[Frontiers in Bioscience 7, d608-618, March 1, 2002]

MOLECULAR ASPECTS OF SCLERODERMA

Maria Trojanowska

Division of Rheumatology and Immunology, Medical University of South Carolina, 96 Jonathan Lucas St., Suite 912, Charleston SC 29425

TABLE OF CONTENTS

1. ABSTRACT

Scleroderma is a complex disease characterized by activation of the immune system, small-vessel vasculopathy, and fibrosis of the skin and other organs. This review is limited to the discussion of scleroderma fibroblast biology and the cellular and molecular mechanisms that contribute to the abnormal deposition of collagen. Selected aspects of abnormal extracellular matrix (ECM) regulation by scleroderma fibroblasts are critically reviewed. These include the role of transforming growth factor-beta (TGF-beta) and connective tissue growth factor (CTGF) and their receptors in the fibrotic process in scleroderma and the overview of the transcription factors involved in regulation of the human alpha2 (I) collagen (COL1A2) gene.

2. INTRODUCTION

Scleroderma is a complex disease characterized by activation of the immune system, small-vessel vasculopathy, and fibrosis of the skin and other organs. Several excellent reviews have recently discussed various pathological aspects of scleroderma (1-4). This review will mainly focus on the mechanisms of ECM dysregulation in scleroderma with emphasis on the presently controversial issues and other aspects of ECM regulation not fully covered by other review articles.

2.1. Scleroderma fibroblasts

Scleroderma fibroblasts derived from skin biopsies of patients with active disease provide a useful *in vitro* model utilized by many scleroderma investigators. Over the years these studies have provided a wealth of information with potential relevance to fibrosis occurring *in vivo*. These fibroblasts produce elevated levels of collagen and other ECM proteins and exhibit altered expression of many other genes. There is an ongoing debate regarding the "origin" of scleroderma fibroblasts. Activation and/or selection of resident fibroblasts have been proposed (5,6). Transdifferentiation of other cell types, such as pericytes (7,8) or monocytes (9) into fibroblastic cells with an

activated phenotype has also been considered. Interestingly, studies from other experimental systems, including other fibrotic diseases, provide increasing evidence that epithelial (or endothelial) cells have the ability to transdifferentiate into fibroblasts (10). In addition, peripheral blood fibrocytes are a recently described population of cells that localize to areas of connective tissue deposition, including wound healing and fibrotic tissues (11). Together, these new developments suggest that activated fibroblasts isolated from scleroderma lesions may have originated from other cell types.

3. GROWTH FACTORS AND RECEPTORS

3.1. TGF-beta *vs* CTGF

TGF-beta and CTGF have emerged as leading candidates responsible for overproduction of ECM proteins in different fibrotic diseases. As discussed in this section, despite extensive studies, their specific contributions to the process of fibrosis, including scleroderma, are not fully understood.

3.1.1. TGF-beta may be responsible for the initiation of fibrosis

A number of studies analyzed the presence of TGF-beta in lesional and non-lesional skin of patients with scleroderma. Elevated levels of TGF-beta have been reported in involved skin lesions of SSc patients (12-15). Furthermore, TGF-beta expression was shown to co-localize with collagen type I mRNA in involved skin of SSc patients (14). Subsequent studies, which analyzed TGF-beta1 expression during disease progression, indicated that TGF-beta1 is present in early inflammatory stage of the disease, prior to the onset of fibrosis (16). In a recent study, expression of all three TGF-beta isoforms at mRNA and protein levels were reexamined. It was found that all three isoforms were expressed in the inflammatory skin areas, but could not be detected in the sclerotic skin (17). Consistent with these observations, TGF-beta1 mRNA expression was shown to precede collagen expression in murine sclerodermatous graft-*vs*-host disease (Scl GVHD) considered an animal model of scleroderma (18). In the latter study, fibrosis was prevented by a neutralizing anti-TGF-beta antibody administered at day 1 and 6 after bone marrow transplant (18). In other experimental models of fibrosis, blockade of the TGF-beta pathway at the time of administration of the fibrotic stimulus was also successful in preventing development of fibrosis (19). Collectively, these *in vivo* studies support a critical role for TGF-beta in the initiation of the fibrotic process. However, the nature of the processes affected by TGF-beta that subsequently contribute to the development of fibrosis is presently unknown. One recently proposed possibility is that TGF-beta contributes to selection of an apoptosis resistant subset of fibroblasts (20, 21). These fibroblasts, which are also characterized by collagen overexpression, may persist in the lesional tissue and contribute to matrix deposition.

3.1.2. CTGF expression correlates with the sclerotic phase of the disease

The elevated expression of CTGF has been universally observed in various fibrotic disorders including scleroderma, keloids, glomerulorsclerosis, hepatic fibrosis, atherosclerosis and others (22, 23). Significantly, a strong correlation between CTGF expression by lesional fibroblasts and skin sclerosis was observed in skin biopsies obtained from scleroderma patients (24, 25). Based on this observation, it was proposed that CTGF contributes to the maintenance of fibrosis. However, these studies did not examine whether fibroblasts that expressed CTGF also produced increased collagen levels. Taking into consideration the recently described properties of CTGF as an inducer of matrix metalloproteinases (MMPs, see below) and the fact that sclerotic lesions diminish spontaneously in scleroderma, the role of CTGF in the late stages of scleroderma is unclear. It is possible that persistent presence of CTGF in the late stages of the disease is associated with matrix degradation rather than with matrix deposition. To strengthen the case of CTGF as the pro-fibrotic agent in scleroderma, co-localization of CTGF with cells actively producing collagen needs to be demonstrated.

The spatial and temporal relationship between TGF-beta and CTGF expression *in vivo* has not been adequately addressed. In the limited number of studies that investigated this issue, both growth factors appear to act in concert. For example, expression of CTGF overlaps with that of TGF-beta during wound healing (24). It is possible that under physiological conditions such as wound healing these two growth factors cooperate to effectively repair damaged tissue. However, this process appears to be dysregulated in fibrosis. As mentioned above, in scleroderma lesions, TGF-beta is expressed in the early inflammatory stage, while CTGF is highly expressed in the late sclerotic stage, where it may persist for many years (25). The inducer of CTGF in the late sclerotic stage is unknown. While initial *in vitro* studies have suggested that TGF-beta is an exclusive inducer of CTGF (24), recent studies have uncovered a number of additional stimuli acting via distinct signaling pathways capable of CTGF induction. These include high glucose (26), dexamethasone (27), Factor VIIa and thrombin (28,29), serotonin and LPA (30), and VEGF (31). Our recent work also suggests that a human cytomegalovirus immediate early gene product (HCMV IE1) is a potent inducer of CTGF (32). Consistent with these *in vitro* observations, a case of pseudoscleroderma was reported in which expression of CTGF colocalized with the COL1A1 in the absence of detectable TGF-beta (33). Together, *in vivo* observations suggest that TGF-beta is associated with the early stages of scleroderma, whereas CTGF is associated with late disease. However the specific roles of these pleiotropic growth factors in the pathological process remain elusive.

3.1.3. TGF-beta is a potent inducer of ECM in cultured fibroblasts

The role for TGF-beta as a potent inducer of ECM synthesis in a variety of cells, including human fibroblasts, is well established. TGF-beta stimulates ECM accumulation through several mechanisms including direct induction of collagen transcription and mRNA stability (34,35) as well as inhibition of ECM degradative pathways (36). Recent studies have characterized some of the specific transcription factors contributing to collagen gene

regulation by TGF-beta (37-39). This aspect of collagen gene regulation is discussed in section 5.

3.1.4. The role of CTGF in ECM deposition and turnover is controversial

The role of CTGF as an inducer of ECM is less clear. Original studies by Grotendorst and collaborators using embryonic rat kidney cells (NRK fibroblasts) have demonstrated that CTGF is a mediator of the fibrogenic effects of TGF-beta including proliferation and ECM production (40). Using NRK fibroblasts these investigators have shown that blocking CTGF either by neutralizing antibody or by antisense oligonucleotides abolishes the stimulation of collagen by TGF-beta. Similar effects were observed in a wound healing model (40). Furthermore, it was shown that both TGF-beta induction of CTGF and subsequent CTGF induction of collagen are negatively regulated by cAMP. More recently Stratton *et al* (41) confirmed these observations using TGF-beta stimulated scleroderma and normal fibroblasts. Induction of cAMP with Iloprost resulted in inhibition of TGF-beta-induced CTGF and collagen. Surprisingly, in the latter study, increased CTGF production by scleroderma fibroblasts did not correlate with increased basal collagen production (41). Thus, CTGF may only function as a stimulator of collagen synthesis in conjunction with activation of TGF-beta signaling. In fact, recent studies using different approaches have suggested that CTGF and a closely related factor Cyr61 are inducers of MMPs (42). Adhesion of human dermal fibroblasts to either CTGF or Cyr61 resulted in formation of focal complexes and activation of FAK, paxillin and Rac kinases. In addition, a prolonged activation of MAPK and induction of MMP-1 (collagenase-1) and MMP-3 (stromelysin-1) mRNAs and proteins were observed. The effects of CTGF were mediated by alpha 6 beta 1 integrin and cell surface HSPGs (heparan sulphate proteoglycan).

Taken together, cell culture studies suggest that CTGF could have both pro-fibrotic and anti-fibrotic modes of action. There are a few possible explanations for these seemingly contradictory observations. **i)** As discussed earlier, induction of MMPs is mediated via the integrin receptors. A separate cell surface receptor(s) may mediate CTGF stimulation of ECM. However, despite intensive efforts, a classic, signaling CTGF-specific receptor has not been characterized. **ii)** The action of CTGF as either pro-fibrotic or anti-fibrotic agent may depend on the simultaneous activation of other signaling pathways such as TGF-beta or insulin/IGFs. For example, the original study by Grotendorst's laboratory describing CTGF effects on ECM production utilized growth medium containing ITS (Insulin-Transferin-Selenium) and IGF-II (insulin growth factor-II) (40). We have also observed that human dermal fibroblasts incubated in ITS-supplemented medium demonstrate increased basal ECM production and increased responses to CTGF (43). On the other hand, studies by Chen *et al* (42) examining MMP induction by CTGF were performed in serum free IMDM medium. **iii)** Finally, specific responses to CTGF may depend on unidentified intrinsic properties of the target cell. For example, scleroderma fibroblasts respond to CTGF with increased collagen type I mRNA levels and an increased collagen promoter/reporter activity (43,44). It may also be relevant that scleroderma fibroblasts produce higher levels of endogenous CTGF (41,44,45). Whether autocrine expression of CTGF by scleroderma fibroblasts contributes to constitutive ECM overexpression by these cells remains to be determined. However, as mentioned in the previous section, there was no correlation between elevated collagen production and CTGF overexpression in a recent study by Stratton *et al* (41).

The mechanisms of action of CTGF, especially its possible profibrogenic effects, are still poorly understood. Lack of commercially available CTGF hampers the progress in this field and may be partially responsible for the inconsistent results reported by different laboratories.

3.2. TGF-beta receptors and Smads

3.2.1. TGF-beta receptors are upregulated in scleroderma fibroblasts

There is evidence that the components of the TGF-beta signaling pathway may be dysregulated in scleroderma. Elevated mRNA levels of TGF-beta receptors type I and II (TGF-betaRI and -II) were observed in scleroderma fibroblasts, which correlated with elevated levels of collagen mRNA (46). Based on these results, it was suggested that elevated production of collagen by scleroderma fibroblasts is dependent on autocrine TGF-beta signaling. This possibility was further supported by demonstration of increased protein levels of TGF-betaRI and TGF-betaRII in scleroderma fibroblasts (47). Furthermore, the latter study has shown that a neutralizing anti-TGF-beta antibody significantly decreased collagen production by scleroderma fibroblasts, without an appreciable effect on collagen production by healthy dermal fibroblasts. Elevated TGF-beta receptor expression has also been observed in skin biopsies from patients with localized scleroderma (48). Our ongoing extended analyses of TGF-beta receptor expression in scleroderma and control fibroblasts confirmed the elevated levels of the TGF-betaRI protein in a large proportion of scleroderma fibroblasts. In contrast, the levels of TGF-betaRII were not consistently elevated (Gore and Trojanowska, unpublished data). To gain additional insights into the role of autocrine TGF-beta pathway in collagen upregulation by scleroderma fibroblasts, our laboratory utilized a kinase-deficient TGF-betaRII (deltakRII) overexpressed via an adenoviral vector. This treatment effectively diminished (by about 50%) basal collagen and fibronectin production by healthy fibroblasts, but was effective only in selected scleroderma fibroblast cell lines (49). Together, these findings suggest that the autocrine TGF-beta signaling may be responsible for the elevated production of ECM only in a proportion of scleroderma fibroblasts. Moreover, these data suggest that in some scleroderma cell lines constitutive collagen production may be independent of TGF-beta signaling. Alternatively, collagen levels in scleroderma cells may be decreased via a kinase-deficient TGF-betaRI (deltakRI) as it was recently demonstrated that only deltakRI abrogates TGF-beta-induced collagen expression in rat mesangial cells (50). Interestingly, discoordinated expression of TGF-beta receptors has also been observed in other fibroproliferative disorders

including hepatic fibrosis and atherosclerosis (51,52). Further studies are needed to delineate the functional significance of the altered expression levels of the TGF-beta receptor subunits in fibrotic disorders.

3.2.2. The role of Smads in scleroderma is unknown

Smads are downstream mediators of the TGF-beta signaling pathway (53). Smad3 has been shown to mediate induction of COL1A2 (37-39) and CTGF (54) in normal dermal fibroblasts. However, the Smad-response element does not contribute to the elevated expression of the CTGF promoter in scleroderma fibroblasts (54). Moreover, the expression levels of Smad3, Smad4, and Smad7 did not differ between scleroderma and healthy control fibroblasts (54). On the other hand, the increased presence of nuclear Smad3 has been observed in SSc fibroblasts in the absence of exogenous TGF-beta stimulation (4). Whether Smads are specifically involved in the abnormal phenotype of scleroderma fibroblasts remains unknown.

3.3. Other growth factors

Besides TGF-beta and CTGF, other growth factors, including pro-IL-1alpha, PDGF, IL-4, Oncostatin M (OSM), IL-13, and others most likely contribute to the pathogenesis of scleroderma by either directly contributing to ECM production or by affecting other cellular functions (reviewed in 4). Recent studies also suggest that thrombin may directly contribute to induction of the myofibroblast phenotype in scleroderma lung fibroblasts (55, 56).

4. SIGNALING PATHWAYS INVOLVED IN COLLAGEN GENE REGULATION

4.1. MAP kinase family

The three major groups of the mammalian MAP kinase family include extracellular signal-regulated kinases (ERKs), c-jun N-terminal or stress activated protein kinases (JNK/SAPKs), and p38 kinases (57). There are numerous studies that link specific kinases to collagen gene regulation in various experimental systems (58, 59). Since their effects depend on experimental conditions and appear to be cell context specific, no clear conclusions can be made at this point about the role of each of these pathways in ECM deposition and turnover. Studies utilizing human dermal fibroblasts are briefly reviewed here because of their potential relevance to scleroderma. In general, activation of ERK1/2 and JNK/SAPKs inhibit collagen production in dermal fibroblasts albeit via distinct mechanisms, whereas activation of p38 is stimulatory. Thus, ERK 1/2 signaling cascade negatively regulates basal and TGF-beta-induced expression of type I collagen through inhibition of alpha1(I) and alpha2(I) collagen mRNA stability (60). Induction of stress activated protein kinases, JNK/SAPKs, inhibit collagens type I and III at the transcriptional level (61). Furthermore, c-Jun is one of the mediators of UV-induced collagen repression in dermal fibroblasts *in vitro*, and *in vivo* in human skin (61). The role of these inhibitory pathways in scleroderma has not been determined.

In contrast, p38MAPK is activated by TGF-beta and cooperates with TGF-beta in stimulation of collagen protein and mRNA levels, and COL1A2 promoter activities in dermal fibroblasts (62). Interestingly, in human fibroblasts, TGF-beta induction of p38 is prolonged with the early peak at 2 hours and the activated state persisting for up to 24 hours. The kinetics of p38 activation correlate well with the persistent stimulatory effects of TGF-beta on collagen mRNA levels (63), suggesting an important role for p38 in collagen gene regulation. The transcription factors involved in collagen gene regulation that are targeted by p38 are currently unknown. However, p38 levels, as well as kinetics of p38 activation by TGF-beta, are similar in SSc and healthy skin fibroblasts, suggesting that elevated collagen production by SSc fibroblasts is not due to the alteration of this pathway (62).

4.2. PKC-delta

Recent studies have implicated PKC-delta in collagen gene regulation in dermal fibroblasts (64). Using a specific pharmacological inhibitor of PKC-delta, rottlerin, Jimenez et al have demonstrated inhibition of basal collagen type I protein, mRNA, and promoter levels in scleroderma and healthy skin fibroblasts. The *cis*-response element and cognate transcription factor mediating inhibitory effects of rottlerin on the COL1A1 promoter remain to be characterized. Further studies are also needed to directly support the role of PKC-delta in the collagen gene regulation. Significantly, scleroderma fibroblasts express higher levels of PKC-delta suggesting that this pathway may directly contribute to scleroderma phenotype.

4.3. Sphingolipid signaling pathway

Sphingolipids, in addition to their role as structural molecules of the plasma membrane, are now recognized as important bioactive mediators of a variety of cellular processes (65, 66). In particular, biologic functions of ceramide, sphingosine, and their phosphates have been extensively studied. Recent studies have suggested that ceramide may be involved in the regulation of collagen metabolism. It was shown that exogenously added C_2-ceramide inhibits collagen gene expression in hepatic stellate cells and dermal fibroblasts (67, 60). Induction of the ERK pathway by C_2-ceramide was shown to mediate collagen gene inhibition (60). In contrast, our recent observations suggest that endogenous ceramide (or a non-sphingoid metabolite), generated by the action of sphingosine 1-phosphate phosphatase is a coactivator of the TGF-beta signaling pathway (Sato and Trojanowska, unpublished data). These observations suggest that sphingolipids may have a dual role in collagen gene regulation and that metabolites with both stimulatory and inhibitory functions can be formed endogenously. The nature of the interaction between the sphingolipid and the TGF-beta signaling pathways and a possible role of sphingolipid signaling in scleroderma remains to be elucidated.

5. TRANSCRIPTIONAL REGULATION OF COLLAGEN TYPE I GENE

Collagen type I, a triple helix of two COL1A1 and one COL1A2 chains, is a primary component of the connective tissue and one of the most abundant physiologic

proteins. Its overexpression is a hallmark of scleroderma and other diseases characterized by fibrosis. In recent years, our understanding of collagen type I gene regulation has increased significantly. In addition to the mapping of *cis*-response elements in the COL1A2 and COL1A1 promoters, several transcription factors involved in regulation of these promoters have been identified. More importantly, it has been found that in scleroderma fibroblasts several of these transcription factors differ either in their expression levels or in levels of post-translational modifications, strongly suggesting that the alteration of transcriptional control mechanisms contribute directly to the activated state of scleroderma fibroblasts. To fully understand collagen gene regulation, it is necessary to complete characterization of the relevant transcription factors and to delineate the functional significance of their post-translational modifications, as well as the nature of the signaling pathways that induce them. Ultimately, these studies should lead to a better understanding of the specific mechanisms that are responsible for collagen dysregulation in scleroderma.

5.1.The central role of Sp1 in basal collagen gene expression

Sp1 is a ubiquitously expressed zinc finger transcription factor implicated in the regulation of numerous viral and cellular genes (68). Studies using the Sp1 inhibitor mithramycin, as well as other strategies aimed at inhibiting Sp1 expression and function, support the crucial role for this factor in basal collagen gene regulation in healthy and scleroderma fibroblasts (69, 70). While Sp1 universally functions as a transcriptional activator, another ubiquitously expressed member of the Sp/KLF (Kruppel-like factor) family, Sp3, can function either as an activator or as a repressor depending on the promoter and cellular context (68). Extensive analyses of the human COL1A2 and COL1A1 promoters characterized several Sp1/Sp3 binding sites in the promoter regions of both genes (71-74). Interestingly, although the organization of both promoters differs significantly, similar transcription factors seem to be involved in their coordinate regulation. The specific role of Sp1 and Sp3 in regulation of each promoter is not clear yet. For example, both Sp1 and Sp3 activate COL1A2 promoter activity in *Drosophila* Schneider cells (74) and in human dermal fibroblasts (Trojanowska, unpublished observations). On the other hand, Sp3 inhibits Sp1-stimulated COL1A1 promoter activity in *Drosophila* Schneider cells (75).

Scleroderma and control healthy fibroblasts express similar levels of Sp1 and Sp3 proteins. However, Sp1 is more phosphorylated in scleroderma fibroblasts (69). Furthermore, increased Sp1 phosphorylation correlates with increased levels of COL1A2 mRNA. While it is tempting to speculate that increased Sp1 phosphorylation contributes to the scleroderma phenotype, many questions remain unanswered. Where do the differentially phosphorylated sites map within Sp1? What is the functional consequence of this phosphorylation? What is the nature of the signaling pathway involved in increased Sp1 phosphorylation in scleroderma fibroblasts? It may be relevant that recent studies have shown that serum induces Sp1 phosphorylation in dermal fibroblasts, which correlates with increased collagen mRNA levels (76). A novel kinase activity has been postulated to phosphorylate the C-terminal segment of Sp1 in response to serum stimulation (77). It may also be relevant that activity of Sp1 is regulated by serine/threonine phosphatases (78). Since multiple cellular kinases have already been implicated in Sp1 phosphorylation in various experimental systems (78), further studies are clearly needed to define the specific pathway relevant to Sp1 phosphorylation in scleroderma.

5.2. Sp1 mediates collagen gene transcription in response to external stimuli

TGF-beta modulation of gene transcription is mediated by Smads, which act in conjunction with other transcription factors in a tissue and promoter specific manner (53). It has been shown that Smad3 binds to the CAGA motif within the COL1A2 promoter and that overexpression of Smad3 leads to induction of this promoter (37,38). Furthermore, cooperation between Sp1 and Smad3 is required for the full TGF-beta response (39). Other inducers of COL1A2 gene expression such as serum and OSM also require intact Sp1 binding sites within the COL1A2 promoter for their effects (76,79). One may speculate that other factors cooperate with Sp1 to stimulate COL1A2 transcription in response to these signals. The nature of these factors is presently unknown. Thus Sp1 is not only an activator of basal collagen type I transcription, but also the effector of distinct signaling pathways that lead to collagen gene activation. It should be noted that basal collagen gene transcription *in vitro* represents an activated state induced by fibroblast adhesion to plastic and propagation in the presence of serum. Furthermore, recent evidence suggests that Sp1 is also a mediator of the inhibitory pathways regulating COL1A2 transcription (39,80,81). The function of Sp1 as a mediator of inhibitory pathways is discussed in the next section.

5.3. Fli-1 may play a role in abnormal ECM deposition in scleroderma

Ets transcription factors are critical mediators of ECM remodeling with a wide spectrum of target genes (82). Recent studies indicate that Fli-1 is an inhibitor of basal COL1A2 transcription. While the specific mechanisms involved in Fli-1 inhibition of collagen are not fully understood at present, the inhibitory function of Fli-1 appears to be Sp1 dependent (80). Furthermore, both Fli1 and Ets1 are antagonists of the profibrogenic effects of TGF-beta (83). Our recent observations suggest that Fli-1 is underexpressed in scleroderma fibroblasts and in addition exhibits abnormal post-translational modifications (unpublished observations). Since Fli-1 is an inhibitor of basal and TGF-beta-induced ECM production, decreased expression of Fli-1 in scleroderma fibroblasts may directly contribute to the increased collagen production by these cells. The functional significance of post-translational modifications of Fli-1 is currently under investigation. Interestingly, Ets-1 has been shown to be elevated in synovial fibroblasts *in vitro* and *in vivo* and may contribute to the pathology of rheumatoid arthritis (84). We have not observed consistent differences in Ets-1 protein expression

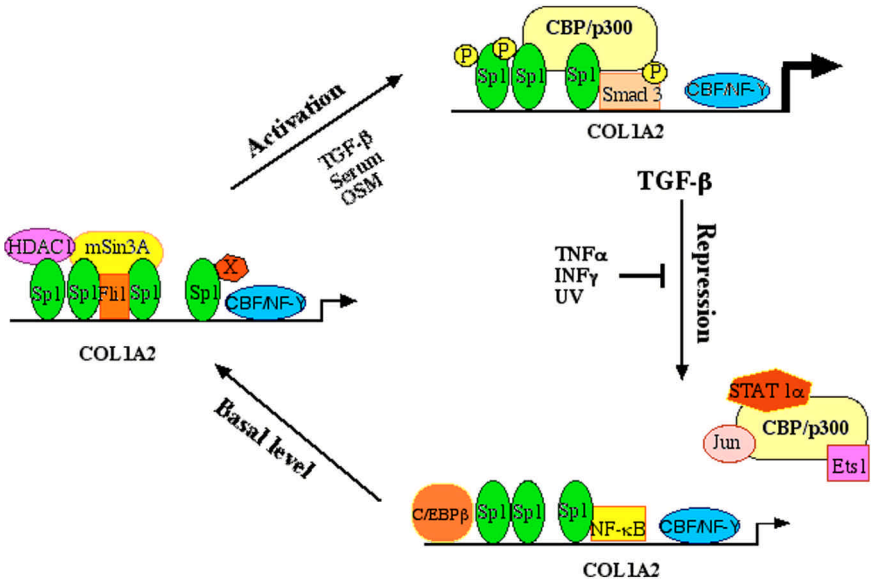

Figure 1. Current model for COL1A2 gene activation and repression. Basal COL1A2 transcription is regulated by the combinatorial action of the protein complexes containing strong activators such as Sp1 and CBF/NF-Y, as well as co-repressors. Fli-1 dependent repression may be mediated via association with co-repressor mSin3A/HDAC (histone deacetylase) complex (96). Another, presently uncharacterized, repressor complex (X) requires Sp1 binding to the pyrimidine-rich motif located at -160 bp within the COL1A2 promoter (73). As discussed in the text, TGF-beta stimulation of the COL1A2 gene involves interaction between Smad3/CBF complex and Sp1. Antagonistic effects of TNF-alpha include both rapid (via activation of NF-kappaB) and sustained responses via *de novo* synthesis of C/EBP-beta, c-Jun/JunB, and Ets1 (97). Ets1, c-Jun/JunB, as well as IFN-gamma-activated STAT-1-alpha may compete with Smad3 for the limiting amounts of CPB/p300.

levels or modification patterns between scleroderma and control fibroblasts (unpublished observations).

5.4. Other transcription factors altered in scleroderma

The heterotrimeric CCAAT binding factor CBF/NF-Y is a ubiquitous transcription factor involved in regulation of many genes (85). Its role in activation of the murine collagen type I gene is well established. CBF/NF-Y is also a strong contributor to the basal promoter activities of human COL1A1 and COL1A2 genes (73,86). Significantly, increased binding of CBF/NF-Y to the proximal CCAAT box in the COL1A1 promoter was observed in nuclear extracts obtained from scleroderma fibroblasts (86).

Another factor, potentially involved in upregulation of the collagen type I gene by scleroderma fibroblasts is c-myb (87,88). C-myb expression is induced by TGF-beta in dermal fibroblasts, and an elevated expression of c-myb has been demonstrated in unstimulated scleroderma fibroblasts. The specific role of c-myb in collagen gene regulation is presently not well defined.

5.5. The role of CBP/p300 in collagen gene regulation

CREB binding protein (CBP) and p300 function as transcriptional coactivators, which are critical to expression of many genes (89). The precise role of these factors in transcriptional process is still not fully understood. One of their proposed functions is to bridge promoter-specific transcription factors to components of the basal transcription machinery. Furthermore, CBP/p300 posses intrinsic histone acetyltransferase (HAT) activity and also associate with other HATs such as PCAF, thus playing a direct role in histone acetylation. Moreover, CBP/p300 may also acetylate transcription factors. Similar to phosphorylation, acetylation of transcription factors has recently been recognized as an important mode of gene regulation.

Another aspect of the CBP/p300 regulatory function is that expression of these multifunctional proteins in limiting quantities leads to competition between different transcription factors involved in specific cellular programs. With regard to the COL1A2 promoter, it has been demonstrated that the antagonistic effects of Interferon-gamma (IFN-gamma) and TGF-beta involve competition between Stat-1-alpha and Smad3 for limiting amounts of CBP/p300 (90,91). The inhibitory effect of the TNF-alpha-induced c-Jun and JunB proteins may also be mediated via sequestration of CBP/p300 (92).

5.6. Current model for COL1A2 gene regulation

Our current knowledge of collagen gene regulation is primarily based on the analyses of the proximal promoter regions. With the recent characterization of the evolutionary conserved far-upstream enhancer of the human COL1A2 gene (93), the spectrum of transcription factors involved in regulation of collagen genes may soon increase. The data presently available indicate that Sp1 (Sp3) and CBF/NF-Y play a major role in activating the proximal COL1A2 promoter (Figure 1). Sp1 also appears to play a critical role in mediating the actions of other co-activators and co-repressors of this promoter. Among the co-activators, only the Smad3/CBP complex

has been relatively well characterized. There are also several known repressors of the COL1A2 gene. In addition to Stat-1-alpha and Jun proteins discussed in the previous section, C/EBP-beta and NF-kappaB have been characterized as mediators of the TNF-alpha repression of the collagen gene (81,94). Furthermore, NF-kappaB has also been shown to inhibit COL1A1 promoter in a Sp1-dependent manner (95). Both IFN-gamma and TNF-alpha are capable of antagonizing the stimulatory effects of TGF-beta and may play important roles in regulating the balance between ECM synthesis and degradation. Finally, Fli-1 appears to repress the basal collagen level (80).

6. CONCLUDING REMARKS AND PERSPECTIVES

It is well recognized that precisely controlled regulation of ECM turnover is critical for the normal function of all tissues. In scleroderma the balance between ECM deposition and degradation is tilted towards enhanced ECM deposition resulting in fibrosis of skin and other internal organs. The activated fibroblast (myofibroblast) is believed to play a critical role in this process. This review has focused on the aspects of ECM regulation which differ in scleroderma. Not discussed here are the influence of the ECM on fibroblast behavior and the contribution of cell-matrix interactions to the scleroderma phenotype. These aspects were recently reviewed by Eckes *et al* (98). The regulation of ECM synthesis and degradation occurs at many levels and involves integration of the signals from cytokines, various matrix molecules, and other cells. Delineation of these pathways should ultimately lead to novel antifibrotic drugs.

7. ACKNOWLEDGMENT

I would like to thank Dr. Tien Hsu and members of the laboratory, Dr. Joanna Czuwara-Ladykowska, Jodi Gore, and Dan Shegogue for critical reading of the manuscript and Dr. Czuwara-Ladykowska for figure preparation. The author's research is supported by National Institutes of Health grants AR42334, AR44883 and by the R. G. Kozmetsky and Scleroderma Foundations.

8. REFERENCES

1. Strehlow, D. & J.H. Korn: Biology of the scleroderma fibroblast. *Curr Opin Rheumatol* 10, 572-578 (1998)

2. Widom, R.L: Regulation of matrix biosynthesis and degradation in systemic sclerosis. *Curr Opin Rheumatol* 12, 534-539 (2000)

3. Jimenez, S.A. & B. Saitta: Alterations in the regulation of expression of the a1(I) collagen gene (COL1A1) in systemic sclerosis (scleroderma). *Springer Semin Immunopathol.* 21, 397-414 (2000)

4. Varga, J. Mori, Y. & S. Takagawa: Molecular and cellular basis of fibrosis in scleroderma. *Clin. Exp. Rheumatol* in press (2001)

5. Botstein, G.R. Sherer, G.K. & E.C. LeRoy: Fibroblast selection in scleroderma. An alternative model of fibrosis. *Arthritis Rheum* 25, 189-1995 (1982)

6. Jelaska, A. Arakawa, M. Broketa, G. & J.H. Korn: Heterogeneity of collagen synthesis in normal and systemic sclerosis skin fibroblasts. Increased proportion of high collagen-producing cells in systemic sclerosis fibroblasts. *Arthritis Rheum* 39, 1338-1346 (1996)

7. Ivarsson, M. Sundberg, C. Farrokhnia, N. Pertoft, H. Rubin, K. & B. Gerdin: Recruitment of type I collagen producing cells from the microvasculature in vitro. *Exp Cell Res* 229, 336-49 (1996)

8. Sundberg, C. Ivarsson, M. Gerdin, B. & K. Rubin: Pericytes as collagen-producing cells in excessive dermal scarring. *Lab Invest* 74, 452-466 (1996)

9. Labat, M.L. Bringuier, A.F. Arys-Philippart, C. Arys, A. & F. Wellens: Monocytic origin of fibrosis. In vitro transformation of HLA-DR monocytes into neo-fibroblasts: inhibitory effect of all-trans retinoic acid on this process. *Biomed Pharmacother* 48, 103-111 (1994)

10. Ng, Y.Y. Huang, T.P. Yang, W.C. Chen, Z.P. Yang, A.H. Mu, W. Nikolic-Paterson, D.J. Atkins, R.C. & H.Y. Lan: Tubular epithelial-myofibroblast transdifferentiation in progressive tubulointerstitial fibrosis in 5/6 nephrectomized rats. *Kidney Int* 54, 864-876 (1998)

11. Chesney, J. & R. Bucala: Peripheral blood fibrocytes: mesenchymal precursor cells and the pathogenesis of fibrosis. *Curr Rheumatol Rep* 2, 501-505 (2000)

12. Gabrielli, A. Di Loreto, C. Taborro, R. Candela, M. Sambo, P. Nitti, C. Danieli, M.G. DeLustro, F. Dasch, J.R. & G. Danieli: Immunohistochemical localization of intracellular and extracellular associated TGF beta in the skin of patients with systemic sclerosis (scleroderma) and primary Raynaud's phenomenon. *Clin Immunol Immunopathol* 68, 340-3499 (1993)

13. Gruschwitz, M. Muller, P.U. Sepp, N. Hofer, E. Fontana, A. & G. Wick: Transcription and expression of transforming growth factor type beta in the skin of progressive systemic sclerosis: a mediator of fibrosis? *J Invest Dermatol* 94, 197-203 (1990)

14. Kulozik, M. Hogg, A. Lankat-Buttgereit, B. & T. Krieg: Co-localization of transforming growth factor beta 2 with alpha 1(I) procollagen mRNA in tissue sections of patients with systemic sclerosis. *J Clin Invest* 86, 917-922 (1990)

15. Sfikakis, P.P. McCune, B.K. Tsokos, M. Aroni, K. Vayiopoulos, G. & G.C. Tsokos: Immunohistological demonstration of transforming growth factor-beta isoforms in the skin of patients with systemic sclerosis. *Clin Immunol Immunopathol* 69, 199-204 (1993)

16. Higley, H. Persichitte, K. Chu, S. Waegell, W. Vancheeswaran, R. & C. Black: Immunocytochemical localization and serologic detection of transforming growth factor beta 1. Association with type I procollagen and inflammatory cell markers in diffuse and limited systemic sclerosis, morphea, and Raynaud's phenomenon. *Arthritis Rheum* 37, 278-288 (1994)

17. Querfeld, C. Eckes, B. Huerkamp, C. Krieg, T. & S. Sollberg: Expression of TGF-beta 1, -beta 2 and -beta 3 in localized and systemic scleroderma. *J Dermatol Sci* 21, 13-22 (1999)

18. McCormick, L.L., Zhang, Y. Tootell, E. & A.C. Gilliam. 1999. Anti-TGF-beta treatment prevents skin and lung fibrosis in murine sclerodermatous graft-versus-host disease: a model for human scleroderma. *J Immunol*. 163:5693-9.

19. Qi, Z. Atsuchi, N. Ooshima, A. Takeshita, A. & H. Ueno: Blockade of type beta transforming growth factor signaling prevents liver fibrosis and dysfunction in the rat. *Proc Natl Acad Sci U S A* 96, 2345-2349 (1999)

20. Jelaska, A. & J.H. Korn: Role of apoptosis and transforming growth factor beta1 in fibroblast selection and activation in systemic sclerosis *Arthritis Rheum* 43, 2230-2239 (2000)

21. Santiago, B. Galindo, M. Rivero, M. & J.L. Pablos: Decreased susceptibility to Fas-induced apoptosis of systemic sclerosis dermal fibroblasts. *Arthritis Rheum* 44, 1667-1676 (2001)

22. Moussad, E.E. & D.R. Brigstock: Connective tissue growth factor: What's in a name? *Mol Genet Metab* 71, 276-292 (2000)

23. Brigstock D.R.: The connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family. *Endocr Rev* 20, 189-206 (1999)

24. Igarashi, A. Okochi, H. Bradham, D.M. & G.R. Grotendorst: Regulation of connective tissue growth factor gene expression in human skin fibroblasts and during wound repair. *Mol Biol Cell* 4, 637-645 (1993)

25. Igarashi, A. Nashiro, K. Kikuchi, K. Sato, S. Ihn, H. Grotendorst, G.R. & K. Takehara: Significant correlation between connective tissue growth factor gene expression and skin sclerosis in tissue sections from patients with systemic sclerosis. *J Invest Dermatol* 105, 280-284 (1995)

26. Murphy, M. Godson, C. Cannon, S. Kato, S. Mackenzie, H.S. Martin, F. & H.R. Brady: Suppression subtractive hybridization identifies high glucose levels as a stimulus for expression of connective tissue growth factor and other genes in human mesangial cells. *J Biol Chem* 274, 5830-5834 (1999)

27. Dammeier, J. Beer, H.D. Brauchle, M. & S. Werner: Dexamethasone is a novel potent inducer of connective tissue growth factor expression. Implications for glucocorticoid therapy. *J Biol Chem* 273, 18185-18190 (1998)

28. Chambers, R.C. Leoni, P. Blanc-Brude, O.P. Wembridge, D.E. & G.J. Laurent: Thrombin is a potent inducer of connective tissue growth factor production via proteolytic activation of protease-activated receptor-1 *J Biol Chem* 275, 35584-3591 (2000)

29. Pendurthi, U.R. Allen, K.E. Ezban, M. & L.V. Rao: Factor VIIa and thrombin induce the expression of Cyr61 and connective tissue growth factor, extracellular matrix signaling proteins that could act as possible downstream mediators in factor VIIa x tissue factor-induced signal transduction. *J Biol Chem* 275, 14632-14641 (2000)

30. Hahn, A. Heusinger-Ribeiro, J.Lanz, T. Zenkel, S. & M. Goppelt-Struebe: Induction of connective tissue growth factor by activation of heptahelical receptors. Modulation by rho proteins and the actin cytoskeleton *J Biol Chem* 275, 37429-37435 (2000)

31. Suzuma, K. Naruse, K.Suzuma, I. Takahara, N. Ueki, K. Aiello, L.P. & G.L. King: Vascular endothelial growth factor induces expression of connective tissue growth factor via KDR, flt1, and phosphatidylinositol 3-kinase-akt-dependent pathways in retinal vascular cells *J Biol Chem* 275, 40725-40731 (2000)

32. Markiewicz, M. Rubinchik, S. Dong, J-Y. Trojanowska, M. & E,C. LeRoy: Human cytomegalovirus immediate early protein 1 (IE1) is a potent activator of connective tissue growth factor in dermal fibroblasts. Abstract. 382 *Arthritis Rheum*. 44 (suppl) (2001)

33. Querfeld, C. Sollberg, S. Huerkamp, C. Eckes, B. & T. Krieg: Pseudoscleroderma associated with lung cancer: correlation of collagen type I and connective tissue growth factor gene expression. *Br J Dermatol* 142, 1228-1233 (2000)

34. Inagaki, Y., Truter, S. & F. Ramirez: Transforming growth factor-beta stimulates alpha 2(I) collagen gene expression through a cis-acting element that contains an Sp1-binding site. *J Biol Chem* 269, 14828-14834 (1994)

35. Raghow, R. Postlethwaite, A.E. Keski-Oja, J. Moses, H.L. & A.H. Kang: Transforming growth factor-beta increases steady state levels of type I procollagen and fibronectin messenger RNAs posttranscriptionally in cultured human dermal fibroblasts. *J Clin Invest* 79, 1285-1288 (1987)

36. Massague, J: The transforming growth factor-beta family *Annu Rev Cell Biol* 6, 597-641 (1990)

37. Chen, S.J. Yuan, W. Mori, Y. Levenson, A. Trojanowska, M. & J. Varga: Stimulation of type I collagen transcription in human skin fibroblasts by TGF-beta: involvement of Smad 3. *J Invest Dermatol* 112, 49-57 (1999)

38. Chen, S.J. Yuan, W. Lo, S. Trojanowska, M. & J. Varga: Interaction of Smad3 with a proximal Smad-binding element of the human alpha2(I) procollagen gene promoter required for transcriptional activation by TGF-beta. *J Cell Physiol* 183, 381-392 (2000)

39. Zhang, W. Ou, J. Inagaki, Y. Greenwel, P. & F. Ramirez: Synergistic cooperation between Sp1 and Smad3/Smad4 mediates transforming growth factor beta 1 stimulation of alpha 2(I)-collagen (COL1A2) transcription. *J Biol Chem* 275, 39237-39245 (2000)

40. Duncan, M.R. Frazier, K.S. Abramson, S. Williams, S. Klapper, H. Huang, X. & G.R. Grotendorst: Connective tissue growth factor mediates transforming growth factor beta-induced collagen synthesis: down-regulation by cAMP. *Faseb J* 13, 1774-1786 (1999)

41. Stratton, R. Shiwen, X. Martini, G. Holmes, A. Leask, A. Haberberger,. T. Martin, G.R. Black, C.M. & D. Abraham: Iloprost suppresses connective tissue growth factor production in fibroblasts and in the skin of scleroderma patients. *J Clin Invest* 108, 241-50 (2001)

42. Chen, C.C. Chen, N. & L.F. Lau. The angiogenic factors Cyr61 and CTGF induce adhesive signaling in primary human skin fibroblasts. *J Biol Chem* 276, 10443-10452 (2001)

43. Pannu, J. Gore, E. Grotendorst, G. & M. Trojanowska: Insulin cooperates with CTGF to stimulate collagen protein synthesis in scleroderma and normal fibroblasts. *Mol Cell Biol* 12 Suppl, Abstract 2537 (2001)

44. Shi-wen, X. Pennington, D. Holmes, A. Leask, A. Bradham, D. Beauchamp, J.R. Fonseca, C. du Bois, R.M. Martin, G.R. Black, C.M. & D.J. Abraham: Autocrine overexpression of CTGF maintains fibrosis: RDA analysis of fibrosis genes in systemic sclerosis. *Exp Cell Res* 259, 213-224 (2000)

45. Abraham, D.J. Shiwen, X. Black, C.M. Sa, S. Xu, Y. & A. Leask: Tumor necrosis factor alpha suppresses the induction of connective tissue growth factor by transforming growth factor-beta in normal and scleroderma fibroblasts. *J Biol Chem* 275, 15220-15225 (2000)

46. Kawakami, T. Ihn, H. Xu, W. Smith, LeRoy,E. C. & M. Trojanowska: Increased expression of TGF-beta receptors by scleroderma fibroblasts: evidence for contribution of autocrine TGF-beta signaling to scleroderma phenotype. *J Invest Dermatol* 110, 47-51 (1998)

47. Ihn, H. Yamane, K. Kubo, M. & K. Tamaki: Blockade of endogenous transforming growth factor beta signaling prevents up-regulated collagen synthesis in scleroderma fibroblasts: association with increased expression of transforming growth factor beta receptors. *Arthritis Rheum* 44, 474-480 (2001)

48. Kubo, M. Ihn, H. Yamane, K. & K. Tamaki: Up-regulated expression of transforming growth factor beta receptors in dermal fibroblasts in skin sections from patients with localized scleroderma. *Arthritis Rheum* 44, 731-734 (2001)

49. Gore, E. E. Smith, S. Rubinchik, J-Y. Dong, & M. Trojanowska: Differential regulation of ECM protein synthesis in scleroderma and normal fibroblasts by autocrine TGF-beta pathway. *Arthr Rheum* 43 (suppl.) Abstract 1151 (2000)

50. Chin, B.Y., A. Mohsenin, S.X. Li, A.M. Choi, & M.E. Choi: Stimulation of pro-alpha(1)(I) collagen by TGF-beta(1) in mesangial cells: role of the p38 MAPK pathway. *Am J Physiol Renal Physiol* 280, F495-F504 (2001)

51. McCaffrey, T.A.: TGF-betas and TGF-beta receptors in atherosclerosis. *Cytokine Growth Factor Rev* 11, 103-114 (2000)

52. Roulot, D., A.M. Sevcsik, T. Coste, A.D. Strosberg, & S. Marullo: Role of transforming growth factor beta type II receptor in hepatic fibrosis: studies of human chronic hepatitis C and experimental fibrosis in rats [see comments]. *Hepatology* 29, 1730-1738 (1999)

53. Massague, J., & Y.G. Chen: Controlling TGF-beta signaling. *Genes Dev* 14, 627-644 (2000)

54. Holmes, A., S. Sa, X. Shiwen, C.M. Black, D.J. Abraham, & A. Leask: CTGF and SMADs: maintenance of scleroderma phenotype is independent of SMAD signalling. *J Biol Chem* 276, 10594-10601 (2001)

55. Tourkina, E., S. Hoffman, J.W. Fenton, 2nd, S. Lipsitz, R.M. Silver, & A. Ludwicka-Bradley: Depletion of protein kinase Cepsilon in normal and scleroderma lung fibroblasts has opposite effects on tenascin expression. *Arthritis Rheum.* 44, 1370-1381 (2001)

56. Bogatkevich, G.S., E. Tourkina, R.M. Silver, & A. Ludwicka-Bradley: Thrombin differentiates normal lung fibroblasts to a myofibroblast phenotype via the proteolytically activated receptor-1 and a protein kinase C-dependent pathway *J Biol Chem*., in press. (2001)

57. Firestein, G.S., & A.M. Manning: Signal transduction and transcription factors in rheumatic disease. *Arthritis Rheum.* 42, 609-621 (1999)

58. Hayashida, T. A.C. Poncelet, S.C. Hubchak, & H.W. Schnaper: TGF-beta1 activates MAP kinase in human mesangial cells: a possible role in collagen expression. *Kidney Int* 56, 1710-1720 (1999)

59. Ivaska, J. H. Reunanen, J. Westermarck, L. Koivisto, V.M. Kahari, & J. Heino: Integrin alpha2beta1 mediates isoform-specific activation of p38 and upregulation of collagen gene transcription by a mechanism involving the alpha2 cytoplasmic tail. *J Cell Biol* 147, 401-416. (1999)

60. Reunanen, N. M. Foschi, J. Han, & V.M. Kahari: Activation of extracellular signal-regulated kinase 1/2 inhibits type I collagen expression by human skin fibroblasts. *J Biol Chem* 275, 34634-34639 (2000)

61. Fisher, G.J. S. Datta, Z. Wang, X.Y. Li, T. Quan, J.H. Chung, S. Kang, & J.J. Voorhees: c-Jun-dependent inhibition of cutaneous procollagen transcription following ultraviolet irradiation is reversed by all-trans retinoic acid. *J Clin Invest* 106, 663-670 (2000)

62.Sato, M. D. Shegogue, E.A. Gore, E.A. Smith, P. J. McDermott & M. Trojanowska: the role of p38MAPK in TGF-beta stimulation of collagen production by scleroderma and healthy skin fibroblasts. *J Invest Dermatol*, in press (2002)

63. Ishikawa, O. A. Yamakage, E.C. LeRoy, & M. Trojanowska: Persistent effect of TGF-beta 1 on extracellular matrix gene expression in human dermal fibroblasts. *Biochem Biophys Res Commun* 169, 232-238 (1990)

64. Jimenez, S.A. Gaidarova, S. Saitta, B. Sandorfi, N. Herrich, D.J. Rosenbloom, J. C. Kucich, U. Abrams, R & J. Rosenbloom: Role of protein kinase C-delta in the regulation of collagen gene expression in scleroderma fibroblasts. *J Clin Invest* 108, 1395-1403 (2001)

65. Liu, G. L. Kleine, & R.L. Hebert: Advances in the signal transduction of ceramide and related sphingolipids. *Crit Rev Clin Lab Sci* 36, 511-573 (1999)

66. Hannun, Y.A. & C. Luberto: Ceramide in the eukaryotic stress response. *Trends Cell Biol* 10, 73-80 (2000)

67. Hernandez-Munoz, I. P. de la Torre, J.A. Sanchez-Alcazar, I. Garcia, E. Santiago, M.T. Munoz-Yague, & J.A. Solis-Herruzo: Tumor necrosis factor alpha inhibits collagen alpha 1(I) gene expression in rat hepatic stellate cells through a G protein. *Gastroenterology* 113, 625-640 (1997)

68. Suske, G.: The Sp-family of transcription factors. *Gene* 238, 291-300 (1999)

69. Ihn, H. & K. Tamaki: Increased phosphorylation of transcription factor Sp1 in scleroderma fibroblasts: association with increased expression of the type I collagen gene. *Arthritis Rheum* 43, 2240-2247 (2000)

70. Verrecchia, F. J. Rossert, & A. Mauviel: Blocking Sp1 transcription factor broadly inhibits extracellular matrix gene expression in vitro and in vivo: implications for the treatment of tissue fibrosis. *J Invest Dermatol* 116, 755-763 (2001)

71. Hitraya, E.G. J. Varga, C.M. Artlett, & S.A. Jimenez: Identification of elements in the promoter region of the alpha1(I) procollagen gene involved in its up-regulated expression in systemic sclerosis. *Arthritis Rheum* 41, 2048-2058 (1998)

72. Tamaki, T. K. Ohnishi, C. Hartl, E.C. LeRoy, & M. Trojanowska: Characterization of a GC-rich region containing Sp1 binding site(s) as a constitutive responsive element of the alpha 2(I) collagen gene in human fibroblasts. *J Biol Chem* 270, 4299-4304 (1995)

73. Ihn, H. K. Ohnishi, T. Tamaki, E.C. LeRoy, & M. Trojanowska: Transcriptional regulation of the human alpha2(I) collagen gene. Combined action of upstream stimulatory and inhibitory cis-acting elements. *J Biol Chem* 271, 26717-2623 (1996)

74. Ihn, H. & M. Trojanowska: Sp3 is a transcriptional activator of the human alpha2(I) collagen gene. *Nucleic Acids Res* 25, 3712-3717 (1997)

75. Li, L. C.M. Artlett, S.A. Jimenez, D.J. Hall, & J. Varga: Positive regulation of human alpha 1 (I) collagen promoter activity by transcription factor Sp1. *Gene* 164, 229-234 (1995)

76. Ihn, H. Y. Ihn, & M. Trojanowska: Spl Phosphorylation Induced by Serum Stimulates the Human alpha2(I) Collagen Gene Expression. *J Invest Dermatol* 117, 301-308 (2001)

77. Black, A.R. D. Jensen, S.Y. Lin, & J.C. Azizkhan: Growth/cell cycle regulation of Sp1 phosphorylation. *J Biol Chem* 274, 1207-1215 (1999)

78. Black, A.R. J.D. Black, & J. Azizkhan-Clifford: Sp1 and kruppel-like factor family of transcription factors in cell growth regulation and cancer. *J Cell Physiol* 188, 143-160 (2001)

79. Ihn, H. E.C. LeRoy, & M. Trojanowska: Oncostatin M stimulates transcription of the human alpha2(I) collagen gene via the Sp1/Sp3-binding site. *J Biol Chem* 272, 24666-24672 (1997)

80. Czuwara-Ladykowska, J. F. Shirasaki, P. Jackers, D.K. Watson, & M. Trojanowska: Fli-1 inhibits collagen type I production in dermal fibroblasts via an Sp1-dependent pathway. *J Biol Chem* 276, 20839-20848 (2001)

81. Greenwel, P. S. Tanaka, D. Penkov, W. Zhang, M. Olive, J. Moll, C. Vinson, M. Di Liberto, & F. Ramirez: Tumor necrosis factor alpha inhibits type I collagen synthesis through repressive CCAAT/enhancer-binding proteins. *Mol Cell Biol* 20, 912-918. (2000)

82. Trojanowska, M: Ets factors and regulation of the extracellular matrix. *Oncogene* 19, 6464-6471 (2000)

83. Czuwara-Ladykowska J, V. Sementchenko, D. Watson & M. Trojanowska. Ets1 is an effector of TGF-beta pathway and an antagonist of profibrotic effects of TGF-beta. *Mol Biol Cell* 12, Abstract 1078 (2001)

84. Redlich, K. H.P. Kiener, G. Schett, M. Tohidast-Akrad, E. Selzer, I. Radda, G.H. Stummvoll, C.W. Steiner, M. Groger, P. Bitzan, P. Zenz, J.S. Smolen, & G. Steiner: Overexpression of transcription factor Ets-1 in rheumatoid

arthritis synovial membrane: regulation of expression and activation by interleukin-1 and tumor necrosis factor alpha. *Arthritis Rheum* 44, 266-274 (2001)

85. Maity, S.N. & B. de Crombrugghe: Role of the CCAAT-binding protein CBF/NF-Y in transcription. *Trends Biochem Sci* 23, 174-178 (1998)

86. Saitta, B. S. Gaidarova, L. Cicchillitti, & S.A. Jimenez: CCAAT binding transcription factor binds and regulates human COL1A1 promoter activity in human dermal fibroblasts: demonstration of increased binding in systemic sclerosis fibroblasts. *Arthritis Rheum* 43, 2219-2229. (2000)

87. Piccinini, G. J. Golay, A. Flora, S. Songia, M. Luchetti, A. Gabrielli, & M. Introna: C-myb, but not B-myb, upregulates type I collagen gene expression in human fibroblasts. *J Invest Dermatol* 112:, 91-96 (1999)

88. Piccinini, G. M.M. Luchetti, M.L. Caniglia, A.M. Carossino, M. Montroni, M. Introna, & A. Gabrielli: c-myb proto-oncogene is expressed by quiescent scleroderma fibroblasts and, unlike B-myb gene, does not correlate with proliferation. *J Invest Dermatol* 106, 1281-1286. (1996)

89. Goodman, R.H. & S. Smolik: CBP/p300 in cell growth, transformation, and development. *Genes Dev* 14, 1553-1577 (2000)

90. Ghosh, A.K. W. Yuan, Y. Mori, S. Chen, & J. Varga: Antagonistic regulation of type I collagen gene expression by interferon-gamma and transforming growth factor-beta. Integration at the level of p300/CBP transcriptional coactivators. *J Biol Chem* 276, 11041-11048 (2001)

91. Ghosh, A.K. W. Yuan, Y. Mori, & J. Varga: Smad-dependent stimulation of type I collagen gene expression in human skin fibroblasts by TGF-beta involves functional cooperation with p300/CBP transcriptional coactivators. *Oncogene* 19, 3546-3555 (2000)

92. Verrecchia, F. M. Pessah, A. Atfi, & A. Mauviel: Tumor necrosis factor-alpha inhibits transforming growth factor-beta /Smad signaling in human dermal fibroblasts via AP-1 activation. *J Biol Chem* 275, 30226-30231 (2000)

93. Antoniv, T.T. S. De Val, D. Wells, C.P. Denton, C. Rabe, B. de Crombrugghe, F. Ramirez, & G. Bou-Gharios: Characterization of an evolutionarily conserved far-upstream enhancer in the human alpha 2(I) collagen (COL1A2) gene. *J Biol Chem* 276, 21754-21764 (2001)

94. Kouba, D.J. K.Y. Chung, T. Nishiyama, L. Vindevoghel, A. Kon, J.F. Klement, J. Uitto, & A. Mauviel: Nuclear factor-kappa B mediates TNF-alpha inhibitory effect on alpha 2(I) collagen (COL1A2) gene transcription in human dermal fibroblasts. *J Immunol* 162, 4226-4234 (1999)

95. Rippe, R.A. L.W. Schrum, B. Stefanovic, J.A. Solis-Herruzo & D.A. Brenner: NF-kappaB inhibits expression of the alpha1(I) collagen gene. *DNA Cell Biol* 18, 751-761. (1999)

96. Deramaudt, T.B. P. Remy & P. Stiegler: Identification of interaction partners for two closely-related members of the ETS protein family, FLI and ERG. *Gene* 274, 169-177 (2001)

97. Gilles, F. M.B. Raes, D. Stehelin, B. Vandenbunder & V. Fafeur: The c-ets-1 proto-oncogene is a new early-response gene differentially regulated by cytokines and growth factors in human fibroblasts. *Exp Cell Res* 222, 370-378 (1996)

98. Eckes, B. P. Zigrino, D. Kessler, O. Holtkotter, P. Shephard, C. Mauch & T. Krieg: Fibroblast-matrix interactions in wound healing and fibrosis. *Matrix Biol* 19, 325-332 (2000)

Key words. Scleroderma, TGF-beta, CTGF, collagen, fibrosis, Review

Send correspondence to: Maria Trojanowska, Division of Rheumatology and Immunology, Medical University of South Carolina, 96 Jonathan Lucas St, Suite 912, Charleston SC 29425, Tel: 843-792-7921; Fax: 843-792-7121, E-mail trojanme@musc.edu