BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **237,** 52–58 (1997)
ARTICLE NO. RC977073

The Nuclear Receptor Corepressor SMRT Inhibits Interstitial Collagenase (MMP-1) Transcription through an HRE-Independent Mechanism

Daniel J. Schroen,* J. Don Chen,† Matthew P. Vincenti,* and Constance E. Brinckerhoff*,‡,[1]

Department of Medicine and ‡Department of Biochemistry, Dartmouth Medical School, HB 7200, Hanover, New Hampshire 03755; and †Department of Pharmacology and Molecular Toxicology, University of Massachusetts Medical Center, 55 Lake Avenue N., Worcester, Massachusetts 01655

Received July 3, 1997

Nuclear receptors inhibit synthesis of collagenase-1 (matrix metalloproeinase-1; MMP-1), an enzyme that degrades interstitial collagens and contributes to joint pathology in rheumatoid arthritis. SMRT (Silencing Mediator for Retinoid and Thyroid hormone receptors) mediates the repressive effect of nuclear receptors at hormone responsive elements (HREs), prompting us to investigate whether this co-repressor could also regulate transcription of MMP-1, which lacks any known HREs. We find that primary synovial fibroblasts express SMRT. When over-expressed by transient transfection, SMRT inhibits MMP-1 promoter activity induced by interleukin-1 (IL-1), phorbol phorbol myristate acetate (PMA) or v-Src. SMRT apparently inhibits MMP-1 gene expression by interfering with one or more transcriptional elements clustered in a region between −321 and +63. We conclude that SMRT negatively regulates MMP-1 synthesis through a novel, HRE-independent mechanism that involves proximal regions of the MMP-1 promoter.

The matrix metalloproteinase (MMP) gene family consists of at least nineteen enzymes that degrade components of the extracellular matrix (ECM) and ensure proper tissue remodelling in normal physiology (1-3). Although MMP over-expression contributes to the pathology of numerous disorders, its role in rheumatoid arthritis is particularly well-documented (3, 4). In this disease, proinflammatory cytokines such as interleukin-1 (IL-1) induce synovial fibroblasts lining the joint to produce excessive amounts of MMP-1 (matrix metalloproteinase-1; interstitial collagenase), which irreversibly cleaves the interstitial collagens types I, II and III and contributes to joint erosion (5-10).

[1] To whom all correspondence should be addressed. Fax: 603-650-1128; E-mail: constance.e.brinckerhoff@dartmouth.edu.

The proximal region of the MMP-1 promoter contains several key elements that regulate transcriptional activation (1, 11). For instance, members of the Fos and Jun family enhance MMP-1 gene expression by binding to the activator protein-1 (AP-1) site at −73 and −77 in the human and rabbit promoters, respectively (12, 13). Proteins that contain an Ets domain bind to a nearby PEA-3 site at −104 (human) and −109 (rabbit) and cooperate physically and functionally with AP-1 (11, 14). The human proximal MMP-1 promoter also contains a functional signal transducer and activator of transcription (STAT) binding element at −53 (15).

In addition to DNA-protein interactions, higher order protein-protein complexes also appear to regulate MMP-1 induction. For example, the co-activator JAB1 specifically interacts with c-Jun or JunD to activate transcription (16). The co-activator p300/CBP binds STAT1α (17) and Fos/Jun family members (18) to stimulate gene expression, potentially through intrinsic histone acetyltransferase (HAT) activity in p300/CBP (19, 20) and HAT activity in the p300/CBP-associated factor (P/CAF) (21). Indeed, histone modifications, through lysine hyperacetylation or ATP-driven mechanisms, have been associated with remodeled chromatin and transcriptionally active DNA (22-25).

The same transcriptional factors and DNA elements that induce MMP-1 transcription can also serve as targets for repression of this gene. Ironically, for a gene highly regulated by nuclear receptors, the MMP-1 promoter lacks any known hormone responsive elements (HREs). Instead, nuclear receptors utilize alternative, diverse mechanisms to inhibit expression of this gene (26). For instance, through their ability to bind p300/CBP, retinoic acid receptors (RARs) may compete for limiting amounts of this co-activator (27). In addition, RARs, glucocorticoid receptor (GR), and thyroid hormone receptor (T_3R) can sequester AP-1 proteins away from their binding sites on the DNA (28-32). RARs and

0006-291X/97 $25.00

GRs also form complexes with AP-1 proteins that are bound to the DNA, perhaps interfering with the ability of AP-1 to transactivate (26, 33-37). Androgen receptors, on the other hand, bind directly to the Ets protein ERM and interfere with MMP-1 activation (38). Thus, complex protein-DNA interactions cooperate to strictly and precisely regulate MMP-1.

Within the last few years, accessory proteins have been shown to bind with nuclear receptors and to act as transcriptional co-repressors (39). For instance, SMRT (Silencing Mediator for Retinoid and Thyroid hormone receptors) and N-CoR (Nuclear receptor Co-Repressor) act in conjunction with RAR/RXR and T_3R/RXR heterodimers in the absence of ligand to silence transcription of HRE-containing genes (40-42). Recently, several laboratories have reported that SMRT and N-CoR form multi-subunit complexes with histone deacetylases, which may alter chromatin structure and repress gene transcription (25, 43-45). While SMRT and other nuclear receptor co-factors exhibit ubiquitous tissue expression, their physiological role in different cell and promoter contexts remains obscure (39, 46).

The ability of nuclear receptors to inhibit MMP-1 gene expression led us to explore the possible involvement of the nuclear receptor co-repressor SMRT in this process. We demonstrate that primary rabbit synovial fibroblasts express a homologue of human SMRT. Overexpression of full length SMRT, but not C-SMRT lacking the transcriptional repression domain, inhibits both basal and induced MMP-1 promoter activities. The induced MMP-1 activity is more sensitive to the repressive effect of SMRT than is basal transcription. Overexpression of the thyroid hormone receptor (TRα) ligand-binding domain, which interacts directly with SMRT, reverses SMRT's repressive capacity, while a TRα mutant that does not bind SMRT fails to do so, further supporting the idea that SMRT is directly involved in suppression of MMP-1 expression. Together, these results suggest that SMRT may contribute to maintaining MMP-1 gene expression in a repressive state, which may have important implications for suppressing development of rheumatoid arthritis.

MATERIALS AND METHODS

Cells. Synovial tissue cells were enzymatically dispersed from the knees of healthy, 4-6 week-old New Zealand White rabbits and cultured in Dulbeco's Modified Eagle's Medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (HyClone, Logan, UT) and antibiotics (50μg/ml penicillin, 50μg/ml streptomycin, and 100μg/ml neomycin; Gibco). Upon achieving confluency, cells were passed 1:3 and were used between passages 3-6. Macrophage-like cells die during passage and the remaining cells consist of a relatively homogeneous population of fibroblast-like cells (7). Human foreskin fibroblasts (HFFs) were obtained from ATCC (Rockville, MD).

RNA extraction and Northern analysis. Cells were grown to confluency in 150mm tissue culture plates, washed three times with Hank's Balanced Salt Solution (Gibco) to remove traces of serum, then cultured 24 hours in serum-free medium (DMEM supplemented with 0.2% lactalbumin hydrolysate and antibiotics; DMEM-LH) or DMEM-LH containing 10^{-6}M all-*trans*- retinoic acid (Sigma, St. Louis, MO). Cells were scraped, pelleted, and lysed in 1 ml of TRIzol total RNA isolation reagent (Gibco), followed by chloroform extraction and precipitation in isopropyl alchohol. RNA (20μg) was separated on a 1% agarose gel containing formaldehyde, transferred to GeneScreen (DuPont-NEN, Boston, MA) and cross-linked using a UV Stratalinker 1800 (Stratagene, LaJolla, CA). A full-length probe for human SMRT (250ng) was labelled using a Randon Primer Fluorescein Labelling Kit (NEL603, DuPont), hybridized to the membrane-bound RNA, and probed with an antifluorescein-alkaline phosphatase conjugate as per instructions of the manufacturer. After addition of a nucleic acid chemiluminescence reagent (DuPont), chemiluminescence was detected by exposing the blot to autoradiography film (NEF-496, DuPont) for 10min.

Plasmids. For the pSG5-SMRT vector, full-length SMRT insert was removed from pCMX-SMRT (40) by cutting 5′ with EcoRV and 3′ with EcoRI. After adding an EcoRI linker to the 5′ end, SMRT was inserted into the EcoR1 site of pSG5 (Stratagene). To create pSG5-C-SMRT, C-SMRT was cut from pCMX-SMRT (40) 5′ with EcoRV and 3′ with BamH1. An EcoRI linker was added to the 5′ end and the insert was directionally cloned into the corresponding restriction sites in pSG5. To make pRSV-$\Delta\Delta$TRα and pRSV-$\Delta\Delta$TRα-R160, thyroid hormone receptor ligand binding domain ($\Delta\Delta$TRα) and its aa160 Pro-Arg mutant ($\Delta\Delta$TRαR160) were cut from pCMX 5′-3′ with HindIII and SmaI and cloned into pBK-RSV (Stratagene). RSV-Luc contains the RSV promoter and enhancer regions in PXP-1 (47). −4614/PXP, −321/PXP and pRSV-Src have been described previously (10, 48). Restriction digestion analysis and/or sequencing validated proper orientation of each clone.

Transient transfections and reporter assays. Briefly, cells were seeded in 6 well cluster plates at 2×10^5 cells/well and transfected with DNA the next day using calcium phosphate co-precipitation and glycerol shock (37). In all cases, the total amount DNA transfected into each well was held constant with empty control vectors. Cells recovered overnight in DMEM-FBS and the following morning were washed 3 times with HBSS followed by addition of unsupplemented DMEM-LH or DMEM-LH supplemented with 10^{-8}M phorbol 12-myristate 13-acetate (PMA; Sigma), 10^{-6}M all-*trans* - retinoic acid (Sigma), or 10ng/ml interleukin-1 (Genzyme). Twenty four hours later, luciferase reporter activity was determined using a model ML2250 microtitier plate luminometer (Dynatech, Chantilly, VA). In some experiments the Hirt's assay was used to monitor transfection efficiency (49), demonstrating consistency in DNA uptake from well to well. Each experiment was performed in triplicate and repeated at least twice. The data are presented as the mean +/− standard deviation from a representative experiment.

RESULTS

Synovial Fibroblasts Express SMRT

To examine role of MMPs in the progression of rheumatoid arthritis, we and others have utilized rabbit models and cultures of synovial fibroblasts obtained from these animals (50-52). Several approaches can be employed to induce rabbit disease, which in many aspects parallels the pathology observed in human rheumatoid arthritis (52). The rabbit cells express a number of MMPs that have close sequence identity to their partners in human cells (53) and several members of the nuclear receptor superfamily inhibit the synthesis of both rabbit and human MMPs (26). The co-repressors SMRT and N-CoR inhibit HRE-driven gene tran-

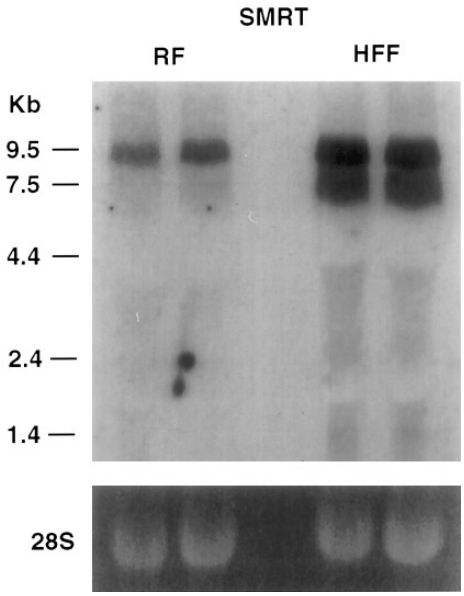

FIG. 1. Rabbit synovial fibroblasts express SMRT. Total RNA (20μg) from primary abbit synovial fibroblasts (RFs) or human foreskin fibroblasts (HFFs) was run out in duplicate, transferred and probed with full-length human SMRT as in the materials and methods. RNA markers are shown at left.

scription through direct interactions with nuclear receptors, quite possibly through higher-order complexes of the co-repressors with histone deacetylases (25). Since nuclear receptors inhibit MMP synthesis (26), and since co-repressors have been implicated in nuclear receptor-mediated repression (39), we sought to establish or exclude a role for SMRT in the suppression of interstitial collagenase (MMP-1).

As a first step in exploring a function for SMRT in MMP-1 regulation, we measured expression of the endogenous SMRT gene in primary rabbit synovial fibroblasts. As with human skin fibroblasts, the rabbit synovial fibroblasts express a major 9Kb transcript, indicating constitutive expression of a rabbit homologue of human SMRT (Figure 1). These data correlate with previous findings, which demonstrated ubiquitous expression of the 9Kb SMRT transcript in fetal human and adult mouse tissues (46). However, it remains unclear whether SMRT plays a regulatory role in all of these tissues or whether SMRT represses gene transcription exclusively through interactions with nuclear receptor superfamily members on HREs. Nonetheless, endogenous SMRT transcript is expressed in synovial fibroblasts, where it may potentially regulate gene expression.

SMRT Differentially Inhibits Basal and IL-1-Induced MMP-1 Promoter Activity

Joint tissues from patients with rheumatoid arthritis display elevated levels of the inflammatory cytokine IL-1 (6, 7), and physiologically relevant concentrations of this cytokine induce MMP-1 transcription in synovial fibroblasts *in vitro* (8-10). Since retinoids and their receptors (RARs and RXRs) inhibit rabbit and human MMP-1 expression induced by IL-1 (8), we investigated whether a nuclear receptor corepressor such as SMRT could influence the transcriptional activity of the MMP-1 gene.

We first co-transfected rabbit synovial fibroblasts with increasing amounts of a human SMRT expression vector (pSG5-SMRT) along with a luciferase reporter plasmid driven by 4614 bp of rabbit MMP-1 promoter (−4614/PXP). As little as 20ng/well of the SMRT expression vector markedly inhibits IL-1-induced transcription (Figure 2A). At 100ng/well of the co-repressor, IL-1-mediated transcription falls to levels similar to those of the untreated cells. However, even 100 ng/well of pSG5-SMRT does not significantly reduce basal transcription, indicating that IL-1-induced promoter activity is much more sensitive to the repressive effects of SMRT than is constitutive MMP-1 transcription. Only at the highest level of repressor, 500ng/well, is basal transcription markedly repressed by over-expression of SMRT. Similar to SMRT repression of MMP-1, other co-repressors have been reported to more effectively inhibit induced, rather than basal transcription (54), suggesting the possibility of a common theme for these inhibitors in the regulation of some genes.

We carried out several control experiments to evaluate the specificity of SMRT's repressive effects on MMP-1 (Figures 2B, 2C, 4). Earlier work identified sites in the SMRT protein that are necessary for repression (40, 46). Deletion of these regions obliterates the ability of SMRT to inhibit gene transcription, while retaining the capacity of SMRT to bind to nuclear receptors. We used a truncation mutant of SMRT (C-SMRT) lacking the repression domain (40, 46) to measure its effects on transcription. Importantly, this construct fails to inhibit basal or IL-1 induced MMP-1 promoter activity (Figure 2B), even when the truncated protein is expressed at high levels (0.5μg/well). In another possibility, over-expressed SMRT might interfere with a component of the transcriptional machinery common to all promoters. However, in opposition to this hypothesis, SMRT does not reduce activity of a constitutively-expressed RSV-luciferase reporter (Figure 2C), indicating SMRT may target a specific protein(s) or element(s) in the MMP-1 promoter that is not present in the promoter of all genes. Together, these data indicate that SMRT, acting through its N-terminal repression domain, specifically inhibits MMP-1 promoter activity.

SMRT Interferes with Proximal MMP-1 Promoter Activity in an HRE-Independent Manner

Several elements within 321 bp of the proximal rabbit MMP-1 promoter play key roles in both induction and

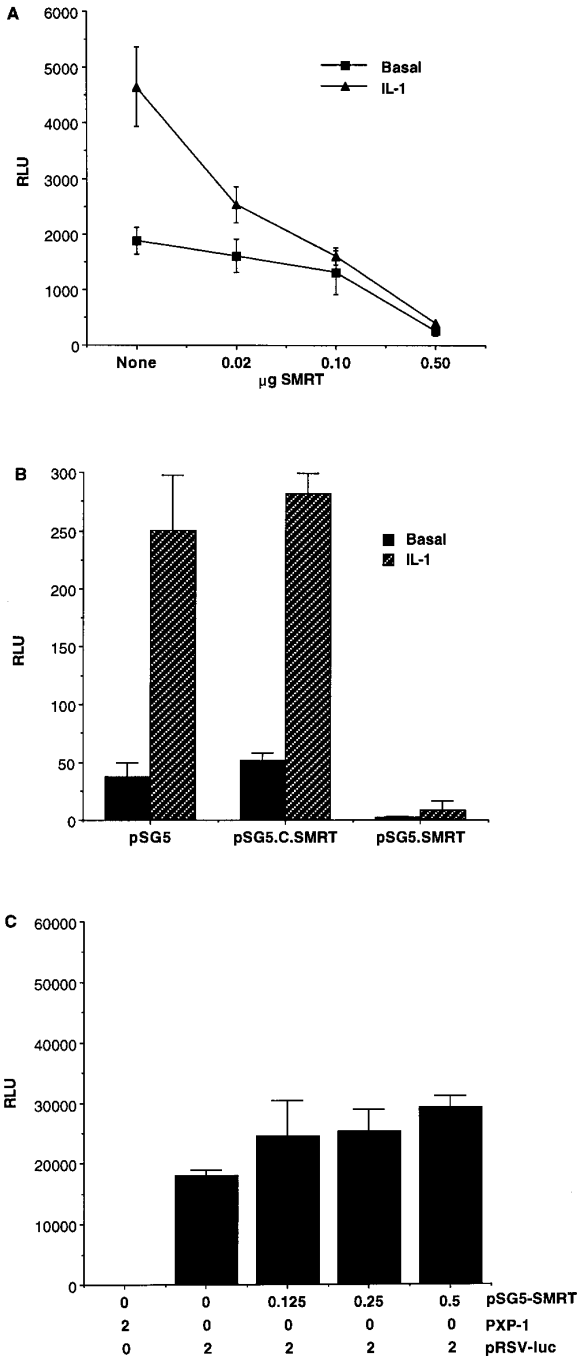

FIG. 2. SMRT inhibits IL-1-induced MMP-1 transcription. **A.** Rabbit synovial fibroblasts were transfected with 2.0μg/well of −4614/PXP alone or −4614/PXP plus increasing concentrations of pSG5-SMRT and were left untreated or were treated 24 hr with 10ng/ml IL-1. **B.** Cells were transfected with 2.0μg/well of −4614/PXP and co-transfected with 0.5μg/well of empty pSG5, pSG5-C-SMRT, or pSG5-SMRT and treated with or without IL-1. C. Cells were transfected with 2.0μg/well emptly PXP-1 luciferase reporter plasmid (PXP-1) or 2.0μg/well of RSV-luc with or without different amounts of pSG5-SMRT.

repression (11, 26). This region contains two AP-1 sites (−186 and −77) and a PEA-3 (Ets) site at −109. A functional STAT-binding element is also found at −53 the human MMP-1 promoter (15). We thus investigated whether SMRT could suppress transcriptional activity of a −321/PXP promoter construct. Figures 3A and 3B show that over-expression of v-Src or treatment with phorbol ester, both of which signal to activate MMP-1 gene expression through proximal regions (9, 10, 12, 13, 55) induce the activity of this shorter promoter fragment (Figure 3A, B). Furthermore, SMRT inhibits both v-Src- and phorbol ester-stimulated reporter activity driven by 321bp of promoter (Figure 3A, B). Nuclear receptors target both AP-1 and Ets proteins to repress MMP-1 transcription, raising the possibility that a co-receptor such as SMRT may target one or more of these elements in the proximal MMP-1 promoter.

To further document SMRT-specific repression of MMP-1 transcription on this shorter promoter fragment, we over-expressed of the ligand-binding domain

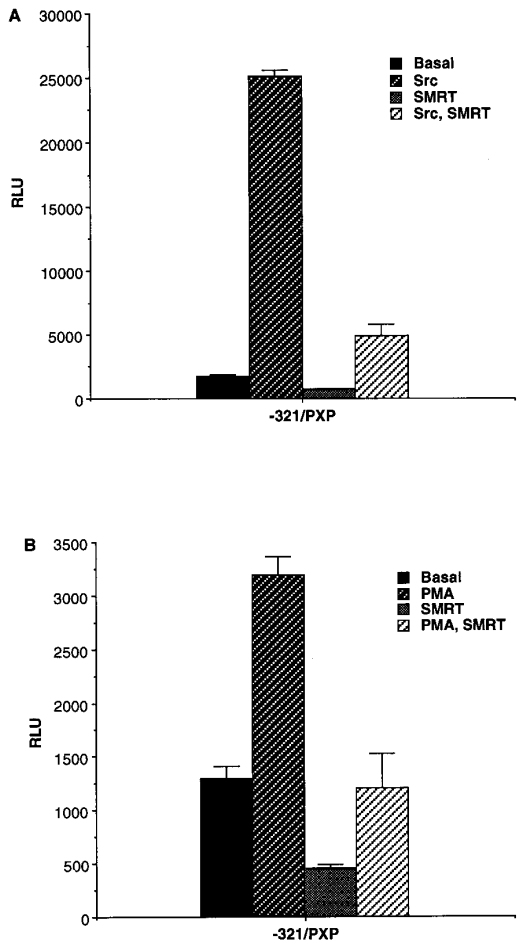

FIG. 3. SMRT inhibits proximal MMP-1 promoter activity induced by v-Src or PMA. **A.** Rabbit synovial fibroblasts were transfected with wt −321/PXP (2.0μg/well) and stimulated by cotransfection with pRSV-Src (0.5μg/well) in the absence or presence of pSG5-SMRT (0.5μg/well). **B.** Cells were transfected with wt −321/PXP in the absence or presence of pSG5-SMRT and stimulated by treatment with 10^{-8}M PMA.

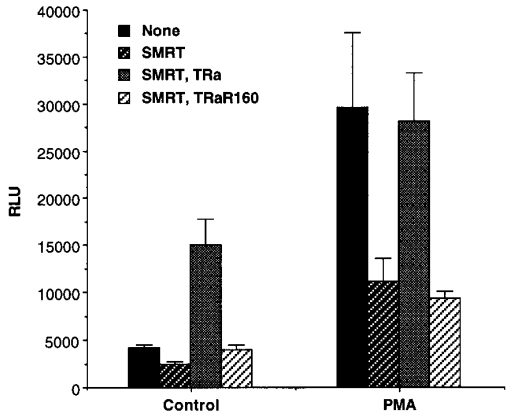

FIG. 4. Thyroid hormone receptor LBD reverses SMRT-mediated MMP-1 repression. Cells were transfected with −321/PXP and co-transfected with pSG5 containing no insert (none) or pSG5 containing SMRT, ΔΔTRα, or ΔΔTRαR160. Cells were left untreated (Control) or were treated with 10^{-8}M PMA.

for the thyroid hormone receptor (pRSV-ΔΔTRα), which binds to SMRT (40). This protein fragment reverses SMRT-mediated repression of phorbol ester-induced −321/PXP activity, presumably by limiting the availability of the co-repressor (Figure 4). However, a point mutation that disrupts binding of the thyroid horomone receptor to SMRT (pRSV-ΔΔTRα-R160) (40) fails to reverse the SMRT-mediated inhibition, indicating that SMRT specifically inhibits the −321/PXP construct, and that limiting available SMRT restores transcriptional activity.

DISCUSSION

In this report we demonstrate that synovial fibroblasts express the nuclear receptor co-repressor SMRT, and when over-expressed, it inhibits transcriptional activity of the proximal MMP-1 promoter. Several experiments demonstrate the specifity of this repression and argue against universal repression of the general transcriptional machinery by SMRT. First of all, at lower amounts of SMRT, the co-repressor drastically reduces IL-1-induced MMP-1 promotor activity without significantly affecting basal transcription (Figure 2A). Only when expressed at the highest levels does SMRT repress basal activity. Secondly, the truncation mutant C-SMRT fails to inhibit MMP-1 transcription (Figure 2B), demonstrating that the amino-terminal repression domain of SMRT is required for the inhibitory effects. As a third point, even at the highest concentrations, SMRT fails to repress transcription of the constitutively-expressed RSV luciferase reporter (Figure 2C). Fourth, the thyroid hormone receptor ligand-binding domain (TRα-LBD), which binds to SMRT (40), reverses the ability of SMRT to inhibit PMA-driven MMP-1 expression (Figure 4). This latter experiment demonstrates that TRα-LBD can limit the amount of SMRT that is available to exert negative effects on MMP-1 transcription.

The lack of any functional HREs in the proximal region of the MMP-1 promoter (37) suggests that SMRT represses this gene through a novel mechanism. SMRT inhibits a proximal region (321bp) of DNA that contains binding sites for several established or putative transcription factors, including Ets and AP-1. It is possible that SMRT represses MMP-1 transcription by interfering with one or more of these sites. For instance, mutation of the proximal AP-1 site at −77 in the MMP-1 promtoter produces the identical effect as strong SMRT over-expression, namely marked reduction of both basal and IL-1- or Src-induced promoter activity (our unpublished observations). While circumstantial, these data suggest that high concentrations of SMRT could functionally obliterate signalling at this AP-1 site. Previously, we provided evidence that RARs/RXRs, through interactions with c-Jun, can bind indirectly to the proximal AP-1 site of the MMP-1 promoter (35, 37). These findings raised the possibility that retinoid receptors in such a complex could inhibit MMP-1 gene transcription, perhaps by interactions with nuclear receptor co-repressors (1, 26, 39, 56). As opposed to HRE-driven genes, where ligand activates transcription by causing SMRT to dissociate from nuclear receptors that are bound constitutively to the DNA, retinoic acid does not reverse or enhance SMRT-mediated repression of MMP-1 (data not shown). Also, the human MMP-1 promoter contains a functional STAT-binding element at −53 (15), and stable transformation by v-Src is known to activite the JAK/STAT pathway (57). It remains to be determined whether the STAT-binding region is functionally conserved in the rabbit promoter or whether it may also serve as a repressive target for SMRT.

With relevance to our current findings, Tagami *et al.* have recently studied the transcriptional regulation of pituitary thyroid-stimulating hormone α-subunit (TSHα) and TSHβ, as well as hypothalamic thyrotropin-releasing hormone (TRH) (58). Thyroid hormone negatively regulates transcriptional activity in the proximal region of these genes, each of which lacks a consensus TRE (thyroid hormone responsive element) but contains binding sites for AP-1 and CREB. Somewhat surprisingly, unliganded thryroid hormone receptor, SMRT and N-CoR actually enhance, rather than repress, basal transcription of these genes. Nonetheless, we agree with the authors' conclusion that nuclear receptors and co-repressors can regulate transcription of genes lacking consensus HREs, and furthermore that complex protein-protein-DNA interactions may be responsible for such regulation.

In the case of MMP-1, endogenous, constitutively-expressed SMRT might contribute to maintaining low, basal gene expression. Furthermore, it is also possible that the recently described interaction of SMRT with

histone deacetylases (25) may play a role in quieting interstitial collagenase gene expression. Signal transduction events initiated by treatment with cytokines, phorbol esters or oncogene over-expression may then result in the activation of proximal transcription factors, which overwhelm the repressive effects of SMRT or cause release of the co-repressor. In line with such a hypothesis, we have observed that a c-Jun expression plasmid incrementally overcomes the repressive effect of SMRT on MMP-1 transcription (our observations). Numerous mechanisms for transcriptional intereference have been proposed, and it is unlikely that a single, isolated pathway accounts for repression of a given gene. Such redundancy and complexity demonstrates strict controls in the regulation of interstial collagenase. In summary, the nuclear receptor co-repressor SMRT inhibits MMP-1 transcription, apparently through a novel, RARE-independent mechanism. We conclude that nuclear receptor cofactors employ diverse strategies to modulate gene expression.

ACKNOWLEDGMENTS

The authors wish to thank members of the Brinckerhoff and Chen laboratories for expert comments, suggestions and technical advice. This work was supported by a postdoctoral fellowship from the Arthritis Foundation (DJS), start-up funds from the Department of Pharmacology and Molecular Toxicology, an Institutional award from the American Cancer Society and a Howard Hughes pilot grant provided by the UMass Medical Center (JDC), NIH grants F32 AR08216 and K01 AR02024 (MPV), and grants from the NIH (AR-26599) and the RGK Foundation (Austin, TX) (CEB).

REFERENCES

1. Vincenti, M. P., White, L. A., Schroen, D. J., Benbow, U., and Brinckerhoff, C. E. (1996) *Crit. Rev. Euk. Gene. Expr.* **6,** 391–411.
2. Birkedal-Hansen, H. (1995) *Curr. Opin. Cell. Biol.* **7,** 728–735.
3. Cawston, T. E. (1995) *Br. Med. Bull.* **51,** 385–401.
4. Vincenti, M. P., Clark, I. M., and Brinckerhoff, C. E. (1994) *Arthritis Rheum.* **37,** 1115–1126.
5. Arend, W. P., and Dayer, J. M. (1995) *Arthritis Rheum.* **38,** 151–160.
6. Feldmann, M., Brennan, F. M., and Maini, R. N. (1996) *Annu. Rev. Immunol.* **14,** 397–440.
7. Firestein, G. S. (1996) *Arthritis Rheum.* **39,** 1781–1790.
8. Lafyatis, R., Kim, S. J., Angel, P., Roberts, A. B., Sporn, M. B., Karin, M., and Wilder, R. L. (1990) *Mol. Endocrinol.* **4,** 973–980.
9. Vincenti, M. P., Coon, C. I., Lee, O., and Brinckerhoff, C. E. (1994) *Nucleic Acids Res.* **22,** 4818–4827.
10. Vincenti, M. P., Coon, C. I., White, L. A., Barchowsky, A., and Brinckerhoff, C. E. (1996) *Arthritis Rheum.* **39,** 574–582.
11. Benbow, U., and Brinckerhoff, C. E. (1997) *Matrix Biol.* **15,** 519–526.
12. Angel, P., Baumann, I., Stein, B., Delius, H., Rahmsdorf, H. J., and Herrlich, P. (1987) *Mol. Cell. Biol.* **7,** 2256–2266.
13. White, L. A., and Brinckerhoff, C. E. (1995) *Matrix Biol.* **14,** 715–725.
14. Buttice, G., Duterque-Coquillaud, M., Basuyaux, J. P., Carrere, S., Kurkinen, M., and Stehelin, D. (1996) *Oncogene* **13,** 2297–2306.
15. Korzus, E., Nagase, H., Rydell, R., and Travis, J. (1997) *J. Biol. Chem.* **272,** 1188–1196.
16. Claret, F. X., Hibi, M., Dhut, S., Toda, T., and Karin, M. (1996) *Nature* **383,** 453–457.
17. Horvai, A. E., Xu, L., Korzus, E., Brard, G., Kalafus, D., Mullen, T. M., Rose, D. W., Rosenfeld, M. G., and Glass, C. K. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 1074–1079.
18. Bannister, A. J., Oehler, T., Wilhelm, D., Angel, P., and Kouzarides, T. (1995) *Oncogene* **11,** 2509–2514.
19. Bannister, A. J., and Kouzarides, T. (1996) *Nature* **384,** 641–643.
20. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H., and Nakatani, Y. (1996) *Cell* **87,** 953–959.
21. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) *Nature* **382,** 319–324.
22. Steger, D. J., and Workman, J. L. (1996) *Bioessays* **18,** 875–884.
23. Werner, M. H., and Burley, S. K. (1997) *Cell* **88,** 733–736.
24. Wade, P. A., Pruss, D., and Wolffe, A. P. (1997) *Trends in Biol. Sci.* **22,** 128–132.
25. Pazin, M. J., and Kadonaga, J. T. (1997) *Cell* **89,** 325–328.
26. Schroen, D. J., and Brinckerhoff, C. E. (1996) *Gene Expression* **6,** 197–207.
27. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Heyman, R. A., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1996) *Cell* **85,** 403–414.
28. Zhang, X. K., Wills, K. N., Husmann, M., Hermann, T., and Pfahl, M. (1991) *Mol. Cell. Biol.* **11,** 6016–6025.
29. Schule, R., Umesono, K., Mangelsdorf, D. J., Bolado, J., Pike, J. W., and Evans, R. M. (1990) *Cell* **61,** 497–504.
30. Yang-Yen, H. F., Chambard, J. C., Sun, Y. L., Smeal, T., Schmidt, T. J., Drouin, J., and Karin, M. (1990) *Cell* **62,** 1205–1215.
31. Schule, R., Rangarajan, P., Yang, N., Kliewer, S., Ransone, L. J., Bolado, J., Verma, I. M., and Evans, R. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6092–6096.
32. Yang-Yen, H. F., Zhang, X. K., Graupner, G., Tzukerman, M., Sakamoto, B., Karin, M., and Pfahl, M. (1991) *New Biol.* **3,** 1206–1219.
33. Jonat, C., Rahmsdorf, H. J., Park, K. K., Cato, A. C., Gebel, S., Ponta, H., and Herrlich, P. (1990) *Cell* **62,** 1189–1204.
34. Konig, H., Ponta, H., Rahmsdorf, H. J., and Herrlich, P. (1992) *Embo. J.* **11,** 2241–2246.
35. Pan, L., Eckhoff, C., and Brinckerhoff, C. E. (1995) *J. Cell. Biochem.* **57,** 575–589.
36. Pfahl, M. (1993) *Endocr. Rev.* **14,** 651–658.
37. Schroen, D. J., and Brinckerhoff, C. E. (1996) *J. Cell. Physiol.* **169,** 320–332.
38. Schneikert, J., Peterziel, H., Defossez, P. A., Klocker, H., Launoit, Y., and Cato, A. C. (1996) *J. Biol. Chem.* **271,** 23907–23913.
39. Horwitz, K. B., Jackson, T. A., Bain, D. L., Richer, J. K., Takimoto, G. S., and Tung, L. (1996) *Molec. Endocrinol.* **10,** 1167–1177.
40. Chen, J. D., and Evans, R. M. (1995) *Nature* **377,** 454–457.
41. Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C. K., and et al. (1995) *Nature* **377,** 397–404.
42. Kurokawa, R., Soderstrom, M., Horlein, A., Halachmi, S., Brown, M., Rosenfeld, M. G., and Glass, C. K. (1995) *Nature* **377,** 451–454.

43. Alland, L., Muhle, R., Hou, Jr., H., Potes, J., Chin, L., Schreiber-Agus, N., and DePinho, R. A. (1997) *Nature* **387,** 49–55.

44. Heinzel, T., Lavinsky, R. M., Mullen, T.-M., Söderström, M., Laherty, C. D., Torchia, J., Yang, W.-M., Brard, G., Ngo, S. D., Davie, J. R., Seto, E., Eisenman, R. N., Rose, D. W., Glass, C. K., and Rosenfeld, M. G. (1997) *Nature* **387,** 43–48.

45. Nagy, L., Kao, H.-Y., Chakravarti, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L., and Evans, R. M. (1997) *Cell* **89,** 373–380.

46. Chen, J. D., Umesono, K., and Evans, R. M. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 7567–7571.

47. Nordeen, S. K. (1988) *Biotechniques* **6,** 454–456.

48. Vincenti, M. P., Schroen, D. J., Coon, C. I., and Brinckerhoff, C. E. (1997) *Oncogene.* [**submitted**]

49. Pan, L., Chamberlain, S. H., Auble, D. T., and Brinckerhoff, C. E. (1992) *Nucleic Acids Res.* **20,** 3105–3111.

50. Brinckerhoff, C. E., and Harris, J., E. D. (1981) *Biochim. Biophys. Acta.* **677,** 424–432.

51. Brinckerhoff, C. E., Nagase, H., Nagel, J. E., and Harris, J., E. D. (1982) *J. Amer. Acad. Dermatol, St. Louis* **6,** 591–602.

52. Hembry, R. M., Bagga, M. R., Murphy, G., Henderson, B., and Reynolds, J. J. (1993) *Am. J. Pathol.* **143,** 628–642.

53. Chamberlain, S. H., Hemmer, R. M., and Brinckerhoff, C. E. (1993) *J. Cell. Biochem.* **52,** 337–351.

54. Kirov, N. C., Lieberman, P. M., and Rushlow, C. (1996) *EMBO J.* **15,** 7079–7087.

55. Auble, D. T., and Brinckerhoff, C. E. (1991) *Biochemistry* **30,** 4629–4635.

56. Beato, M., and Sanchez-Pacheco, A. (1996) *Endocr. Rev.* **17,** 587–609.

57. Campbell, G. S., Yu, C. L., Jove, R., and Carter-Su, C. (1997) *J. Biol. Chem.* **272,** 2591–2594.

58. Tagami, T., Madison, L. D., Nagaya, T., and Jameson, J. L. (1997) *Mol. Cell. Biol.* **17,** 2642–2648.