentration was determined by using the Bradford protein assay reagent (25). Gel retardation assays were performed as described in previous studies (26). The oligonucleotides used in these assays are shown in Table I. For competition experiments, 200-fold excess of unlabeled oligonucleotide was added to binding reactions. Supershift assays were performed as a standard mobility shift assay, except that 1 μl of specific polyclonal antiserum raised against Sp1, Sp3, NF-1, BTEB, Zf9, AP2, or c-Krox was added to the binding reaction for 1 h at 0 °C. Sp1 antibody is specific for Sp1 and does not cross-react with Sp3 or other members of the Sp family. Likewise, Sp3 antibody is specific for Sp3 protein and does not cross-react with Sp1.

**Transient Transfection and Chloramphenicol Acetyltransferase (CAT) Assay**—In general, 5 × 10⁴ HSC were transiently transfected prior to confluence by the LipofectAMINE technique (27). Cell lysates were prepared, and CAT activity was determined as described elsewhere (28). Luciferase activity was determined using the enhanced luciferase assay kit according to the manufacturer’s protocol (Analytical Luminescence, San Diego). Cell lysates were prepared in 125 μl of cell lysis buffer. Luciferase activity was determined using 50-μl aliquots, and protein concentrations were determined by using the Bradford protein assay (Bio-Rad) (25). All reporter genes were normalized for transfection efficiency by co-transfecting a constant amount of pRLSVβ-Gal reporter gene and determining β-galactosidase activity (29).

**Statistical Analysis**—All results are expressed as mean ± S.D. unless otherwise mentioned. Student’s t test was used to evaluate the difference of means between groups, accepting a p value of <0.05 as the level of significance (30).

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**Table I**

| Name | Sequence |
|------|----------|
| FP1  | 5′-CTATCGTATTGCTGGGCGCCGTT-3′ |
|      | 3′-GACTACCAGGCCCCGGCCGACTAG-5′ |
| FP2  | 5′-AGCTTCCAAATGGGGCGGCCTAG-3′ |
|      | 3′-AGTTAAAAACCGGCGGTTCCTAG-5′ |
| FP3  | 5′-ACCTTCCTCCTCCTCCTCCCCCTTCTCC-3′ |
|      | 5′-GGAGGAAAGGGAGGCGGGAGGAAG-3′ |
| FP4  | 5′-TGCgggggggggggctgtgtgaga |
|      | 3′-AACCCCGTCCCGCGGGACcactg-5′ |
| Sp1  | 5′-GggattaGGATAGGGGGGGGAC-3′ |
|      | 3′-TAAGCTTACCGCGCGCGTCTG-5′ |
| NF-1 | 5′-GTtggagattgagccatatagag-3′ |
|      | 3′-AAAACCTTACATGCGGTAAGTCG-5′ |

**RESULTS**

Malondialdehyde Induces col1a1 Gene Expression in Primary Culture of HSC—Treatment of cells with MDA led to a significant increase in the steady-state level of col1a1 mRNA without change in the 18 S RNA. This effect was time- and dose-dependent. Northern blots showed that col1a1 mRNA levels in HSC incubated in HMEM without FBS were increased 1.5-, 2-, 2.3-, 2.8-, and 3.3-fold over the control level after incubation with 25, 50, 100, 200, and 300 μM MDA, respectively, for 24 h (Fig. 1A). Likewise, col1a1 mRNA levels increased 1.1-, 1.0-, 1.8-, 2.2-, and 2.6-fold over the control level in cells treated with 200 μM MDA for 1, 2, 6, 16, and 24 h, respectively (Fig. 1B). This effect of MDA was prevented by pretreating cells with 0.1 mM cycloheximide (Fig. 2A) and with 4 μM PHMB or 10 μM PTP, two inhibitors of the aldehyde-protein adduct formation (Fig. 2B). None of these agents was toxic to the HSC at these concentrations.

MDA Induces the Binding of Trans-Acting Factors Sp1 and Sp3 to FP2 (−129 to −110 bp) and FP3 (−161 to −133 bp) of the col1a1 Promoter—Addition of 200 μM MDA to confluent HSC transiently transfected with a reporter-CAT plasmid driven by the whole col1a1 promoter resulted in a 3.4 ± 0.1-fold increase in the CAT activity (Fig. 3A). This effect was totally abrogated by pretreating HSC with 4 μM PHMB. Deletion of promoter sequences upstream of −220 bp relative to the transcription start site increased the basal CAT activity 4.4-fold (Fig. 3E) but did not abrogate the stimulatory effect of MDA on col1a1 gene expression (Fig. 3B). By contrast, HSC transfected with the construct p(-111)Col1a1, lacking sequences upstream of −110 bp, did not respond to MDA (Fig. 3C), suggesting that the MDA-responsive element is located in sequences between −220 and −111 bp relative to the transcription start site of the col1a1 promoter.

Because the 220 bp upstream of the transcription start point in the col1a1 promoter contains four regions protected from DNase I digestion by nuclear proteins from a number of cell types (16, 17, 26, 31), including HSC (20, 32), we studied whether treatment of HSC with 200 μM MDA induced any change in the binding of nuclear proteins to these elements. Gel retardation experiments showed that incubation of a 5′-end-labeled oligonucleotide containing sequences between −103 and −82 bp (FP1) with nuclear protein extracts from either untreated cells or cells treated with 200 μM MDA for 1–24 h led to the formation of two major specific DNA-protein complexes (Fig. 4). However, few changes in the pattern or intensity of the retarded bands were observed when the nuclear protein...
used in this assay was extracted from control or MDA-treated cells. A 200-fold molar excess of unlabeled Sp1 oligonucleotide abrogated the formation of the upper complex, whereas the same excess of unlabeled NF1 oligonucleotide failed to compete significantly with nuclear proteins for the binding to the FP1 probe (Fig. 4). These results suggest that treatment of HSC with the MDA does not influence the interaction of nuclear proteins with FP1.

To examine the effect of the treatment of cells with MDA on the interaction of nuclear proteins with the element extending from −129 bp to −110 bp (FP2) of the col1a1 promoter, we performed DNA mobility assays using as a radiolabeled probe the FP2 oligonucleotide. Incubation of this probe with nuclear protein extracts from either untreated or treated cells with 200 μM MDA for 1–24 h also led to the formation of two specific DNA-protein complexes. However, the intensity of these bands was clearly more pronounced when nuclear extract was prepared from cells treated with MDA for 6 or more hours (Fig. 5A). The FP2 contains a reverse CCAAT motif and a 12-bp G-C-rich direct repeat (26). Because it has been shown that the trans-acting factors NF-1 and Sp1 interact with this footprinted region in NIH 3T3 fibroblasts (26), we performed competition assays with 200-fold molar excess of unlabeled oligonucleotides with high affinity for either NF-1 or Sp1. As Fig. 5A demonstrates, the excess of NF-1 unlabeled oligonucleotide did not compete significantly with the formation of any of these bands, whereas the 200-fold molar excess of Sp1 oligonucleotide totally competed with the formation of the upper DNA-protein complex and partially with the lower one. To confirm that Sp1 is involved in the formation of the FP2-protein complex, we performed supershift assays by including in the binding reaction an Sp1-specific polyclonal antibody. This in-
incubation led to the formation of a supershifted complex and to a marked decrease in the intensity of the upper band (Fig. 5B). In an attempt to identify other nuclear factors bound to FP2, supershift assays using polyclonal antisera against Sp3, BTEB, NF1, Zf9, AP2, C/EBPβ, or c-Krox were also performed. Whereas BTEB, NF1, Zf9, AP2, C/EBPβ, and c-Krox antibodies failed to supershift any band, Sp3 antiserum supershifted almost completely the lower band (Fig. 5B). In addition, incubation of nuclear extracts and FP2 probe with Sp1 and Sp3 antibodies simultaneously supershifted both bands.

Gel retardation experiments were performed to analyze the effect of MDA treatment on the binding of nuclear proteins to the remaining two DNase I-protected regions. Incubation of nuclear extracts from confluent HSC with a radiolabeled FP3 oligonucleotide (−161 to −133 bp) led to the formation of two major DNA-protein complexes, and the intensity increased markedly with nuclear extracts from cells treated with 200 μM MDA for 6–24 h (Fig. 6A). Competition with a 200-fold molar excess of unlabeled Sp1 (Sp), NF-1 (NF), FP1 (F1) oligonucleotide was used as competitors (Comp.) in a competition assay. NE, nuclear extract.

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nucleotide containing sequence spanning nucleotides –190 to –170 of the col1a1 promoter as a labeled probe also demonstrated two major complexes formed with nuclear extract from HSC. However, the pattern and the intensity of these complexes were not modified by the treatment of cells with 200 μM MDA for 1–24 h (Fig. 7B).

To assess the role of the FP2 and FP3 in the stimulation of col1a1 gene expression, we transfected HSC with plasmids containing mutations either at FP2 [p(−905ΔFP2)ColICAT] or at FP2 and FP3 [p(−905ΔFP2/ΔFP3)ColICAT] or no mutations [p(−905)ColICAT]. As Fig. 8A shows, the stimulatory effect of MDA on p(−905)ColICAT expression disappeared in cells transfected with mutant constructs in which the Sp1/Sp3-binding sites in FP2 have been converted from GGGGCGCCGGG into GGGAACCCTGG and the Sp1/Sp3-binding sites in FP3 from CCCCGCTTCTT into CCAGACTCTT. To determine whether the binding of Sp1 and Sp3 to the col1a1 promoter is involved in the mediation of the MDA-induced expression of the col1a1 gene, we pretreated HSC with 0.1 μM mithramycin for 2 h, a drug that interferes with the binding of transcription factors to G + C-rich promoters (33). These experiments confirmed not only that mithramycin reduced the binding of nuclear proteins to the col1a1 promoter (33). These experiments confirmed not only that mithramycin reduced the binding of nuclear proteins to

Because these data suggested that the stimulatory effect of MDA was mediated through the binding of Sp proteins to the col1a1 promoter, we wanted to determine whether this effect of MDA could be reproduced in other genes containing Sp1-binding sites. Therefore, we transiently transfected HSC using either pSLuc or p(−126)Luc reporter plasmids. Whereas addition of 200 μM MDA to HSC transfected with pSLuc, a deletion construct lacking Sp1-binding site, did not change
significantly the expression of this gene, addition of the same amount of MDA to HSC transfected with p(+126)Luc, a plasmid containing two consensus Sp1-binding elements, resulted in a 3.7-fold increase in gene expression (Fig. 9).

**MDA Increases Sp1 Gene Expression and Immunoreactive Sp1 and Sp3 Proteins in Primary Culture of HSC**—To determine whether the increased binding of Sp1 and Sp3 proteins to the col1a1 promoter induced by the MDA treatment was due to a change in the amount of these proteins in the cell extract, Western blot assays were performed. These assays showed that treatment of cells with 200 μM MDA increased 2- and 3-fold the cell content in Sp3 and Sp1 proteins, respectively (Fig. 10A). Moreover, treatment of cells with 200 μM MDA increased the steady-state levels of Sp1 mRNA 4.8-fold. The maximal expression was reached by 3 h, but declined afterward (Fig. 10B).

**DISCUSSION**

In the present study, we demonstrate that MDA stimulates significantly col1a1 gene expression in primary culture of confluent HSC in a dose- and time-dependent manner (Fig. 1). These results concur with those reported by Tsukamoto and co-workers (5, 6) in HSC and by Chojkier et al. (1) and Maher et al. (2) in human and rat fibroblasts, respectively. On the contrary, Maher et al. (2) found that HSC displayed only a modest increase in collagen synthesis in response to MDA or even failed to produce collagen in response to oxidants or their by-products (34). The reasons for this discrepancy are unknown, although differences in the glutathione levels or conditions of the experiments may have an impact in the response to MDA (34).

Moreover, in a previous study (21), we showed that incubation of HSC with a pro-oxidant combination of iron, ascorbic acid, and citric acid induced a time- and dose-dependent stimulation of the col1a1 gene expression.

A large number of studies have suggested that aldehyde-
protein adducts may play a role in the regulation of collagen gene expression and in the pathogenesis of liver fibrosis (1, 6, 12, 14, 15). Our study adds new evidence supporting the role of these adducts in the mediation of these effects, because pretreatment of HSC with pHMB or P5P abolished the effect of MDA on col1a1 mRNA levels (Fig. 2B). Likewise, the activating effect of the pro-oxidant combination of iron with ascorbic and citric acids on col1a1 gene expression was abrogated by pre-treating HSC with pHMB or P5P (21). These two agents have many biological activities but share a common inhibitory effect on aldehyde-protein adduct formation (35, 36).

We also show that MDA activated col1a1 gene expression acting on DNA elements located in the 200-bp segment upstream of the transcription start site (Fig. 3). This segment contains four sites protected from DNase I digestion by nuclear proteins from HSC and other cell lines, named footprinting 1 (FP1) to footprinting 4 (FP4) (16, 17, 20, 26, 32, 37). Mobility shift assays suggested that MDA activates col1a1 gene expression by acting on the footprinted regions, FP2 and FP3 (Fig. 5 and 6), located between nucleotides −110 and −220 bp of the col1a1 promoter.

The FP2 contains a G + C-rich 12-bp direct repeat and a reverse CCAAT motif that have been shown to be a binding site for transcription factors Sp1 and NF-1 in a variety of cell extracts (16, 26). Supershift and competition assays demonstrated that the increased binding activity of nuclear proteins extracted from MDA-treated cells may be ascribed to Sp1 and Sp3 (Fig. 5). On the contrary, these assays also showed that NF-1, C/EBPβ, two CCAAT box-binding proteins, Zf9, BTEB, AP2, or c-Krox, four transcription factors binding to G + C-rich regions are not involved in the formation of any FP2-protein complex in HSC.

Our study also shows that MDA increased significantly the interaction of nuclear proteins from HSC with a radiolabeled probe containing FP3 sequence (nucleotide −165 to −133) (Fig. 6). This footprinted region contains two consecutive CCCTCC sequences that are considered consensus sequences for the transcription factor c-Krox (38). Although our study confirms an interaction between c-Krox and FP3, we showed that MDA decreases this binding without modifying the cellular content of c-Krox (Figs. 6B and 7A). Because this factor is considered an inhibitor of collagen gene transcription (37, 39, 40), this reduced DNA binding may contribute to enhance col1a1 gene expression. On the other hand, supershift assays showed that Sp1 and Sp3 are the main binding proteins responsible for the formation of the two major FP3-protein complexes in HSC (Fig. 6B). This interaction of Sp1 with FP3 has also been reported by other authors (41, 42). Although the CCCTCC sequence is an unusual Sp1-binding site, it has also been found in other gene promoters (43, 44). Moreover, Ihn and Tamaki (45) reported that the affinity of Sp1 or Sp3 for the CCCTCC motif found in FP3 was greater that that for the Sp1 consensus sequence. The MDA-induced binding of Sp1 and Sp3 to FP2 and FP3 seems to be involved in the stimulation of the col1a1 gene expression as suggested by the abrogation of the effect of MDA on the expression of this gene by mithramycin (Fig. 8). This drug inhibits the binding of transcription factors, including Sp1 and Sp3, to genes containing G + C-rich promoters (33). These effects of MDA were also confirmed on a different gene driven by two Sp1-binding sites (Fig. 9). The role played by the binding of Sp proteins to FP3 and mainly to FP2 in the mediation of the effects of MDA on col1a1 gene expression was also supported by mutation analysis at the Sp1/Sp3 sites in FP2 and FP3 (Fig. 8A).

Transcriptional control of col1a1 gene expression may be exerted either by varying levels of Sp proteins or by posttranslational modifications of these proteins (46). Our study clearly demonstrates that MDA increases Sp1 gene expression and Sp1 and Sp3 protein levels (Fig. 10), which might be responsible for both the increased FP2 and FP3 binding activity and the enhanced col1a1 gene expression observed in MDA-treated
cells. Sp1 and Sp3, like other members of the Sp family, recognize the G + C-rich repeats (47) and act as positive transcription factors on a wide variety of cellular genes (48), including collagen genes (49–51).

The results of the present study led us to consider whether this mechanism of action is specific for MDA or, on the contrary, whether it is shared by other stimulating factors of collagen gene expression such as transforming growth factor-β1, acetaldehyde, or iron salts (1, 21, 52, 53). The molecular mechanisms by which transforming growth factor-β1 and acetaldehyde increase colla1 gene expression are controversial, but in any case, they seem to be different from those used by MDA (54–57). On the contrary, the results of our study parallel those obtained when HSCs were exposed to an iron-containing solution (21), which led to the binding of transcription factors Sp1 and Sp3 to the FP2 and FP3 of the colla1 promoter and to the activation of this gene.

Acknowledgment—We are indebted to Dr. Philippe Galéa (Laboratoire de Biochimie du Tissu Conjontif, Caen, France) for the generous gift of the rabbit c-Krox antiserum and of the recombinant mouse c-Krox protein.

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*J. Biol. Chem. 2002, 277:30551-30558.*
doi: 10.1074/jbc.M203368200 originally published online June 7, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M203368200

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