SP3 Represses the SP1-mediated Transactivation of the Human COL2A1 Gene in Primary and De-differentiated Chondrocytes*

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SP1 and SP3 effects on the transcription of the human α1(II) procollagen gene (COL2A1) were investigated in both differentiated and de-differentiated rabbit articular chondrocytes. Transient transfection with constructs of deleted COL2A1 promoter sequences driving the luciferase reporter gene revealed that the region spanning −266 to +121 base pairs showed SP1-enhancing effects, whatever the differentiation state. In contrast, SP3 did not influence COL2A1 gene transcription. Concomitant overexpression of the two SP proteins demonstrated that SP3 blocked the SP1 induction of COL2A1 promoter activity. Moreover, inhibition of SP1/SP3 binding to their target DNA sequence decreased both COL2A1 gene transcription and SP1-enhancing effects. DNase I footprinting and gel retardation assays revealed that SP1 and SP3 bind specifically to cis-sequences of the COL2A1 gene promoter whereby they exert their transcriptional effects. SP1 and SP3 levels were found to be reduced in de-differentiated chondrocytes, as revealed by DNA-binding and immunochemical study. SP1 specifically activated collagen neosynthesis whatever the differentiation state of chondrocytes, suggesting that this factor exerts a major role in the expression of collagen type II. However, our data indicate that type II collagen-specific expression in chondrocytes depend on both the SP1/SP3 ratio and cooperation of SP1 with other transcription factors, the amounts of which are also modulated by phenotype alteration.

Differentiation of mesenchymal cells into chondrocytes results in the synthesis and secretion of a series of proteins characteristic of the cartilage matrix, including type II, IX, XI, and X collagens, the proteoglycan aggrecan, link protein, and cartilage matrix protein 1. Type II collagen is considered as a critical phenotypic marker gene for analysis of molecular events involved in chondrogenesis process as well as in chondrocyte phenotype maintenance. Alteration of type II collagen expression in cartilage may be due to a variety of genetic, inflammatory, or degenerative circumstances and may lead to a variety of chondrodysplasias and joint diseases such as osteoarthritis (3–8). In osteoarthritis, chondrocytes undergo de-differentiation and synthesize types I and III collagens at the expense of type II (9–11). Similarly, when chondrocytes are subcultured in vitro as monolayers, they progressively reduce their synthesis of type II collagen (12–14), mimicking the behavior of osteoarthritic chondrocytes. However, they can recover the chondrocytic phenotype by transfer to three-dimensional culture systems (14–16). Therefore, in vitro analysis of the molecular mechanisms that regulate COL2A1 gene expression can be an approach to understand the process of phenotype alteration in chondrocytes, and its impact on joint diseases.

A 48-bp DNA element has been identified as an enhancer that directs chondrocyte-specific expression of the COL2A1 gene in transgenic mice (17–19). Such an element was also found in the rat COL2A1 gene (20, 21). The mouse enhancer appears as a large and abundant complex with chondrocyte nuclear proteins named chondrocyte-specific enhancer proteins, including SOX9, a new long form of SOX5 (L-SOX5), and SOX6 (19). The three SOX genes are coexpressed and cooperate in COL2A1 gene activation. Although SOX9 is absolutely required for cartilage formation (22), it is unable by itself to induce the chondrocyte phenotype on 10T1/2 cells, suggesting that other factors are required to induce the cartilaginous phenotype (23).

We have previously shown that C-Krox, a member of the Krox family (24, 25), activates COL2A1 gene expression in primary rabbit articular chondrocytes (RAC) through a 458-bp enhancer containing C-Krox-responsive elements present in the first intron (26). By contrast, in de-differentiated chondrocytes, C-Krox levels decrease and the factor acts as an inhibitor of COL2A1 gene expression through the promoter region, in which C-Krox-DNA-binding sites were also identified. Based on these findings, C-Krox could appear as a major factor in maintenance of chondrocyte phenotype, albeit other proteins are likely to participate in the control mechanism (26).

SP1 appears as a good candidate to act in concert with SOX9 and C-Krox to control type II collagen-specific expression. This factor is a ubiquitous zinc finger protein, homologue to C-Krox, that was originally identified by its role in SV40 gene expression (27). SP1 recognizes GC- or GT-rich motifs widely distrib-

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The abbreviations used are: bp, base pair(s); BLOTTO, bovine lacto transferase inhibitor buffer; CIIZFP, CII zinc finger protein; EMSA, electromobility shift assay; FCS, fetal calf serum; HSV-7K, herpes simplex virus-thymidine kinase; kb, kilobase(s); RAC, rabbit articular chondrocytes; DMEM, Dulbecco’s modified Eagle’s medium; RT-PCR, reverse transcriptase-polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline.

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**EXPERIMENTAL PROCEDURES**

**Cell Cultures**—RAC, HCS-2/8 human chondrosarcoma cell line (43), and NIH-3T3 fibroblasts were cultured as previously described (26).

**Transfection Experiments**—Chondrocytes were plated at 1 × 10⁶ cells per 100-mm dishes or 2 × 10⁶ cells per 9.6-cm² dishes in DMEM + 10% FCS. Cells at 80% confluency were transiently transfected by the calcium phosphate/DNA co-precipitation as described (26). Reporter plasmids (10 µg of each) were co-transfected with various amounts (0.1–30 µg) of Sp1 or Sp3 expression vectors (pEVR2/Sp1 or pRC/CMV/Sp3) (33). 10–15 h after transfection, the medium was changed and cells were harvested 24 h later. Luciferase activity was assayed on total cell extracts using a commercial kit (Promega), and corrected for protein amount (Bradford colorimetric method, Bio-Rad). Data are expressed in relative luciferase activity and represented mean ± S.D. of three inde-
Nuclear Extracts and DNA Binding Analysis

Nuclear extracts were prepared as described (26). EMSAs were performed with the oligonucleotides shown in Table I in the conditions previously described (26).

For mithramycin treatment, DNA probes were incubated for 1 h at 4 °C in the presence of the drug (100 nM) and the nuclear extracts or Sp1 recombinant protein (Promega) were then added to the binding reaction mixture.

In the antibody interference assays, 2 µl of preimmune serum or anti-Sp1 or anti-Sp3 antibodies (Santa Cruz Biotechnology) were added to each reaction mixture for 10–15 h at 4 °C in the presence of the oligonucleotide probe. At the end of the incubation, the samples were run on a 5% polyacrylamide gel, in nondenaturing conditions.

In DNase I footprinting experiments, labeling of the 150-bp BamHI–XhoI (−2889/+2842), 305-bp XhoI–BamHI (−2584/+2689), −391/−21 bp StyI–NarI, and −266/−21 bp Smal–NarI fragments of COL2A1 gene, gel purification, and elution were processed as previously described (26).

For Southwestern analysis, 20–40 µg of nuclear extracts were run on a 10%–polyacrylamide gel electrophoresis gel. After electrotransfer, the proteins fixed on the membranes were processed according to the method of Singh et al. (47), and submitted to a denaturation/renaturation protocol. Briefly, filters were incubated in binding buffer (25 mM HEPES, pH 7.9, 25 mM NaCl, 5 mM MgCl2, 0.5 mM dithiothreitol) containing 125 µM of calf thymus DNA and 125 µM of poly(dI-dC). Filters were then washed four times with the binding buffer, including 125 µM of calf thymus DNA and 125 µM of poly(dI-dC). Filters were then washed four times with the binding buffer, each for 40 min, and finally subjected to autoradiography at −80 °C.

RT-PCR Analysis—Total RNA was extracted as previously described (26). One-microgram samples of total RNA were reverse transcribed into cDNA in the presence of 50 pmol of oligo(dT), 40 units of RNaseOut (Life Technologies, Inc.), 10 mM MgCl2, 0.5 mM of each dNTPs and Moloney murine leukemia virus reverse transcriptase (Promega). The reaction was carried out at 42 °C for 15 min and followed by a 5-min step at 99 °C. Amplification of the generated CDNAs was performed in an Omni E Hybaid thermocycler using a PCR kit (Life Technologies, Inc.), in the presence of both sense and antisense primers (50 pmol) as follows: COL2A1: sense, 5′-GACCCCATGCAGTACATGGTTAC-3′; antisense, 5′-GACGGTCTTGCCCACTT-3′; Sp1: sense, 5′-CTACCCATCTAAGAGGAAGGAAAGAGG-3′; antisense, 5′-CTTCCCTTTCCTTTGTGCTGG-3′; and Sp3: sense, 5′-TAAGGTGATTGCGCTTCT-3′; antisense, 5′-T-
Fig. 3. Sp1 binds specifically to the +2817/+2845 1(II) cis-acting element. DNA binding was analyzed by EMSA. A labeled +2817/+2845 1(II) double-stranded oligonucleotide was incubated for 15 min with the recombinant Sp1. DNA competition experiments were performed with the indicated molar excess of wt. +2817/+2845 1(II) oligonucleotide (panel A, lanes 2–4), +2817/+2845 1(II) mut. (panel A, lanes 5–7), wt. +2440/+2485 1(II) oligonucleotide (panel A, lanes 8–10), mut.2 +2440/+2485 1(II) oligonucleotide (panel A, lanes 11–13), wt. −360/−337 1(II) oligonucleotide (panel A, lanes 14–17), mut. −360/−337 1(II) oligonucleotide (panel A, lanes 18–20), wt. and mut. −96/−69 1(II) oligonucleotides (panel A, lanes 21–23 and 24–26, respectively), wt. and mut. −305/−297 1(II) oligonucleotides (panel B, lanes 2–4 and 5–7, respectively), wt. and mut. −107/−135 1(II) oligonucleotides (panel B, lanes 8–10 and 11–13, respectively), and wt. and mut. −188/−225 1(II) oligonucleotides (panel B, lanes 14–16 and 17–19, respectively). In panel A, lane 1 represents the control of lanes 2–13, lane 14 the control of lanes 15–20, and lane 27 the control of lanes 21–26. In panel B, lane 1 corresponds to the control of lanes 2–19.

GAGGTGCTTAAAGAT-3’ (49); β-actin, sense, 5’-GTGGGGCGCCCAGGAGCACCA-3’/antisense, 5’-CTCGTTAAGTCGACGCGATTTCC-3’ (50).

A variable number of PCR cycles were done as follows: 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Then, an additional step at 72°C for 10 min was included. cDNAs were analyzed in 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The amplification reaction yielded expected cDNA sizes (COL2A1, 648 bp; Sp1, 821 bp; Sp3, 541 bp; and β-actin, 548 bp). After photography of the gels, the intensity of the corresponding bands was quantified by densitometry using the ImageQuant software (Molecular Dynamics) and normalized to β-actin cDNA levels.

Western Blotting—RAC were cultured in 100-mm Petri dishes for characterization of the different maturation forms of type II collagen by SDS-PAGE followed by Western blot analysis. The cell layer-associated proteins (50 μg) were resolved on a 10% polyacrylamide gel in denaturing conditions using Tris glycin buffer containing 1% SDS (40 mA, 2.5 h). The gel was then equilibrated for 30 min in the transfer buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine, 20% methanol (v/v)), and the proteins were electrophoresed on a nitrocellulose membrane (30 min, 10 V), using a Bio-Rad transfer apparatus (Semi-Dry Transfer Cell). Free protein-binding sites were blocked by incubating the filters for 30 min in PBS buffer containing 10% nonfat dry milk. Then, the membranes were rinsed three times in PBS, and the filters were incubated for 24 h in the presence of an anti-human type II collagen antibody raised in rabbit (kindly provided by D.-J. Hartmann, Faculte de Pharmacie, Lyon) (1/400 dilution in PBS-containing 10% nonfat dry milk).

Filters were then incubated for 2 h with a secondary antibody (horse radish peroxidase-conjugated guinea pig anti-IgG, 1/10,000 dilution in PBS + 10% nonfat dry milk). The blots were rinsed six times for 5 min in PBS. Finally, type II collagen expression was revealed using an ECL + Plus Western blot detection kit (Amersham Pharmacia Biotech).

The Western blot analysis of Sp1 and Sp3 in RAC nuclear extracts was performed as previously described (26). Briefly, the filters were incubated with primary anti-Sp1 and Sp3 antibodies (1/10000 dilution) (Santa Cruz Biotechnologies) and then treated with a secondary antibody (goat anti-rabbit, 1/1000 dilution) coupled to peroxidase. Sp1 and Sp3 expression was revealed with the ECL kit as above.

Collagen Labeling and Assay—RAC were seeded in 10% FCS-containing DMEM, in 9-cm dishes, and transiently transfected with the Sp1 and Sp3 expression vectors or the corresponding insertless plasmids in DMEM + 10% FCS supplemented with sodium ascorbate (50 μg/ml) for 15 h. Then, the medium was replaced by the same fresh medium supplemented with β-aminopropionitrile (100 μg/ml) and [3H]proline (2 μCi/ml) (PerkinElmer Life Sciences), and the cultures were pulsed for 24 h.

At the end of the incubation, the culture medium was collected and the labeled collagen was assayed with pure bacterial collagenase (51). The cell layer-associated collagen was also assayed after scraping and sonication of the cells, since that fraction generally contains large amounts of type II collagen (13, 52). Levels of collagen and noncollagenous proteins were corrected for protein amount determined by the Bradford colorimetric assay.

RESULTS

Delineation of Sp1-DNA-binding Sites in the Human COL2A1 Gene—We previously characterized a 458-bp enhancer in the first intron of the human COL2A1 gene (+2384/+2842) and a 266-bp short promoter, both involved in the specific expression of the gene in RAC. They include DNA-binding sites for a zinc finger protein, C-Krox, which was shown to differentially regulate the transcription of COL2A1 gene according to the differentiation state of the chondrocytes (26). As Sp1 and C-Krox belong to the same family of transcription factors, it was of interest to determine the binding sites and potential recognition sequences for these proteins on the two sequences responsible for type II collagen-specific expression. This may provide clues for understanding mechanisms governing cartilage-specific COL2A1 gene transcription.

To determine whether these promoter and first intron sites interacted with other transcription factors, in vitro DNase I footprinting assays were performed on both regions. Several regions of the promoter were found to be protected by proteins present in nuclear extracts from RAC (Fig. 1, A and B). They were located between −66/−103 bp, −107/−135 bp, −225/


-305 bp, and -305/-360 bp. These sequences also bound Sp1 recombinant protein, except for the -329/-360-bp region (Fig. 1, A and B). Sp1 interacts equally with the -188/-219 sequence that was not protected in the presence of nuclear extracts.

Similar experiments performed on a fragment from the first intron of COL2A1 gene (+2689/+2842), previously shown to take part in the enhancer activity, revealed a protected area between +2817/+2842 (Fig. 1C). Moreover, Sp1 was found to bind this sequence (Fig. 1C).

The analysis was also extended to another part of the enhancer, in a region covering the +2384/+2689-bp sequence. The +2440/+2485 DNA fragment was protected by factors present in nuclear extracts of RAC and by exogenous Sp1 (Fig. 1D). Thus, Sp1-DNA-binding sites are present in both the promoter and first intron enhancer of the human COL2A1 gene.

EMSA analysis was then performed using the wild-type oligonucleotide sequences previously identified in the COL2A1 gene and their mutant counterparts (Table I). In direct binding experiments, the recombinant Sp1 protein bound to all the wild-type DNA-binding sites of the first intron and promoter of COL2A1 gene (Fig. 2 and data not shown for the -107/-135 α1(II) oligonucleotide). This interaction is specific since mutant oligonucleotides did not bind Sp1. For the +2440/+2485 α1(II) and -225/-265 α1(II) oligonucleotides, two mutants were generated because these sequences include two potential Sp1-binding motifs. The mutants correspond to the mutation of the first potential Sp1 site localized 5' to the probe, and the mutants included a mutation in the two Sp1 cis elements. For the two concerned oligonucleotides, mutation of the upstream Sp1-DNA-binding motifs did not prevent Sp1 binding to the probe, suggesting that this transcription factor binds to the two sites.

Competition experiments performed with a +2817/+2845 oligonucleotide demonstrated that Sp1 bound in a specific manner since the binding was suppressed by molar excess of the same cold wild-type site and not by the respective mutant oligonucleotide (Fig. 3A). Moreover, addition of cold molar excess of the +2440/+2485 wild-type oligonucleotide did not alter Sp1 binding to the +2817/+2845 probe, indicating that the transcription factor bound with high affinity to this probe. On the other hand, some doubt remains about the potential transcription function of the +2440/+2485 sequence (Fig. 3A). EMSAs performed with the same probe, but competed with wild-type and respective mutant oligonucleotides identified in the COL2A1 gene promoter region demonstrated that Sp1 binding is specific, since the wild-type oligonucleotides decreased Sp1 binding, but not the corresponding mutant competitors (Fig. 3A). The binding of Sp1 to the +2817/+2845 site was also inhibited by excess amounts of -305/-327, -107/-135, or -188/-225 wild-type oligonucleotides but not by the same molar amounts of the respective mutant oligonucleotides (Fig. 3B). This indicates therefore that Sp1-binding sites of the COL2A1 promoter region bind this factor with a strong affinity, albeit to a lesser extent compared with the +2817/+2845 site (except for the -305/-327 which shows also a very high affinity) (Fig. 3B).

As further proof of Sp1 binding specificity, DNA competition experiments demonstrated that the binding of Sp1 to the -66/96 and -107/-135 oligonucleotides was inhibited by a molar excess of the wild-type -66/-96, -107/-135, or -188/-225 oligonucleotides, but not by the respective mutants (Fig. 4, A and B).

To obtain additional information on nuclear proteins that could bind to Sp1-binding sites of the COL2A1 gene, EMSAs were carried out with RAC nuclear extracts and the +2817/+2845 oligonucleotide as a probe (Fig. 5A). Two retarded protein-DNA complexes were observed, one major designated “Sp1, Sp3” and a minor involving Sp3, as demonstrated in antibody interference analysis (Fig. 5B). A faint third complex with higher electrophoretic mobility was detected which was previously shown to involve C-Krox (26). C-Krox bound to the probe with lower affinity compared with the members of the Sp family. The specificity of the Sp1- and Sp3-DNA interactions was demonstrated by disappearance of the shifted bands in the presence of molar excess of unlabeled wild-type +2817/+2845 and -305/-327 oligonucleotides (Fig. 5A). In contrast, incubation with 25- and 75-fold molar excess of mutant +2817/+2845 and -305/-327 oligonucleotides did not reduce the intensity of the shifted bands (Fig. 5A). Sp1 and Sp3 bound with lower affinity to the -66/-96 oligonucleotide compared with the +2817/+2845 cis element since the binding was not completely abolished when the wild-type -66/-96 oligonucleotide was used as the competitor (Fig. 5A).

Antibody interference assays were carried out, using Sp1 and Sp3 antibodies, to further characterize the two major protein-DNA complexes observed in EMSAs (Fig. 5B). Nuclear extracts from HCS-2/8 chondrosarcoma cells were incubated with a +2440/+2485α1(II) probe together with preimmune serum or the antibodies. As shown in Fig. 5B, Sp1, Sp3, and
In conclusion, Sp1 and Sp3 bound to all the different cis elements identified in the promoter and the enhancer regions of the human COL2A1 gene. Three specific DNA-binding sites for the two Sp proteins were present in the first intron enhancer of the COL2A1 gene (+2817/+2845, +2440/+2485, and +2459/+2485). Nine specific Sp-binding sites have been found in the promoter of that gene (−360/−337, −327/−305, −304/−274, −265/−225 (two sites), −225/−188, −135/−107 (two sites), and −96/−66). All the promoter sites display a similar affinity for Sp proteins, albeit lower when compared with the +2817/+2845 site.

Sp1/Sp3 Levels and DNA Binding Activities Decrease during Chondrocyte De-differentiation—Type II collagen biosynthesis is significantly reduced during in vitro de-differentiation of chondrocytes (12). We previously demonstrated that the process is accompanied by decreased DNA binding activity of C-Krox, Sp1, and Sp3 transcription factors (26).

Further evidence of this effect is provided here by Southwestern experiments performed with both the wild-type and mutated probes representing two of the identified cis elements present in the human COL2A1 gene. When nuclear extracts of primary RAC and NIH-3T3 were used, three major polypeptides of relative molecular mass 100, 65, and 55 kDa (a, b, and c), bound specifically to the two wild-type probes and not to the mutated counterparts (Fig. 6A). A single faint band of ~55 kDa is detected with HCS-2/8 and de-differentiated RAC nuclear extracts (Fig. 6A). This suggested that the proteins of molecular mass 100, 65, and 55 kDa are present in lower amounts in HCS-2/8 and de-differentiated RAC than in primary RAC and confirmed that the amounts ofand the binding activity of transcription factors recognizing the different GC-rich sequences in both the promoter and intronic enhancer of COL2A1 gene were decreased during RAC phenotypic modulation. The fact that the same polypeptides were detected with the −337/−360a(III) probe in both chondrocytes and fibroblasts suggests that these factors are probably not cell-specific.

Although the electrophoretic pattern was quite similar for the two different cis elements, a difference was observed with the wild-type +2440/+2485a(III) probe which binds two proteins of ~65 kDa whereas only one protein of that molecular mass seemed to interact with the −337/−360a(III) wild-type probe (Fig. 6A). Moreover, the binding of the different polypeptide species was more modest with the wild-type COL2A1 intronic sequence compared with the wild-type COL2A1 promoter cis element.

The complex a (95–105 kDa) probably corresponds to Sp1 and Sp3 as they were shown to bind these cis elements in EMSAs performed in the absence or presence of Sp1 and Sp3 antibodies (Fig. 5B). The faster migrating species most probably reflects Sp3 binding to the probes (complex b) since a Sp3 antibody recognized 97, and about 60- and 58-kDa polypeptides (33). In this regard, it is of interest to note that Sp3 is actually present in chondrocytes and binds to the −337/−360a(III) probe. The complex c (~55 kDa) could implicate C-Krox, previously shown to bind to the two cis-acting elements in EMSA experiments (26). Additional support to this interpretation is also provided by Western blot data showing that this protein reacted with a C-Krox antibody (26).

To determine whether Sp1/Sp3 decrease of DNA binding activity resulted from a transcriptional and/or a translational regulation, RT-PCR and Western blot analyses were performed. More precisely, we wanted to determine if there was a correlation between the phenotypic alteration of chondrocytes and a reduced level or DNA binding of Sp factors, in particular, Sp1. In EMSAs, Sp1/Sp3 binding activities were significantly decreased after one or two passages of the chondrocyte cultures (Fig. 6B). Western blot performed on the same nuclear extracts from primary cultures demonstrated that a Sp1 antibody reacted with two polypeptides of an apparent molecular mass of 95 and 105 kDa, corresponding, respectively, to the nonphosphorylated and phosphorylated Sp1 proteins (Fig. 6C). The amounts of both forms of Sp1 were decreased in passaged cells,
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i.e. in de-differentiated chondrocytes (Fig. 6C). With primary RAC nuclear extracts, the Sp3 antibody revealed a protein of ~100 kDa and a doublet of ~60 kDa similar to that already described (33) (Fig. 6D). As the RAC de-differentiate in vitro, a very slight, but still present, decrease in the different forms of Sp3 expression was observed (Fig. 6D). However, Sp3 decrease is minimal compared with Sp1 decrease.

RT-PCR analysis showed that the steady-state level of COL2A1 mRNA was reduced, as expected, after two culture passages (~53%, Fig. 7A). At the same time, Sp1 and Sp3 mRNA amounts were, respectively, reduced by 35 and 20% (Fig. 7A and B). This suggests that the decreased amounts and DNA binding activities of Sp1/Sp3 during RAC de-differentiation in vitro can be due, at least in part, to a transcriptional control of the cognate gene expression, with a more marked effect on Sp1.

**Effects of Sp1 and Sp3 on the Transcription Activity of COL2A1 Gene in Primary and De-differentiated RAC**—To determine the contribution of Sp DNA-binding sites from the promoter and the intrinsic enhancer regions, several constructs from both regions were co-transfected with equal amounts of Sp1 and Sp3 plasmids (pEVR2/Sp1 and pRC/CMV/Sp3) and their corresponding insertless plasmids (pEVR2 and pRC/CMV). First, we verified that transfection of the Sp1 expression plasmid led to overexpression of the transcription factor: a 2-fold induction of Sp1 mRNA level was observed after transfection of 10 µg of Sp1 plasmid (data not shown). Sp1 overexpression in primary RAC leads to a substantial increase in relative luciferase activity of all the constructs (Fig. 8A). Nevertheless, Sp1 augmented by 13–14-fold the transcription level of pGL2–3.059 kb reporter vector and only by 2-fold those of the pGL2–3.774 kb, pGL2–3.059 kb, and pGL2–1.167 kb constructs. This could be due to the presence of a potential silencer element in the last three constructs (26). When the silencer element was deleted in the pGL2–3.059 kb construct, Sp1 maximal transactivating action became effective. Additionally, in the absence of Sp1 overexpression and according to the values presented in the legend of Fig. 8, the COL2A1 promoter deletion data demonstrated that the 266-bp promoter is the highest transcriptionally active promoter in these experimental conditions, indicating that this region contains DNA-binding motifs which are necessary for high basal transcription activity. As Sp1 induction of human COL2A1 gene expression appeared to be mediated through the short 266-bp promoter, we performed dose-response experiments which actually showed a Sp1 transactivating effect, increasing with the concentration of expression plasmid (Fig. 8B).

The preceding experiments illustrated the transcriptional activity of COL2A1 reporter vectors, evaluated under both endogenous and exogenous Sp1 influence, but they did not provide information on the endogenous role played by Sp1. Therefore, EMSAs and transfection analysis were performed in the presence of mithramycin, an inhibitor of Sp1 binding (53). Mithramycin was found to prevent Sp1 and Sp3 binding in EMSAs of primary RAC nuclear extracts and therefore abolished their transcriptional effects on COL2A1 gene (Fig. 9A).
Moreover, the use of recombinant Sp1 as a positive control confirmed that Sp1 binding is completely blocked under mithramycin treatment (Fig. 9A). Addition of mithramycin induced a 50% decrease of basal COL2A1 gene expression, as shown by co-transfection of the most representative construct, pGL2–3.774 kb, and the Sp1 cDNA insertless vector in primary chondrocyte cultures (Fig. 9B). Furthermore, Sp1 overexpression led to a 6-fold increase in the transcription of the pGL2–3.774 kb reporter vector while mithramycin treatment reduced by 2-fold the Sp1-induced expression (Fig. 9B). These results demonstrate that mithramycin repressed both basal and amplified COL2A1 gene transcriptions induced by Sp1 and/or Sp3. However, other cofactors are probably involved in that expression since mithramycin does not completely abolish basal transcription activity. Decoy experiments were also performed to discriminate between Sp1/Sp3 endogenous and exogenous effects on COL2A1 gene expression in primary RAC. Chondrocytes were co-transfected with the pGL2–3.774 kb construct and the wild-type Sp1 multicopy double-stranded oligonucleotide (mcSp1wt: two copies of the −107/−135α1(II) oligonucleotide, Table I) as an endogenous Sp1-DNA-binding site competitor, in order to block endogenous Sp1 and Sp3 effects on COL2A1 gene transcription. The data indicated that excesses of the transfected mcSp1wt oligonucleotide decreased by 3-fold the basal transcriptional activity of the pGL2–3.774 kb construct and Sp1 induction of COL2A1 gene expression was completely abolished (Fig. 9C). Therefore, an endogenous Sp1-like activity functions, in a specific manner, as an enhancer of COL2A1 gene transcription in primary chondrocytes.

As Sp1 is a transcriptional activator of COL2A1 gene in differentiated RAC, and that its amount/binding activity decreased during phenotype alteration, we then investigated whether this factor could exert the same effect in de-differentiated RAC, with the objective of testing the hypothesis that Sp1 overexpression could prevent phenotype loss or restore the chondrocytic phenotype after de-differentiation. We co-transfected the same reporter vectors as above and Sp1 expression plasmid in cells de-differentiated by culture passages. Transcriptional activities observed with the COL2A1 reporter plasmids were lower than in primary RAC, as previously reported (26), reflecting the reduced collagen type II synthesis of these cells. This reduced transcriptional activity could result from a diminished expression of the major transactivating factors controlling COL2A1 gene expression. As shown in Fig. 10, overexpressed Sp1 activates in a dose-dependent manner the transcription of all the COL2A1 constructs. However, the maximal Sp1 effect was detected with the 266-bp promoter, as in primary RAC, indicating that the optimal effect on COL2A1 gene expression requires several functional Sp1-DNA-binding motifs of this short promoter. It must be noticed that the presence of the intronic enhancer sequence in this 266-bp promoter (26) exerted a transactivating effect under Sp1 overexpression which is not significantly different from that of the promoter alone, whatever the amount of Sp1 expression vector used (Fig. 10). These data suggest that the first intron enhancer of the COL2A1 gene, despite the presence of a Sp1-DNA-binding motif of very high affinity (Figs. 1–3 and 5; +2817/+2845 site), is not involved in the transcriptional Sp1

FIG. 7. The steady-state levels of COL2A1, Sp1, and Sp3 mRNAs are decreased during in vitro de-differentiation of RAC. Total RNAs (1 μg/lane) from primary RAC (RAC P0) and RAC P2 (2 passages) were reverse-transcribed into cDNA by using specific antisense primer for COL2A1, Sp1, Sp3, and β-actin mRNAs. 15–35 PCR cycles were performed under conditions described under “Experimental Procedures.” The products were analyzed in 2% agarose gel electrophoresis in the presence of ethidium bromide and photographed under UV light. After photography of the gels, a densitometric analysis was performed and the amounts of COL2A1, Sp1, and Sp3 cDNAs were normalized to β-actin cDNA levels, and shown as histograms.
induction of the gene, at least in our experimental conditions.

To determine whether the binding of Sp1 or Sp3 to the Sp-DNA-binding motifs identified in the COL2A1 gene has functional consequences and to further analyze the contribution of each Sp protein to the constitutive and stimulated activities of COL2A1, we co-transfected Sp3 expression vector with various COL2A1 gene constructs in differentiated RAC. Overexpression of increasing amounts of Sp3 (1, 5, and 10 µg of pGL2–0.387 kb reporter construct and 10 µg of the Sp1 insertless expression vector pEVR2 for the experimental control) activated the transcription of the Sp1 insertless expression vector (pEVR2) for the experimental control. 1, 5, and 10 µg of pEVR2 expression plasmid were co-transfected together with 10 µg of pEVR2 expression plasmid.

Add the expression of Sp1 and Sp3 to the Sp-DNA-binding motifs identified in the COL2A1 gene has functional consequences and to further analyze the contribution of each Sp protein to the constitutive and stimulated activities of COL2A1, we co-transfected Sp3 expression vector with various COL2A1 gene constructs in differentiated RAC. Overexpression of increasing amounts of Sp3 (1, 5, and 10 µg of pGL2–0.387 kb reporter construct and 10 µg of the Sp1 insertless expression vector pEVR2 for the experimental control) activated the transcription of the Sp1 insertless expression vector (pEVR2) for the experimental control. 1, 5, and 10 µg of pEVR2 expression plasmid were co-transfected together with 10 µg of pEVR2 expression plasmid.

![Image](https://via.placeholder.com/150?text=Image)

**FIG. 8.** Sp1 stimulates human COL2A1 gene transcription in primary RAC through the 266-bp region located upstream the transcription start site. Panel A, effect of Sp1 overexpression on the regulatory sequences of human COL2A1 gene. Primary RAC in 9.6 cm² dishes were co-transfected with 10 µg of different COL2A1 reporter plasmids along with 10 µg of pEVR2 expression vector that either contained (pEVR2/Sp1) or not (pEVR2/Sp1) CDNA. Luciferase activities of a representative experiment are presented as the mean ± S.D. of three independent samples. Relative luciferase unit (RLU) values upon Sp1 expression vector co-transfection are expressed as percentage relatively to that of the respective reporter construct co-transfected by the Sp1 insertless expression vector pEVR2. For pGL2–3.774 kb, pGL2–3.059 kb, pGL2–1.167 kb, and pGL2–0.387 kb constructs indicating that this factor, by binding to its DNA motifs, prevents Sp1 binding through a competition mechanism for DNA interaction. These data demonstrate that mul-
copies of Sp1-DNA binding motifs found in the COL2A1 gene promoter activate transcription of a nonspecific promoter in primary and de-differentiated chondrocytes, this effect reflecting the transcriptional effects exerted on the COL2A1 gene.

**Fig. 9.** Consequence of inhibition of Sp1 binding to its target DNA sequence on COL2A1 gene transcription. Panel A, EMSA of nuclear extracts from primary RAC and recombinant Sp1 incubated in vitro with or without mithramycin. A labeled +2817/+2845a1(II) Sp1-binding site was incubated with 10 μg of primary RAC nuclear extracts (lanes 1–3) or 5 fmol of recombinant Sp1 (lanes 4 and 5). Nuclear extracts and Sp1 were incubated with the mithramycin vehicle, methanol (lanes 2 and 4), or with mithramycin (lanes 3 and 5). Panel B, primary RAC in 55-cm² dishes were co-transfected with the pGL2–3.774 kb reporter construct along with 10 μg of pEVR2 expression vector that either contained (pEVR2/Sp1) or not (pEVR2) the Sp1 cDNA. Then, the cells were incubated for 24 h in the presence or absence of methanol or mithramycin. Relative luciferase units (RLU), expressed as arbitrary units (AU/μg of protein, represent the mean ± S.D. of three independent experiments.

Panel C, primary RAC cultures in 5.6-cm² dishes were co-transfected with the pGL2–3.774 kb construct (15 μg) along with the pEVR2 expression vector, or the pEVR2/Sp1 plasmid in the presence or absence of the double-strand mcSp1 wt. oligonucleotide (0.15 μM). RLU represents the mean ± S.D. of three independent samples from a representative experiment.

**Fig. 10.** Sp1 activates the transcription of the COL2A1 gene in a dose-dependent manner through the 266-bp short promoter in de-differentiated chondrocytes. Chondrocytes de-differentiated by four passages (9.6-cm² dishes) were co-transfected with some of the COL2A1 gene reporter constructs (10 μg) along with 1, 5, or 10 μg of pEVR2/Sp1 expression plasmid in the same conditions as in Fig. 8D. Relative luciferase unit (RLU) values upon Sp1 overexpression vector co-transfection are expressed as percentage relative to that of the reporter vector co-transfected with the Sp1 insertless expression plasmid pEVR2. RLU represents the mean ± S.D. of three independent samples of a representative experiment. The numbers indicated on the top of the histograms represent the RLU percentages obtained after Sp1 overexpression versus respective control vectors. The indication US in the construct name refers to the upstream cloning positions of the first intron regions, 5' of the 266-bp COL2A1 promoter.
effects of the two Sp proteins were reflected at the level of collagen protein, the synthesis was assayed after \([3H]\)proline labeling of primary and passaged cultures. Primary chondrocytes were previously transfected with pEVR2, pEVR2/Sp1, pRC/CMV, or pRC/CMV/Sp3 plasmids, before metabolic labeling, to estimate newly synthesized collagen amounts. As shown in Fig. 14A, Sp1 overexpression increased total collagen neo-synthesis (essentially collagen type II) by 90%. This increase was mainly observed for the cell layer-associated fraction, which was augmented by 7-fold under Sp1 overexpression (Fig. 14A). In contrast, overexpressed Sp3 produced a slight inhibition of total collagen production (\(-34\%\)), affecting the cell layer compartment (3-fold decrease, Fig. 14A). Sp1 and Sp3 overexpression induced specific effects on collagen synthesis since the production of non-collagenous proteins was not altered in these experiments (data not shown).

In de-differentiated RAC, Sp1 overexpression led to a 2–3-fold increase of total collagen neo-synthesis which affected the cell layer fraction, while Sp3 was without effect (Fig. 14B). As above, the Sp1 effect was specific of collagen synthesis since non-collagenous protein synthesis was not altered by the transcription factor (data not shown).

Thus, there is a correlation between the transcriptional Sp1 effects on the COL2A1 gene and the level of total collagen production, whatever the differentiation state of the chondrocytes. However, the collagen neo-synthesis was modestly inhibited when Sp3 was overexpressed in primary chondrocytes, suggesting that post-transcriptional events are implicated in the synthesis process.

In addition to total collagen synthesis assays, type II collagen levels were evaluated by Western blotting. Only cell layer-associated fraction was studied as it contains the bulk of type II collagen formed by monolayer cultures. At least three proteins of apparent molecular mass 170, 150, and 95 kDa reacted with the type II collagen antibody in primary cultures, corresponding, respectively, to pro-\(\alpha1(II)\), pC-\(\alpha1(II)\), and the mature \(\alpha1(II)\) chains (52) (Fig. 14C). Transient transfection of the primary RAC with the insertless expression vectors pEVR2 and pRC/CMV did not alter the chondrocyte phenotype since the patterns of the different maturation forms of type II collagen in transfected and non-transfected cells were closely similar (Fig. 14C). When Sp1 was overexpressed in the differentiated RAC, a slight increase in pro-\(\alpha1(II)\) and pC-\(\alpha1(II)\) chains was observed, whereas the amount of the mature \(\alpha1(II)\) form remained unchanged (Fig. 14C). By contrast, Sp3 overexpression was shown to decrease the levels of all the maturation forms of type II collagen (Fig. 14C). The de-differentiated chondrocytes were found to still synthesize substantial amounts of type II...
The chondrocyte is a cell known to de-differentiate easily. Our data reveal the implication of Sp1 and Sp3 in regulation of the human COL2A1 gene. The chondrocyte is a cell known to de-differentiate easily, producing type I collagen together with type II collagen, one of the cartilage-specific markers. This phenomenon occurs in the osteoarthritic process and can be mimicked in vitro by subculturing monolayer cultures of chondrocytes. This culture model provides a way to understand some of the molecular mechanisms responsible for activation of type I collagen synthesis and decreased production of type II collagen in articular chondrocytes, and may offer a clue to elucidate the transition from healthy to osteoarthritic cartilage.

Our data reveal the implication of Sp1 and Sp3 in regulation of COL2A1 gene expression in articular chondrocytes and demonstrated that these two factors bound with a similar affinity to the same COL2A1 gene sequences, and suggesting that their functions can be mutually dependent. Sp1 was shown to activate the transcription of all the human COL2A1 gene constructs in differentiated and de-differentiated chondrocytes. Apparently, the human COL2A1 intronic enhancer is not involved in transcriptional activation induced by Sp1, although the control of the specific regulation of mouse and rat COL2A1 genes involves an interaction between promoter and enhancer elements, respectively, a chondrocyte-specific enhancer-binding protein complex including SOX9, L- SOX5, SOX6 (18, 19), and an heteromeric complex in which was found CIIZFP (CII zinc finger protein) and Sp1 (41). From our results, Sp1 induction of the human COL2A1 gene transcription was shown to be mediated by the 266-bp promoter, but we cannot exclude that Sp1 could be involved in transcriptional activation of that gene induced by other transcription factors such as SOX9, CIIZFP, or C-Krox, through heteromeric complex formation. Indeed, the maximal Sp1 effect is mediated by the 266-bp promoter region immediately located upstream the transcription start site. Since the constructions containing sequences between −544 to −266 were moderately transactivated by Sp1 compared with the −266-bp short promoter, this suggests that the region −544 to −266 contains an effective CIIS2 silencer element previously characterized in the rat gene (54). This putative trans-inhibiting element localized in the human gene between −440 and −458 may prevent the transcriptional activation of the COL2A1 gene mediated by the intrinsic enhancer (26), and Sp1 induction of that gene in the chondrocytes. Although the silencer region within the human COL2A1 gene has not been characterized precisely, it is likely that the E2 box localized in that sequence may mediate the transcriptional suppression induced by 5¢EF1, since it has been demonstrated that promoter 5¢EF1 recognition sites negatively regulate rat COL2A1 gene expression (55).

Additionally, deletion data indicated that the 266-bp COL2A1 promoter is the most active small promoter. Shorter promoters including 63- and 35-bp sequences upstream the transcription start site displayed a smaller activity (2–4-fold) (data not shown) as it is the case for constructs containing −544-bp and −932-bp sequences (this study). Therefore, the sequences from −63 to −266 contain cis-elements necessary for high basal transcription of the gene. This region was found to include six Sp1-binding motifs which are crucial for promoter activity.

Most reports on Sp3 transcriptional properties are based on transient transfection experiments in the Drosophila SL2 cell line. By contrast, few studies deal with experimental analysis on the physiological role of Sp3 on a promoter activity. In our model, Sp3 was not able to affect COL2A1 gene transcription. Nevertheless, Sp3 can prevent Sp1 transcriptional induction of the gene in both primary and de-differentiated chondrocytes by interacting with a cis-acting element common to Sp1. The transcriptional function of Sp1 and Sp3 in COL2A1 gene expression have been confirmed by using constructs containing multimerized Sp1-DNA-binding sites (mcSp1wt and mcSp1mut) cloned upstream of a nonspecific promoter. Indeed, the Sp1-DNA-binding sites remained efficient whatever the differentiation state of the RAC, indicating that there was a close correlation between Sp1 and Sp3 transcriptional functions on a nonspecific promoter and on the human COL2A1 gene. The function of Sp3 in chondrocytes is not surprising since it was found to block the transactivating effect of Sp1 on a target gene in other systems. For example, Sp1 and Sp3 can bind to common cis elements in c-myc gene. When Sp1 interacts with c-myc promoter, it activates its transcription. In contrast, when Sp3 is bound, there is no activation of the transcription. Moreover, Sp3 overexpression suppresses the Sp1-induced activation of transcription (32, 56, 57). Therefore, Sp1 is likely to be a common transcriptional...
A

![Graph A](image)

Supernatant
Cell layer
Super. + Cell layer

B

![Graph B](image)

Supernatant
Cell layer
Super. + Cell layer

C

![Graph C](image)

Fig. 14. Effects of Sp1 and Sp3 overexpression on collagen synthesis in primary and de-differentiated chondrocytes. Panel A, primary chondrocytes (9.6-cm² dishes) were transiently transfected with 10 µg of pEVR2, PEVR2/Sp1, pRC/CMV, or pRC/CMV/Sp3. After 16 h of transfection, the medium was changed and the cells were pulsed for 24 h in DMEM + 10% FCS containing ascorbic acid (50 µg/ml), β-aminopropionitrile (100 µg/ml), and 2 µCi/ml tritiated proline. At the end of the experiment, the amount of radiolabeled collagen was assayed in both medium and cell layer as collagenase-digestible radioactivity. The values, normalized to total protein amount assayed by the Bradford colorimetric method, are expressed as counts/min/µg of protein and represent the mean ± S.D. of triplicate dishes. Panel B, chondrocytes de-differentiated by four passages (9.6-cm² dishes) were transiently transfected as in panel A. The values are expressed as counts/min/µg of protein and represent the mean ± S.D. of triplicate dishes. Panel C, primary chondrocytes (RAC P0) and chondrocytes de-differentiated by four passages (RAC P4) (65-cm² dishes) were transiently transfected as activator in chondrocytes, as in most of the systems studied to date, while Sp3 effect would depend on the Sp1/Sp3 ratio and the cellular context. Our results partially agree with those of Dennig et al. (35) who demonstrated that when a promoter contains only one Sp1 site, Sp3 activates the transcription of the corresponding gene whereas it has no effect on transcriptional activity, or prevents Sp1 activation of transcription when the promoter includes several Sp1-DNA binding motifs. We cannot exclude also that other transcription factors could participate in the transcriptional regulation, acting as co-repressors, since it has been demonstrated that the SIF-1 protein (Sp3-interacting protein-1) interacts specifically with Sp3 (30).

During chondrocyte in vitro de-differentiation, a correlation was observed in the decrease of both type II collagen production and Sp1/Sp3 amounts/DNA binding activities, this latter being due to transcriptional control of the corresponding Sp-protein genes. However, the production at the level of Sp1 protein is much more pronounced than that observed at the Sp1 mRNA level and this could be due to several reasons. First, there may be a very low level of expression of the Sp1 gene in primary cells such as RAC. This could be reinforced by the fact that recent cloning and characterization of the 5′-flanking region of the Sp1 gene revealed the absence of both CAAT and TATA boxes and an initiator element indicating that Sp1 is encoded by a house-keeping gene (58). One of the major characteristics of this gene family is to present a continuous, but a very low transcription level. Another possible explanation is that Sp1 mRNA is rapidly translated into nonphosphorylated Sp1 protein or that this transcript is rapidly degraded in the RAC. Further studies will have to clarify these points.

Because of the correlation in the decrease of both type II collagen synthesis and Sp1/Sp3 amounts and DNA binding activity occurring as RAC de-differentiate in vitro, it was of interest to determine if the type II collagen synthesis could be restored under Sp1 and/or Sp3 overexpressions. Only Sp1 overexpression was shown to increase type II collagen production through transcriptional control in de-differentiated chondrocytes, as it was the case in differentiated chondrocytes. All together, these data demonstrate that Sp1 is the major factor, capable of restoring by itself the chondrocyte phenotype, at least partially. This is not surprising since inactivation of the mouse Sp1 gene revealed that Sp1−/− embryos are retarded in development and die around 11 days post-coitum, indicating that Sp1 function is crucial for cellular differentiation (29).

Therefore, it could be suggested that Sp1 is also essential for cartilage phenotype maintenance.

We previously reported that C-Krox, Sp1, and Sp3 amounts/DNA binding activities were specifically decreased as chondrocytes de-differentiate in vitro, while the binding activity of a NF-κB complex was elevated (26). Preliminary studies from our laboratory indicate that this NF-κB complex implies at least the p65 subunit (not shown). Moreover, transient transfection of both differentiated and de-differentiated chondrocytes showed that p50 overexpression enhances COL2A1 gene transcription by a short promoter whereas overexpression of the p65 subunit does not modify transcriptional activity (not shown). The short promoter region contains Sp1-binding sites indicated on the figure with 10 µg of pEVR2, PEVR2/Sp1, pRC/CMV, or pRC/CMV/Sp3. NT represents primary RAC that were not transfected and used as a control. After 16 h of transfection, the medium was changed and the cells were incubated for 24 h in DMEM + 10% FCS containing ascorbic acid (50 µg/ml) and β-aminopropionitrile (100 µg/ml). At the end of the experiment, samples were processed as described under “Experimental Procedures,” and the different maturation forms of type II collagen were detected by using anti-type II collagen antibody. Numbers on the left correspond to the molecular mass of prestained marker proteins (kDa).
and a NF-κB-like element. Since the binding activity of the NF-κB complex containing p65 is increased during chondrocyte de-differentiation, it could be suggested that this complex could preferentially bind to the Sp1-binding sites or the NF-κB-like motif, or that Sp3, the amount/binding activity of which are less decreased than that of Sp1, interacts with these cis elements, the overall effect leading to reduced expression of the COL2A1 gene.

Several promoters contain multiple G-rich motifs, often found adjacent to binding sites for other transcription factors than Sp1/Sp3, suggesting that the factors may act in conjunction with each other to modulate transcription. Physical and/or functional interaction between Sp1 and other factors has been revealed. For example, cooperative interaction between Sp1 and NF-κBp65 is necessary for HIV-1 enhancer activity, which is mediated by adjacent Sp1 and NF-κB-binding sites (59, 60). Interactions between Sp1, Sp3, and NF-κB or other transcription factors could provide the chondrocytes with additional means of regulating COL2A1 gene expression than interactions solely at specific Sp- or NF-κB-binding sites. For example, NF-κB p65 physically interacts with Sp1, both in vitro (59) and in nuclear extracts (61). In other cases, the effects of NF-κB are mediated through critical Sp1-binding sites: the inhibitory effect of NF-κB p65 on the transcriptional activity of the COL2A1 gene in fibroblasts involves Sp1-cis elements of the promoter region (61). Since Sp1 amount/binding activity are strongly reduced during chondrocyte de-differentiation while those of NF-κB increased, we can hypothesize that the p65 subunit preferentially binds to Sp1-binding sites, preventing the activation of COL2A1 gene by endogenous Sp1.

Our study demonstrates that COL2A1 gene expression is controlled by a set of trans factors that differ in their relative amount and binding activity according to the differentiation state. When chondrocytes start de-differentiating after their first passage, Sp1, Sp3, and C-Krox functions decrease (Ref. 26; this study). However, this finding is not in agreement with a previous study showing that Sp1 binding activity was increased during in vitro de-differentiation of human chondrocytes (42). The discrepancy could be due to the fact that we use another cellular model. Furthermore, these authors showed that Sp1 overexpression increased the transcription of two COL2A1 promoters (−577/+63, −131/+63) in Drosophila Schneider line L2 cells. These data are difficult to interpret since the Sp1 binding activity is increased during phenotypic modulation of human chondrocytes. Therefore, the transcriptional Sp1 function observed in their model should have led to an increased transcription of type II collagen gene in the de-differentiated chondrocytes, which is not the case. Another explanation would be that post-transcriptional regulatory mechanisms are responsible for down-regulation of type II collagen synthesis following Sp1 overexpression in their system.

Several lines of evidence strongly suggest that Sp1 and C-Krox, because of their transactivating functions on the COL2A1 gene expression in differentiated chondrocytes (Ref. 26; this work) are involved in the chondrocytic phenotype loss since their amounts were strongly decreased during the process. Nevertheless, NF-κB could also take part in that modulation because the inflammatory cytokines such as interleukin-1 and tumor necrosis factor-α decrease the production of cartilage-specific proteins and activate catabolism of matrix molecules through AP-1 and NF-κB cis elements of metalloprotease genes (4, 62–66). These effects contribute to chondrocyte de-differentiation in osteoarthritis. Interestingly, it has been recently reported that the expression of SOX9, a major trans factor responsible for cartilage differentiation, is down-regulated by interleukin-1 and tumor necrosis factor-α (67). Expression of p50 and p65 NF-κB subunits is induced by the two cytokines, and mediates the transcriptional inhibition of mouse COL2A1 gene constructs through a region including the intronic enhancer (67).

In summary, together with SOX9 and C-Krox, Sp1 appears as a crucial factor in the maintenance of chondrocyte phenotype since high Sp1 binding activity and transcriptional function are associated with a strong type II collagen expression. By contrast, Sp3 and a NF-κB complex involving the p65 subunit are likely to promote chondrocyte de-differentiation since we have shown that they prevent the activation of type II collagen expression. More precisely, Sp3 could be considered as a regulating factor exerting a repressive effect on Sp1-induced COL2A1 gene expression that depends on the environmental context of promoter-binding sites.

Further investigation is required to better define the transcriptional machinery responsible for expression of the cartilage marker collagen type II in articular chondrocytes and its modulation by cytokines and growth factors. However, the present data may offer the possibility to manipulate transcription factors as potential tools to prevent cartilage degradation and promote repair of the tissue.

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SP3 Represses the SP1-mediated Transactivation of the Human COL2A1 Gene in Primary and De-differentiated Chondrocytes
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