A GT-rich Sequence Binding the Transcription Factor Sp1 Is Crucial for High Expression of the Human Type VII Collagen Gene (COL7A1) in Fibroblasts and Keratinocytes*

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Type VII collagen is the major component of anchoring fibrils, structural elements that stabilize the attachment of the basement membrane to the underlying dermis. In this study, we have dissected the human type VII collagen gene (COL7A1) promoter to characterize the cis-elements responsible for the expression of the gene in cultured fibroblasts and keratinocytes. Using transient cell transfections with various 5′ end deletion COL7A1 promoter/chloramphenicol acetyltransferase reporter gene plasmid constructs, we determined that the region between nucleotides −524 and −456, relative to the transcription start site, is critical for high promoter activity in both cell types studied. Gel mobility shift assays using several DNA fragments spanning this region identified a GT-rich sequence between residues −512 and −505, necessary for the binding of nuclear proteins to this region of the promoter. Point mutations abolished the binding of nuclear proteins in gel shift assays and drastically diminished the activity of the promoter in transient cell transfections. Supershift assays with antibodies against various transcription factors including Sp1, Sp3, c-Jun/AP-1, and AP-2, and competition experiments with oligonucleotides containing consensus sequences for Sp1 and AP-1 binding identified Sp1 as the transcription factor binding to this region of the COL7A1 promoter. Indeed, recombinant human Sp1 was shown to bind the COL7A1 promoter GT-rich element but not its mutated form in gel mobility shift assays. In addition, co-transfection of pPacSp1, an expression vector for Sp1, together with the COL7A1 promoter/chloramphenicol acetyltransferase construct into Sp1-deficient Drosophila Schneider SL2 cells unequivocally demonstrated that Sp1 is essential for high expression of the COL7A1 gene. These data represent the first in-depth analysis of the human COL7A1 promoter transcriptional control.

The collagens comprise a family of proteins that play a crucial role in the maintenance of the extracellular matrix integ.

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MATERIALS AND METHODS

Cell Cultures—Human dermal fibroblast cultures, established by explanting tissue specimens obtained from neonatal foreskins, were utilized in passages 3–6. The cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal calf rity. The more abundant collagens, such as types I and III, have a widespread distribution throughout the body, whereas some minor collagens have a strictly limited tissue location. Among the latter, type VII collagen is the predominant, if not the exclusive, component of anchoring fibrils, attachment structures that play a critical role in ensuring stability to the association of the basement membrane zone to the underlying papillary dermis (3, 4). Although it is generally believed that the basement membrane is of epithelial origin, differentiated fibroblasts adjacent to epithelial tissue in vivo produce basement membrane components in general and type VII collagen in particular and assist in basement membrane assembly and anchoring fibril formation on the dermal side of the basement membrane (5). Therefore, it is considered that the two main cell types producing type VII collagen are keratinocytes and fibroblasts. Type VII collagen is a homotrimer, a1/VIIα3, and each α-chain consists of a central collagenous domain of −145 kDa flanked by noncollagenous segments (6, 7). The tissue form of type VII collagen has been suggested to be an anti-parallel dimer, associated through overlapping carboxyl-terminal regions between the individual molecules. These anti-parallel dimers then aggregate laterally to form the anchoring fibrils (4).

Synthesis of functional anchoring fibrils is of critical importance in providing integrity to the cutaneous basement membrane zone, and abnormalities in these structures clinically manifest as dystrophic forms of epidermolysis bullosa, a group of bullous diseases characterized by cutaneous fragility and tendency to sub-basal lamina densa blister formation (8). In fact, recent cloning of COL7A1 genomic sequences in our laboratory (9, 10) allowed us to demonstrate that mutations within this gene are associated with different forms of dystrophic epidermolysis bullosa (11, 12). Analysis of the 5′ end sequences of both human and murine COL7A1 genes has revealed a promoter without either a canonical TATA or a CAAT box, upstream of the transcription initiation site (13), a feature usually associated with so-called housekeeping genes.

In this study, we have investigated the molecular mechanisms regulating the activity of human COL7A1 in dermal fibroblasts and epidermal keratinocytes, two principal cell types in the skin expressing the type VII collagen gene (2, 4).

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serum, 2 mM glucose, and antibiotics (100 units/ml penicillin, 50 μg/ml streptomycin-G, and 0.25 μg/ml Fungizone®). Human epidermal keratinocyte cultures, initiated by explanting foreskin specimens, were grown in serum-free, low calcium (0.15 mM) keratinocyte growth medium (Clonetics Corp., San Diego, CA), supplemented with epidermal growth factor, 50 μg/ml insulin, 10% heat-inactivated fetal calf serum, and antibiotics. Keratinocyte cultures were utilized in passages 1 and 2 to avoid differentiation inherent to prolonged subculturing of these cells. Drosophila melanogaster Schneider SL2 cells (14), kindly provided by Dr. James B. Jaynes (Jefferson Medical College), were grown in Schneider medium (Life Technologies, Inc.) supplemented with antibiotics (100 units/ml penicillin, 50 μg/ml streptomycin-G, 0.25 μg/ml Fungizone®) and 12% heat-inactivated fetal calf serum.

**Plasmid Constructs—**To study the transcriptional regulation of human type VII collagen gene (COL7A1) expression, transient transfection experiments were performed with various COL7A1 promoter 5'-deletion fragments cloned into promoterless pBSoCAT vector (15). A mutated promoter construct, −524m, was generated by polymerase chain reaction amplification using a 5' end primer containing a double point mutation (in bold) in the GT box located between nucleotides −512 and −505, GGTTGGGG GTTGGGG, and a Xhol restriction site. At the 3' end, a primer upstream of position +92 containing a HindIII restriction site was utilized. The resulting amplification product was cloned as a Xho/HindIII fragment into pBSoCAT. The fidelity of both polymerase chain reaction and subcloning procedure was ensured by automated sequencing (ABI).

pPacSp1, an expression vector for Sp1 driven by the actin promoter (16), a kind gift of Dr. Robert Tjian (University of California, Berkeley, CA), was used to express Sp1 in Drosophila SL2 cells deficient in Sp1. Empty pPac0 was used as a control.

**Transient Cell Transfections and Chloramphenicol Acetyltransferase Assays—**Transient cell transfections of human dermal fibroblasts were performed with calcium phosphate/DNA co-precipitation procedure (17). Briefly, cultured cells were transfected with 10 μg of plasmid DNA and 2 μg of the pRSV-β-galactosidase plasmid DNA to monitor the transfection efficiencies (18). After glycerol shock, the cells were placed in Dulbecco’s modified Eagle’s medium containing 1% fetal calf serum. After 40 h of incubation, the cells were harvested by scraping and lysed in 200 μl of Reporter Lysis Buffer (Promega, Madison, WI).

Basal keratinocytes grown in keratinocyte growth medium were transiently transfected with a liposome-based method (DOTAP, Boehringer Mannheim), according to the manufacturer’s protocol. Sixteen hours after transfection, medium was replaced and cells were incubated for 48 h. The cells were then collected by scraping, rinsed twice in phosphate-buffered saline, harvested by scraping, and lysed by three cycles of freeze-thawing in 200 μl of reporter lysis buffer. Aliquots corresponding to identical β-galactosidase activity were used for each CAT assay, using [3H]chloramphenicol as substrate (19).

Drosophila SL2 cells were transfected with 2 μg of −524 COL7A1 promoter/CAT reporter gene plasmid construct together with 6 μg of either pPacSp1 or pPacSp1 by the calcium phosphate/DNA co-precipitation method. The DNA precipitates were left on the cells for 48 h. The protein concentration in the extracts was determined using a commercial assay kit (Bio-Rad).

Nuclear extracts (5 μg) were incubated for 20 min on ice in binding reaction buffer (10 mM HEPES-KOH, pH 7.5, at 4 °C, 4 mM glycerol, 40 mM KCl, 0.4 mM EDTA, and 0.4 mM diethiothreitol) in the presence of 1 μg of poly(dI-dC) prior to the addition of gel-purified, 5’ end-labeled oligomers (0.05–0.1 pmol, 2 × 10⁶ cpm) for another 20-min incubation at 4 °C. For competition experiments, a 1–60-fold molar excess of unlabeled DNA was added to the binding reaction. For supershift experiments, nuclear extracts were incubated overnight at 4 °C with 2 μg of polyclonal antibodies specific for c-Jun, AP-2, Sp1, or Sp3 (Trans-Cruz® Gel Supershift Reagents, Santa Cruz Biotechnologies, Santa Cruz, CA) prior to the binding reactions. In experiments with human recombinant Sp1 (Promega Corp., Madison, WI), the amount of poly(dI-dC) per reaction was decreased to 500 ng, and bovine serum albumin (10 μg) was added to the samples. Samples were then separated by electrophoresis on a 4% polyacrylamide gel in 0.5 × Tris borate-EDTA buffer at 200 V for 2 h at 4 °C, fixed for 1 h in 30% methanol-10% acetic acid, vacuum-dried, and autoradiographed.

**RESULTS**

**Deletion Analysis of the Human COL7A1 Promoter—**To localize the cis-acting elements involved in the basal activity of the COL7A1 promoter, human dermal fibroblast and epidermal keratinocyte cultures were transiently transfected with several 5’ end deletion/CAT reporter gene constructs spanning the COL7A1 promoter region from positions −722 to +92 relative to the transcription start site, +1. A high level of activity was detected with constructs −722 and −524 in both cell types (Fig. 2, panels A and C). Subsequent 5’ deletion of 68 bp to position −456 led to a dramatic reduction (70–90%) of the promoter activity, whereas additional deletions to positions −396 and −230 had no further effect (Fig. 2, panels B and D). These data suggest that the DNA sequences located between residues −524 and −456 are essential in providing high basal activity to the human COL7A1 promoter constructs in both dermal fibroblasts and epidermal keratinocytes.

**Binding of Nuclear Factors to the −524/−491 Region of Human COL7A1 Promoter—**To elucidate the transcriptional mechanisms of type VII collagen gene expression, and in particular, the role played by the region of promoter comprised between residues −524 and −456, we analyzed the binding of nuclear factors to this region. For this purpose, we designed a series of oligonucleotides spanning this whole region. Their sequences and relative positions are depicted in Fig. 1. Gel mobility shift assays were performed either with the oligonucleotide WT, containing the entire sequence of the −524/−457 region of the COL7A1 promoter, or with three shorter oligonucleotides, I, II, and III, spanning the regions −524/−491, −490/−457, and −500/−475, respectively (see Fig. 1). Incubation of the radio-labeled WT oligonucleotide with fibroblast nuclear extracts resulted in the formation of two major DNA/protein complexes, identified as shifts 1 and 3 (Fig. 3, lane 2). The formation of these two complexes could be abolished by the addition of a 60-fold molar excess of unlabeled oligonucleotide I (Fig. 3, lane 3), but there was no competition by the addition of the same molar excess of the unlabeled oligonucleotides II or III (Fig. 3, lanes 4 and 5, respectively). Together, these data indicate that binding of nuclear proteins to the −524/−456 region of human COL7A1 promoter occurs within the region spanned by oligonucleotide I, from position −524 to −491.

In gel mobility shift assays, 1 μg was used as a probe in gel mobility shift assays. An electrophoretic pattern similar to that obtained with radiolabeled WT was observed, with the exception that the slowest migrating complex appeared as a doublet instead of a single band; these bands were identified as shifts 1 and 2 (Fig. 3, lanes 6–9). The formation of all three distinct DNA-protein complexes 1, 2, and 3 was abolished by the addi-
tion of a 60-fold molar excess of unlabeled oligonucleotide I (Fig. 3, lane 7) but not by a 60-fold molar excess of unlabeled oligonucleotides II or III (Fig. 3, lanes 8 and 9, respectively), further indicating that these three complexes specifically bind to fragment I. No specific DNA-protein complex was detected when either fragment II (Fig. 3, lanes 10–13) or fragment III (data not shown) was used as a probe. Similar results were obtained when nuclear proteins extracted from either dermal fibroblasts (see Fig. 3) or epidermal keratinocytes (data not shown) were used in gel mobility shift assays with the various radiolabeled probes. It should be noted that the electrophoretic pattern generated by nuclear extracts from fibroblasts is similar to that of keratinocyte extracts, both qualitatively and quantitatively (Fig. 4, lanes 3 and 6 versus lanes 2 and 5), suggesting that the regulatory mechanisms controlled by this region of COL7A1 promoter may be similar in both cell types.

A GT-rich Element Is Critical for Binding to the −524/−491 Region of COL7A1 Promoter—The next set of experiments was designed to further identify the cis-acting element(s) responsible for the binding of fibroblast nuclear extracts to the 34-bp segment of the COL7A1 promoter located between residues −524 and −491 (oligonucleotide I). Computer analysis for sequence homologies to known transcription factor binding sites was performed (DNASIS software, Hitachi Software Engineering Co., Yokohama, Japan), revealing two distinct areas of interest: (a) a 8-bp GT-rich sequence (GGGTGGGG) located between nucleotides −512 and −505, referred to as box A and (b) a potential γ-interferon response element, CAGGAGG, located between nucleotides −502 and −495, referred to as box B. To determine whether these two boxes were involved in the binding of nuclear proteins to fragment I, two oligonucleotides were designed spanning the whole region covered by oligomer I.

**FIG. 1. Oligonucleotides used in this study.** A, sequences of the various double-stranded oligonucleotides used in gel mobility shift assays, spanning the region from −524 to −457 of the human COL7A1 promoter. Only the upper strand is shown. Oligomers IA, IB, and ImA, respectively, correspond to fragment I devoid of box A or box B or mutated in box A (see “Results”). Mutated bases are indicated by asterisks. IA and IB were synthesized based on a computer search for potential transcription factor binding sites within the sequence spanned by oligonucleotide WT. ImA was synthesized after DNA-protein interaction assays showed the importance of box A. Consensus motifs for Sp1 and AP-1 transcription factors are underlined. NA, not applicable. B, schematic representation of the oligonucleotides. Note that fragments I and II collectively cover the entire WT sequence but do not overlap and that oligonucleotide III does not contain box A. NA, not applicable.
but devoid of either box A (ΔA) or box B (ΔB), respectively (see Fig. 1). Gel mobility shift assays performed in parallel with probes I, ΔA, or ΔB indicated that deletion of the 8-bp region encompassing box A (probe ΔA) abolished the formation of DNA-protein complexes detected with probe I (Fig. 5A, lane 4 versus lane 2), whereas deletion of box B (probe ΔB) did not alter the binding pattern obtained with probe I (Fig. 5A, lane 6 versus lane 2). These data demonstrate that the formation of all three DNA-protein complexes, shifts 1, 2, and 3, require box A between nucleotides −512 and −505.

The critical role of box A was further confirmed in competition experiments with unlabeled oligonucleotides ΔA and ΔB (Fig. 5B). Specifically, the binding activity of nuclear extracts to probe I (Fig. 5B, lane 2) was abolished by the addition of a 60-fold molar excess of cold oligonucleotide ΔB (Fig. 5B, lane 5), but it was not altered by the addition of the same molar excess of oligonucleotide ΔA (Fig. 5B, lane 8).

**Mutation of Box A Abolishes Transcription Factor Binding and Reduces COL7A1 Promoter Activity—**To further understand the importance of box A in the transcription of COL7A1, an oligonucleotide, ImA, containing a double mutation in box A (in bold), GGGTGGGG→GTTTGGGG, was generated for use in gel mobility shift assays. A 60-fold molar excess of unlabeled oligomer ImA used as a competitor in a binding reaction with radiolabeled oligomer I failed to compete with the binding of nuclear proteins to probe I (Fig. 6A, lane 4), whereas an identical amount of unlabeled oligonucleotide I abolished the binding of all three complexes 1, 2, and 3 (Fig. 6A, lane 3 versus lane 2). Furthermore, no DNA-protein complexes were detected when the mutated oligonucleotide ImA was used as a probe in a parallel DNA-protein binding assay (Fig. 6A, lane 6 versus lane 2). Collectively, these experiments demonstrate that box A is responsible for the formation of three separate DNA-protein complexes in the −524/−491 region of the human COL7A1 promoter and that the two adjacent guanosine nucleotides mutated within box A are critical for DNA-protein interaction. Also, these data indicate that box B, identified by computer sequence analysis, is not critical for basal expression of COL7A1.

To evaluate the contribution of the GT box identified between residues −512 and −505 to the basal activity of the human COL7A1 promoter, a nucleotide substitution identical to that introduced into oligomer ImA (see above and Fig. 1) was created in the −524 COL7A1 promoter/CAT construct, generating the construct −524m (see “Materials and Methods”). The basal activity of the two constructs was compared in transient cell transfections. Mutation of the GT box led to a dramatic drop, −70%, of the basal promoter activity as compared with that of the wild type −524 CAT construct, both in fibroblasts (Fig. 6B) and in keratinocytes (Fig. 6C). In fact, the CAT activity of the −524m construct was comparable with that of the −456 construct, suggesting that the 8-bp GT-rich sequence,
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FIG. 3. Binding of fibroblast nuclear proteins to WT, I and II regions of human COL7A1 promoter. Gel mobility shift assays were performed with labeled oligonucleotides spanning the region from −524 to −457 of the COL7A1 promoter. WT, lanes 1–5; probe I (−524 to −491), lanes 6–9; and probe II (−490 to −457), lanes 10–13. Details on the sequences and relationships of the different probes are provided in the legend to Fig. 1. Competition assays were performed with a 60-fold molar excess of unlabeled oligonucleotides, as indicated at the top of the figure. DNA-protein complexes were separated from unbound oligonucleotides by nondenaturing 4% acrylamide gel electrophoresis. Shifts 1, 2, and 3 indicate major DNA-protein complexes observed in this experiment. Free probes refer to the unbound radiolabeled oligonucleotides. Note the presence of several fast migrating bands with a pattern identical with all three probes used. Because probes I and II, which span WT, do not share any overlapping sequence, these bands are likely to represent nonspecific binding.

FIG. 4. Comparison of fibroblast and keratinocyte nuclear protein binding to fragments WT and I of the human COL7A1 promoter. Gel mobility shift assays were performed as described in the legend to Fig. 3 using 5 μg/lane of nuclear extracts of either fibroblasts (F) or keratinocytes (K) with either probe WT (lanes 1–3) or I (lanes 4–6).

Located between nucleotides −512 and −505 and designated as box A, is crucial in providing high basal activity of COL7A1 promoter in both cell types.

**Sp1 Is the Transcription Factor Binding to Box A—GT boxes**, such as found in box A, have been previously described in other gene promoters and shown to be potential binding sites for the transcription factor Sp1 (21), although more classical Sp1-binding sites generally consist of GC boxes (22, 23). Further, the electrophoretic pattern observed in mobility shift assays with radiolabeled oligonucleotide I as a probe closely resembles that of Sp1 binding. To ascertain that the GT box between residues −512 and −505 of the COL7A1 promoter is an Sp1-binding site, an oligonucleotide containing a high affinity Sp1 recognition site (GC box) was used as a competitor in gel mobility shift assays with probe I (Fig. 7A). The binding of nuclear factors to probe I (Fig. 7A, lane 2) was competed away by unlabeled homologous DNA (COL7A1 GT box), in a dose-dependent manner (Fig. 7A, lanes 3–5), as well as the consensus Sp1 (GC box) oligonucleotide (Fig. 7A, lanes 6–8). In contrast, the binding was not affected by a 60-fold molar excess of a consensus AP-1 oligonucleotide (Fig. 7A, lane 9), attesting to the specificity of the competition by the Sp1 oligonucleotide.

These observations prompted us to explore the possibility that Sp1 or an Sp1-related protein may interact with the GT box located between nucleotides −512 and −505 of the human COL7A1 promoter. Toward this end, supershift experiments were performed with polyclonal antiserum specific for either Sp1, AP-2, or c-Jun. As shown in Fig. 7B, the two major complexes observed with the WT oligonucleotide were replaced by a unique supershifted complex when using the polyclonal anti-Sp1 antiserum (Fig. 7B, lane 2 versus lane 1). In contrast, no supershift was observed when using specific antibodies directed against AP-2 (Fig. 7B, lane 3) or c-Jun (Fig. 7B, lane 4). Similarly, the Sp1 antibody supershifted all three complexes formed with oligonucleotide I (Fig. 7B, lane 6 versus lane 5), whereas neither AP-2 nor c-Jun antibodies had an effect on the electrophoretic mobility of the DNA-protein complexes (Fig. 7B, lanes 7 and 8, respectively). These data indicate that all three complexes are immunologically related to Sp1.

Recently, several novel factors binding to either GC or GT boxes have been identified by cDNA cloning, and many of these proteins are closely related to Sp1 (24–27). Indeed, three of these proteins have been characterized in detail and designated Sp2, Sp3, and Sp4, putative new members of the Sp1 multigene family. These proteins are predicted to contain zinc finger and trans-activation domains similar to those found in Sp1. Among these factors, Sp3 is strikingly homologous to Sp1 (26). However, a polyclonal antibody against Sp3 did not supershift any of the DNA-protein complexes formed by incubating nuclear extracts from fibroblasts with either WT or I oligonucleotides, indicating that Sp3 does not participate in the formation of these shifts (data not shown). Taken together, these results suggest that Sp1 is the transcription factor involved in the binding to the GT box located between nucleotides −512 and −505 of the COL7A1 promoter.
Recombinant Sp1 Binds Box A in Gel Mobility Shift Assays—To formally prove that Sp1 binds to the 8-bp GT-rich box of the \textit{COL7A1} promoter (box A), gel mobility shift assays were performed using human recombinant Sp1 protein (Fig. 8). Indeed, recombinant Sp1 effectively bound to probes WT (Fig. 8, lane 2) and \(I\) (Fig. 8, lane 4) and to the consensus Sp1-binding site oligonucleotide (Fig. 8, lane 8). However, this DNA-Sp1 interaction was not noted when oligonucleotide \(I\)mA, in which the GT box has been altered, was used as a probe (Fig. 8, lane 6).

**Fig. 5.** Box A is critical for binding to fragment I. A, [\(^{32}\)P]ATP-labeled oligonucleotides I (lanes 1 and 2), \(I\)\(\Delta\)A (lanes 3 and 4), and \(I\)\(\Delta\)B (lanes 5 and 6) were used in gel mobility shift assays in the absence or the presence of 5 \(\mu\)g/lane of fibroblast nuclear extract as indicated to study their respective abilities to bind transcription factors. Note the total absence of binding to probe \(I\)\(\Delta\)A, lacking box A, and the binding to \(I\)\(\Delta\)B in a manner identical to that to probe I. B, binding of nuclear proteins to probe I was competed against a 1-, 20-, or 60-fold molar excess of unlabeled fragment \(I\)\(\Delta\)B (lanes 3–5) or \(I\)\(\Delta\)A (lanes 6–8). Note that only \(I\)\(\Delta\)B, which contains box A, successfully competes nuclear protein binding to probe I.

Recombinant Sp1 Binds Box A in Gel Mobility Shift Assays—To formally prove that Sp1 binds to the 8-bp GT-rich box of the \textit{COL7A1} promoter (box A), gel mobility shift assays were performed using human recombinant Sp1 protein (Fig. 8). Indeed, recombinant Sp1 effectively bound to probes WT (Fig. 8, lane 2) and \(I\) (Fig. 8, lane 4) and to the consensus Sp1-binding site oligonucleotide (Fig. 8, lane 8). However, this DNA-Sp1 interaction was not noted when oligonucleotide \(I\)mA, in which the GT box has been altered, was used as a probe (Fig. 8, lane 6). Together with the results of competition and supershift assays described above, these data unequivocally demonstrate that Sp1 is the protein binding to the GT-rich sequence located between nucleotides \(-512\) and \(-505\) of \textit{COL7A1}. Also, these
data indicate that integrity of box A is required for Sp1 binding. These results clearly identify that all major DNA-protein complexes observed with either probe WT (shifts 1 and 3) or probe I (shifts 1 and 2) are due to Sp1 binding. Because the formation of multiple complexes between Sp1 and a single DNA probe has been previously reported (24, 28), we speculate that the fastest migrating complex (shift 3) is a minor degradation product of the Sp1 protein, as described previously (29). The two larger complexes observed with radiolabeled oligonucleotide I (shifts 1 and 2) are likely to represent two states of differential phosphorylation of Sp1 (30). At this point, we cannot explain the presence of a single upper band when oligonucleotide WT is used as a probe.

**FIG. 7.** Sp1 is the transcription factor binding to box A. A, gel mobility shift assays were performed by incubating fibroblasts nuclear extracts (5 μg/lane) with probe I (lane 2). Competitions were carried out with increasing concentrations (1-, 20-, and 60-fold molar excess) of unlabeled fragment I (lanes 3–5) with consensus Sp1 (lanes 6–8) or with a 60-fold molar excess of unlabeled oligonucleotide containing a consensus AP-1 binding site (lane 9). B, gel mobility supershift assays were performed by incubating fibroblast nuclear extracts (5 μg/lane) with polyclonal antibodies for Sp1, AP-2, or c-Jun prior to the binding reaction with radiolabeled probes WT or I. Note that the Sp1 antibody displaces all three complexes bound to probes WT and I. Similar observations were obtained in experiments utilizing keratinocyte nuclear extracts (not shown).

**FIG. 8.** Recombinant Sp1 binds the COL7A1 promoter fragment containing box A. Probes WT, I, and Ima and a radiolabeled consensus Sp1 oligonucleotide were used in parallel gel mobility shift assays with human recombinant Sp1 as described under “Materials and Methods.” Samples were separated on a 4% acrylamide gel under non-denaturing conditions. Note that Sp1 binds all but Ima probes.

not explain the presence of a single upper band when oligonucleotide WT is used as a probe.

**Sp1 trans-Activates the COL7A1 Promoter in Sp1-deficient Drosophila Cells**—As a critical test for the requirement of Sp1 in COL7A1 promoter activity, the −524 COL7A1 promoter/CAT construct was co-transfected into Drosophila SL2 cells in the presence of either pPacSp1 or pPac0 (see “Materials and Methods”). As shown in Fig. 9, the COL7A1 promoter had negligible basal activity in Sp1-deficient SL2 cells. In contrast, the promoter activity was highly induced in response to Sp1 expression, demonstrating that Sp1 is a transcription factor essential for high expression of the COL7A1 gene.

**DISCUSSION**

The interest in studying the regulation of COL7A1 gene expression is motivated by the fact that this gene product is essential for the integrity of the attachment of the epidermis to the underlying dermis. Indeed, structural abnormalities, paucity, or even absence of type VII collagen/anchoring fibrils manifest clinically as the dystrophic forms of epidermolysis bullosa, a group of blistering skin diseases characterized by cutaneous fragility and tendency to sub-basal lamina densa blister formation (31). In the present study, we provide compelling evidence for the role of the transcription factor Sp1 in maintaining high expression of COL7A1 in both fibroblasts and keratinocytes, the two principal cell types expressing type VII collagen gene in the skin (2, 4).

It has been previously assumed that the transcription initiation of eukaryotic genes could be directed either by the TATA sequence as in tissue-specific genes or by GC/GT boxes as is the case in several TATA-less housekeeping genes. In the latter case, the TATA-binding protein, TFIIID, does not directly interact with the DNA but is positioned indirectly to the correct initiation site via protein-protein interactions. In this context, Sp1 has recently been found to be able to recruit TATA-binding protein, thereby positioning the initiation complex to the correct start site (32–34). The Sp1 binding site crucial for the
Fig. 9. Expression of Sp1 in Sp1-deficient Drosophila SL2 cells induces COL7A1 promoter activity. −524 COL7A1 promoter/CAT plasmid construct was transfected in Drosophila SL2 cells with either empty pPac0 or pPacSp1 expression vector. CAT activity, representing the promoter activity, was determined 48 h later using [14C]chloramphenicol as a substrate as described under “Materials and Methods.” A representative experiment is shown. AC, acetylated chloramphenicol; C, unacetylated chloramphenicol.

maintenance of the high transcriptional activity of human COL7A1 is located at position −505, away from the transcription start site, and may not be involved in the initiation of the transcription. In this context, three other Sp1 sites (GGGCGGG) are located at positions −150, −121, and −25 upstream of the transcription initiation site (13). The fact that the COL7A1 promoter not only lacks a typical TATA element but also contains a Sp1 motif at a position usually occupied by the TATA box, around position −20, supports the notion that Sp1 may be involved in the initiation of the transcription of TATA-less promoter genes.

Although Sp1 was originally described as a ubiquitous transcription factor regulating housekeeping genes, recent observations indicate that its expression can be regulated during development (35) and that it may be important for the cell type-specific regulation of gene expression, as exemplified by studies on the keratinocyte-specific keratin K3 gene (36), demonstrating a direct correlation between the levels of Sp1 and the expression of K3. In the case of type VII collagen, Sp1 binding to box A was identical in both fibroblasts and keratinocytes (see Fig. 4), contrasting with the results of Regauer et al. (36) on K3 gene expression. Both cell types have been shown to express and synthesize type VII collagen (5, 36–38), and the latter observation being in agreement with another recent study (43). Also, it should be noted that Sp1 was recently shown to be essential for the basal expression of other extracellular matrix genes, including syndecan-1 (44), COL1A1 (45), COL6A1 (46), and the collagen/laminin receptor α2 integrin (47), and may therefore be an important factor in repair processes, such as wound healing.

In summary, using a series of 5′ deletion constructs of the COL7A1 promoter, we have identified a functional region within the human COL7A1 promoter necessary for relatively high level of expression of the gene in both fibroblasts and keratinocytes. This region located between nucleotides −524 and −456 contains a functional GT box that binds Sp1 between residues −512 and −505. Disruption of the nucleotide sequence of this 8-bp element prevents the binding of Sp1 and reduces the promoter activity by ~70%. In addition, recombinant expression of Sp1 in Sp1-deficient Drosophila SL2 cells results in high expression of the COL7A1 promoter. Collectively, these results provide the first in-depth characterization of the transcription mechanisms regulating the expression of COL7A1.

Also, our study provides further evidence that Sp1 is an essential transcription factor for the expression of extracellular matrix genes.

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A GT-rich Sequence Binding the Transcription Factor Sp1 Is Crucial for High Expression of the Human Type VII Collagen Gene (COL7A1) in Fibroblasts and Keratinocytes

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