Differential regulation of transcription and transcript stability of pro-α1(I) collagen and fibronectin in activated fibroblasts derived from patients with systemic scleroderma

Beate ECKES*, Cornelia MAUCH, Gabriele HÜPPE and Thomas KRIEG

Department of Dermatology, University of Cologne, Joseph Stelzmann Strasse 9, D-50924 Cologne, Germany

Activated fibroblasts were derived from the skin of patients with systemic scleroderma (SSc), used as a model for fibrosis. Such cells are characterized by increased production of collagens and other matrix constituents. Increased collagen and fibronectin production has been correlated with similarly elevated mRNA steady-state levels. In the present study we analysed the contribution of transcriptional activity and post-transcriptional transcript stability to the increases in pro-α1(I) collagen and fibronectin mRNA steady-state levels in activated (scleroderma) fibroblasts. Fibroblasts, when cultured in close contact with a three-dimensional collagenous matrix, down-regulate collagen synthesis. Culture of skin fibroblasts from two patients with SSc in three-dimensional collagen lattices, however, showed 4-fold elevated pro-α1(I) collagen mRNA levels over fibroblasts from healthy donors. Transcription of the *COL1A1* gene in SSc fibroblasts was induced 2–3-fold over that in controls in both monolayer and lattice cultures, accounting in part for the elevated steady-state level. A 50 % decrease in transcription rate in lattice compared with monolayer culture occurred, as in control cells. In contrast, whereas control cells in lattices responded with decreased (50 %) pro-α1(I) collagen mRNA stability, in SSc cells these transcripts were found to be more stable (half-life of 5 h compared with 2 h in control cells). Fibronectin steady-state mRNA levels, in contrast, were not significantly regulated by the three-dimensional environment. In SSc fibroblasts, fibronectin mRNA levels were induced 1.5–4.9-fold over controls. In part, this increase appears to be due to elevated transcription, and an increase in fibronectin transcript stability was also detected. We therefore conclude that activated fibroblasts such as those derived from scleroderma patients utilize transcriptional and post-transcriptional mechanisms to maintain increased collagen and fibronectin production, which contribute to the pathogenesis of the disease.

INTRODUCTION

Systemic scleroderma (SSc) is a generalized disease which affects connective tissue in the skin and internal organs, mainly the lungs, kidneys, oesophagus and myocardium. Although the initial pathogenesis is still under debate, excessive production as well as reorganization of connective tissue constituents determines the development of the disease [1]. Increased levels of procollagen peptides have been found in the serum of patients with the active disease [2,3], and in the skin increased mRNA steady-state levels as well as increased activity of prolyl and lysyl hydroxylases have been reported [4,5]. Fibroblasts derived from affected skin areas of patients are considered to be in an activated state, synthesizing increased amounts of connective tissue components, mainly collagens I, III [6–8] and VI [9], fibronectin [3,10,11] and proteoglycans [12]. This activation in the initial stages of the disease has been shown to be related to changes in cytokine production and responsiveness which involve in particular the transforming growth factor-β (TGF-β) family [13,14]. Other parameters that are altered in SSc fibroblasts include the platelet-derived growth factor receptor [15,16] and matrix environment-recognizing integrin receptors [17,18]. Cytokine- and cell surface receptor-mediated signalling is thought to lead to nuclear events which result in increased production of transcripts for collagens, fibronectin and other matrix constituents found in excess amounts in the affected tissues. Using monolayer culture systems, previous reports indicated that increased collagen synthesis is due to enhanced mRNA steady-state levels [19,20]. In addition, transcriptional activity of the α2(I) collagen gene was shown to be elevated in fibroblasts from a patient with localized scleroderma [21].

We have shown that fibroblasts embedded into a three-dimensional collagen network reflect the *in vivo* situation more closely than those in monolayer culture, and that collagen synthesis is regulated profoundly by this surrounding extracellular matrix (ECM) [22]. We therefore used a three-dimensional culture system to study altered collagen metabolism in activated fibroblasts. Under these conditions SSc fibroblasts, which we use as a model for activated cells found in fibrotic processes, were found not to respond to the ECM by down-regulating collagen mRNA steady-state levels to the extent observed in control fibroblasts [23]. We had previously reported that down-regulation of pro-α1(I) collagen mRNA steady-state levels in control fibroblasts involves transcriptional as well as post-transcriptional mechanisms [24]. In order to determine at which level this regulation might be impaired in SSc fibroblasts, in the present study we used run-on transcription assays and pulse-chase labelling to analyse the transcription rates of the *COL1A1* and fibronectin genes and the stability of the corresponding transcripts.

MATERIALS AND METHODS

Cell culture

Primary cultures of human dermal fibroblasts were established by outgrowth from skin biopsies in Dulbecco's modified Eagle's

Abbreviations used: DMEM, Dulbecco's modified Eagle's medium; SSc, systemic scleroderma; TGF-β, transforming growth factor-β; UTR, untranslated region.

* To whom correspondence should be addressed.

medium (DMEM) containing 10% (v/v) fetal calf serum, 50 μg/ml sodium ascorbate, 300 μg/ml glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin in 5% CO_2/air at 37 °C [24]. SSc strain S1 was derived from a clinically involved site on the forearm of a 49-year-old female patient with puffy hands, initial lung involvement and antibodies directed against topoisomerase I. SSc strain S2 was obtained from the forearm of a 53-year-old female patient with acrosclerosis (limited disease; anticentromere antibodies positive). Both patients were in an active state of the disease and had not been treated. Control fibroblasts (C1–C3) were obtained from identical locations of healthy, age-matched female volunteers. Cells were used while in a subconfluent stage, as the transcription rate of the pro-α1(I) collagen gene is severely affected by cell density [25]. Preparation of collagen lattice cultures was as described earlier [24]. No significant differences in the contractile capacity of collagen lattices between control and SSc fibroblasts were observed.

Pro-α1(I) collagen and fibronectin mRNA steady-state levels

Total RNA isolated from monolayer and lattice cultures [24] was subjected to denaturing agarose gel electrophoresis [26], transferred to nylon membranes (Du Pont), UV cross-linked and hybridized to ^{32}P-labelled [27] cDNA sequences specific for pro-α1(I) collagen (Hf677) [28] and fibronectin (FN771) [29] as described in [24]. The final washing stringency was $0.1 \times$ SSC/0.1% SDS (where $1 \times$ SSC = 0.15 M NaCl and 0.015 M sodium citrate) at 65 °C (collagen) or 55 °C (fibronectin). Following autoradiography, signal intensity was determined densitometrically using ImageQuant software (Molecular Dynamics).

In order to confirm equal RNA loading per lane, filters were stained following UV cross-linking for 5 min in 0.04% Methylene Blue in 0.5 M sodium acetate (pH 5.2). Excess stain was removed in 25% ethanol.

Nuclear run-on transcription

Preparation of nuclei, run-on transcription and hybridization were performed as described in [24]. Briefly, fibroblast nuclei were prepared according to the method of Marzluff and Huang [30] by ultracentrifugation through 2.4 M sucrose. To be certain that comparable amounts of nuclei were present in each aliquot, the DNA content in lysed aliquots was determined. A sample of 40 μl of nuclei per reaction was used in a total volume of 100 μl. Transcripts were labelled with 1.85 MBq of [α-^{32}P]UTP (Amersham; 3000 Ci/mmol) per reaction.

Samples containing 1×10^7 c.p.m. of reactions that had yielded comparable total counts were hybridized to slot-blotted cDNA (1 μg/slot of Hf677 and FN771) for 36 h in $5 \times$ SSC, 1% sodium laurylsarcosine at 65 °C and washed as described [24]. Signal intensities were determined densitometrically and values were normalized to tubulin signals.

Determination of transcript stability by pulse–chase labelling

The procedure used was modified according to Hämäläinen et al. [31] and is described in detail in [24]. Collagen lattices were prepared by seeding 10^7 cells in a total volume of 30 ml per gel in 150 mm-diam. bacteriological dishes and allowed to contract for 7 h at 37 °C. In parallel, 3×10^6 cells/100 mm culture dish were incubated for 7 h. For pulse-labelling, contracting lattices were transferred to plastic tubes, and monolayers and lattices were incubated for 1 h at 37 °C in 5 ml of serum-free DMEM containing 15.4 MBq of [5,6-^{3}H]uridine (Du Pont). The chase medium contained DMEM, 10% (v/v) fetal calf serum, and uridine and cytidine at 10 mM each. Parallel monolayer and lattice cultures were lysed at 0, 2, 4, 6, 8 and 12 h and processed for RNA isolation and hybridization to dot-blotted cDNA (0.2 μg/dot of Hf677 and FN771) precisely as described in [24]. Decay curves were drawn. The decay constant, which is inversely proportional to the half-life of an mRNA, was calculated from the slopes of these straight lines. From these, mRNA half-lives were deduced.

RESULTS

Differential regulation of steady-state levels of pro-α1(I) collagen and fibronectin mRNAs by a three-dimensional matrix environment

Activated fibroblasts, obtained from involved skin specimens from SSc patients, were analysed for their capacity to down-regulate pro-α1(I) collagen steady-state mRNA levels in response to culture within a three-dimensional collagen lattice. We had previously reported [23] that this environment leads to the down-regulation of pro-α1(I) collagen steady-state mRNA levels. However, in contrast to control fibroblasts in which levels are reduced to 10% or less within 2 days of culture, several SSc strains maintained elevated collagen mRNA levels which were paralleled by correspondingly increased collagen protein production. In the present study, two out of eight strains were indistinguishable from controls after 2 days of gel culture, whereas six showed heterogeneously increased pro-α1(I) collagen mRNA levels (results not shown). Of these, two (S1 and S2) were further investigated as a model to study how activated fibroblasts regulate collagen synthesis and adapt to the surrounding ECM: S1 as a distinctive overproducer of ECM, and S2 as a moderate overproducer (Figures 1 and 2). After 2 days, pro-α1(I) collagen mRNA levels were increased 3.7-fold (S1) and 1.8-fold (S2) over controls; after 4 days the increase over the control was > 4-fold (Figure 1). As already shown for healthy fibroblasts [22], matrix-induced down-regulation differentially affected the two transcript species of pro-α1(I) collagen in SSc fibroblasts. Both transcripts

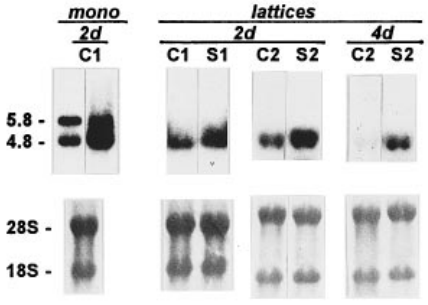

Figure 1 Pro-α1(I) collagen mRNA steady-state levels

Human fibroblasts from healthy volunteers (C1, C2) and SSc patients (S1, S2) were cultured as monolayers (mono) or within three-dimensional collagen gels (lattices). RNA was extracted after 2 or 4 days of culture and hybridized to cDNA sequences specific for pro-α1(I) collagen. The upper panel shows the autoradiographic signals of the two transcripts, 5.8 and 4.8 kb in length. For monolayer cultures, two exposures are shown: the left one is a short exposure, shown to clearly identify the two collagen-specific transcripts, and the right one has been exposed for the same time as the 'lattices' lanes, thus allowing direct comparison of signals. The lower panel depicts Methylene Blue-stained filters showing 28 S and 18 S rRNA.

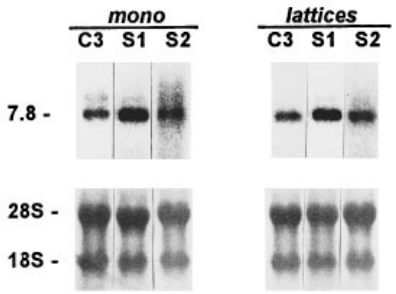

Figure 2 Fibronectin mRNA steady-state levels

Human fibroblasts from a healthy volunteer (C3) and from SSc patients (S1, S2) were cultured as monolayers (mono) or within three-dimensional collagen gels (lattices) for 2 days. RNA was extracted and hybridized to fibronectin cDNA sequences. The upper panel shows the autoradiographic signals of the 7.8 kb transcript, and the lower panel represents the Methylene Blue-stained membranes showing 28 S and 18 S rRNA.

were present at equal intensity in monolayer culture; however, in lattices the shorter (4.8 kb) transcript was readily detected, whereas the longer one (5.8 kb) was decreased more severely, being almost undetectable after 48 h of lattice culture (Figure 1).

In addition, after 2 days of culture both S1 and S2 displayed elevated fibronectin mRNA steady-state levels as compared with control fibroblasts (Figure 2). In contrast to the regulation of collagen mRNA levels, fibronectin mRNA was equally increased in monolayer and lattice cultures, suggesting that negligible regulatory effects are exerted by the ECM. Strain S2 revealed a moderate increase of 1.5-fold over controls, whereas the up-regulation in S1 was exceptionally high: 4.9-fold in monolayer and 4.0-fold in gel culture.

Increased *de novo* transcription of pro-α1(I) collagen and fibronectin mRNAs in SSc fibroblasts

In order to determine the contribution of transcriptional activity to the elevation in collagen and fibronectin steady-state mRNA levels, nuclear run-on assays were performed using control and SSc fibroblast (S1) nuclei isolated from monolayer and lattice cultures. In comparison with monolayer cultures, control cells grown within collagen lattices showed a 50 % decrease in *de novo* transcription of the *COL1A1* gene, but no significant alteration in fibronectin gene transcription (Figure 3). In SSc fibroblasts, transcription of *COL1A1* was elevated 2.6-fold in monolayers compared with controls. In lattice cultures, transcription was decreased to 50 % of that in monolayers (Figure 3), which is the same extent of decrease found with control fibroblasts.

In comparison, fibronectin gene transcription was only moderately affected by culture of both control and SSc fibroblasts within a three-dimensional collagen lattice. Transcription rates of fibronectin in SSc cells were elevated (Figure 2), although the high steady-state levels shown in Figure 2 were not reflected at the transcriptional level.

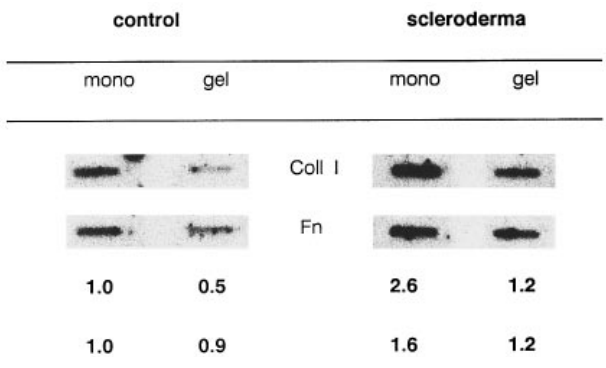

Figure 3 *De novo* transcription of the *COL1A1* and fibronectin genes

cDNAs specific for pro-α1(I) collagen (Coll I) and fibronectin (Fn) were fixed to a nylon membrane and hybridized to 1×10^7 c.p.m. of ^{32}P-labelled run-on transcripts of control and scleroderma (patient S1) fibroblast nuclei from monolayer (mono) and collagen gel (gel) cultures. Signals were quantified densitometrically and normalized to β-tubulin signals (not shown). The value obtained for control cells in monolayer culture was set as 1.0.

Alterations of pro-α1(I) collagen and fibronectin mRNA stability in SSc fibroblasts

Initially, fibroblasts were cultured as monolayers as well as within three-dimensional collagen matrices, and incubated for up to 12 h in the presence of 5, 7.5 or 10 μg/ml actinomycin D. However, under these conditions stabilization of the collagen I mRNA occurred which lasted for at least 6 h. In lattices this effect persisted even longer. Similar data have been published previously [32,33]. We therefore used pulse-labelling of RNA for the following experiments.

Pulse-labelling and chase over 12 h revealed that pro-α1(I) collagen transcripts in SSc fibroblasts (S1 and S2) decayed at the same rate (half-life of 4.1–4.6 h) (Figure 4, Table 1) as in control fibroblasts when the cells were grown as monolayers [24]. In lattice culture, where the half-life in control cells is reduced to 2.2 h [24], pro-α1(I) collagen transcripts in SSc fibroblasts were not destabilized but displayed even longer half-lives of 5.0–5.4 h (Figure 4, Table 1), indicating a further control mechanism acting post-transcriptionally which contributes to elevated collagen steady-state mRNA levels. Interestingly, stabilization of pro-α1(I) collagen transcripts was observed in both strains S1 and S2.

Fibronectin transcript stability was also increased in SSc fibroblasts. In the 'high-producing' strain S1 the fibronectin mRNA decayed with a half-life of 5.5 h in both monolayer and lattice culture (Figure 4, Table 1). In strain S2, which displayed moderate overexpression of matrix proteins, the fibronectin mRNA half-life was 3.6 h in monolayers and 3.2 h in lattice culture (Table 1). In agreement with the comparable steady-state mRNA levels and transcription rates for fibronectin in monolayer and lattice cultures, no differential regulation of mRNA half-life was exerted by the contact of cells with the collagenous matrix (Table 1).

DISCUSSION

In agreement with previous reports [34–36], the initial characterization of fibroblasts derived from patients with SSc revealed considerable heterogeneity. Six out of eight strains studied were found to be collagen overproducers to various extents; of these, two were further investigated: S1 as the strain displaying highest production, and S2 as a moderate intermediate. In the present study we show that elevated transcript levels for pro-α1(I) collagen and fibronectin in these two activated fibroblast strains from patients with SSc, used as a model to study fibrotic processes, are caused by increased *de novo* transcription of the genes and, in addition, by stabilization of the transcripts. This was investigated in three-dimensional lattice cultures, in which the activated state of scleroderma fibroblasts is more obvious

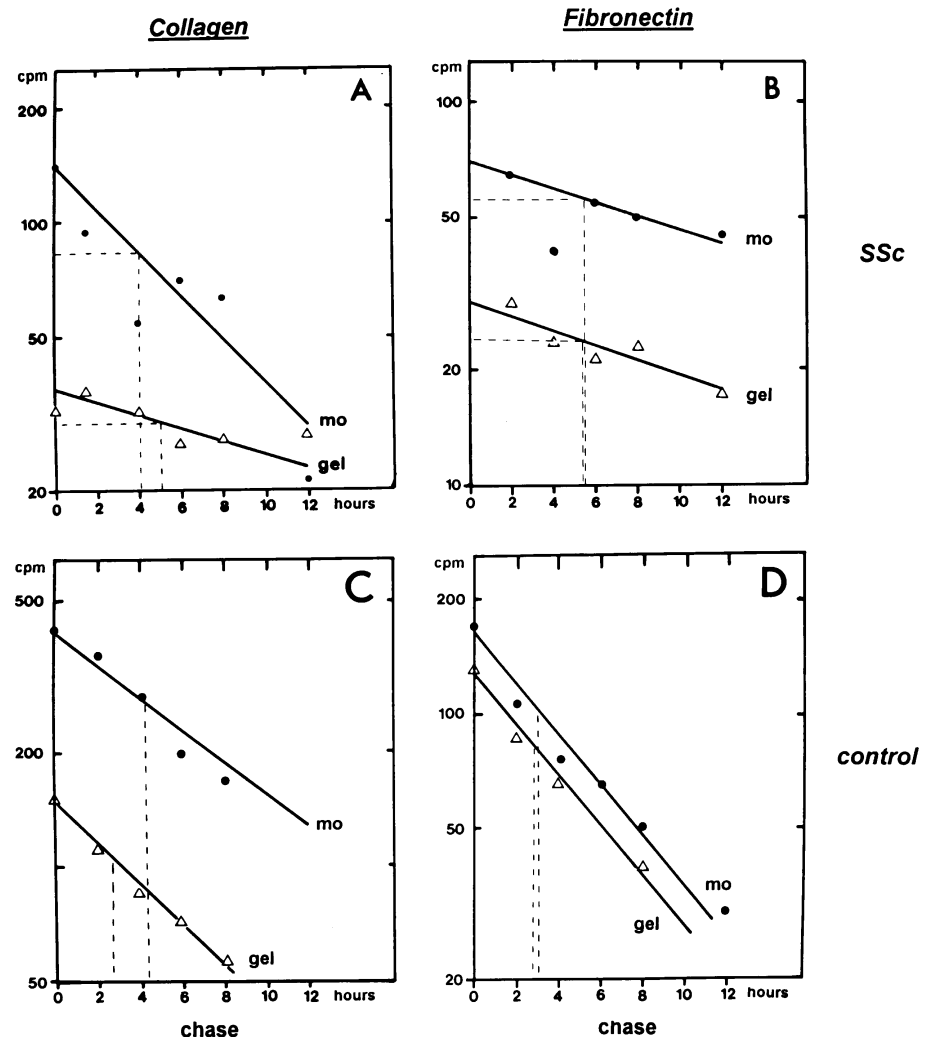

Figure 4 Stability of pro-α1(I) collagen and fibronectin transcripts in control and SSc fibroblasts

Fibroblasts obtained from SSc patients (**A** and **B**) and from healthy donors (**C** and **D**) were cultured as monolayers (mo) or in collagen lattices (gel), and pulse-labelled and chased as described in the Materials and methods section. At each time point, 20 μg of total [^{3}H]RNA was hybridized to dot-blotted cDNA sequences specific for pro-α1(I) collagen (**A** and **C**) and fibronectin (**B** and **D**), and processed for liquid scintillation counting. Results are expressed as radioactivity (c.p.m.).

Table 1 Pro-α1(I) collagen and fibronectin mRNA transcript stability in control and SSc fibroblasts

Half-lives of pro-α1(I) collagen and fibronectin transcripts in control and SSc fibroblasts, cultured as monolayers or within three-dimensional collagen matrices (gel), were derived from decay curves such as those shown in Figure 4. Values are means $\pm$ S.D. of three measurements for control and SSc fibroblast strains S1 and S2.

	Half-life (h)			
	Pro-α1(I) collagen		Fibronectin	
Fibroblasts	Monolayer	Gel	Monolayer	Gel
Control	4.4 $\pm$ 0.4	2.2 $\pm$ 0.3	2.6 $\pm$ 0.8	2.4 $\pm$ 0.6
S1	4.6 $\pm$ 0.8	5.4 $\pm$ 0.6	5.5 $\pm$ 0.5	5.5 $\pm$ 0.4
S2	4.1 $\pm$ 0.5	5.0 $\pm$ 0.7	3.6 $\pm$ 0.4	3.2 $\pm$ 0.4

than in monolayer culture. Most noteworthy is the differential effect exerted by a three-dimensional matrix environment on collagen, which is distinctly regulated at the transcriptional, post-transcriptional and steady-state levels, in contrast to fibronectin where results in monolayer and lattice cultures do not differ significantly.

We report here that transcription of the *COL1A1* gene in fibroblasts from SSc patients in a three-dimensional collagen lattice is increased compared with that in control fibroblasts. In agreement with previous reports, we demonstrated that this increase is more marked in three-dimensional culture than in monolayer culture [23]. Kähäri et al. [21] demonstrated that transcription of the *COL1A2* gene was increased 2–4-fold in monolayer culture in cells from a patient with the localized form of scleroderma. Our data confirm, therefore, the concept of co-ordinated transcriptional regulation of the two genes that contribute to the formation of heterotrimeric type I collagen fibrils. Analysis of the *COL1A1* promoter and first intron in mouse and

human fibroblasts has identified several elements that include binding sites for regulatory proteins [37–39]. So far, no elements or protein factors have been demonstrated to specifically contribute to the regulation of α1(I) collagen transcript levels by a collagenous matrix or in scleroderma fibroblasts.

We showed previously that decreased pro-α1(I) collagen steady-state mRNA levels in control fibroblasts grown in lattice culture are achieved by decreases in the transcription rate (50 %) and the transcript half-life (50 %) [24]. In SSc fibroblasts which do display elevated transcription, in both monolayer and lattice culture, the extent of transcriptional down-regulation in response to the ECM is identical to that in controls (i.e. 50 %), indicating that this control level functions normally in SSc fibroblasts. These observations imply increased promoter activity in SSc fibroblasts but a normal response to regulatory signals transduced from the external environment. This is in contrast to the response controlling pro-α1(I) collagen mRNA stability, which is down-regulated in control cells but increased in SSc cells. Interestingly, S1 and S2 cells both display the increase only when cultured in contact with the three-dimensional matrix, suggesting that the cell–ECM interaction triggers a signal leading to post-transcriptional control which cannot be detected in monolayer culture. In addition, these findings might be an indication of different signals being transduced through a collagen integrin receptor (possibly VLA-1), with one pathway leading to transcriptional control in the nucleus, which functions normally in controls and SSc fibroblasts, and a second pathway ending in post-transcriptional control in the cytoplasm, which is altered in SSc cells.

This is in contrast with the findings with fibronectin, where the transcription rate and transcript stability did not differ significantly between monolayer and lattice culture.

The extent of the increase in the mRNA half-life appears to correlate with the steady-state mRNA level: high collagen and fibronectin mRNA steady-state levels were detected in strain S1, which displayed the most pronounced increase in the stability of the pro-α1(I) collagen and fibronectin transcripts, whereas intermediate (between control and S1) levels for steady-state mRNA levels and transcript half-life were observed in S2. Thus maintenance of elevated transcript stability may possibly represent a metabolic marker of scleroderma cells, representing an activated phenotype of fibroblasts.

It is not well understood at present which part of the transcripts is involved in regulating transcript stability. Work by Määttä and Penttinen [40,41] has focused on studying the 3′ untranslated region (3′ UTR) of the shorter (4.8 kb) pro-α1(I) collagen transcript. A cytoplasmic factor termed α1-RBF$_{67}$ is described which binds to a highly conserved region which, according to a computer-based model simulating the secondary structure, resides in a bulge structure, i.e. a prominent topological marker likely to represent a recognition target for protein–RNA interactions. The binding activity of this protein is presumably correlated with decreased pro-α1(I) mRNA stability in glucocorticoid-treated fibroblasts. The role of α1-RBF$_{67}$ in scleroderma fibroblasts has not yet been clarified. In addition, Määttä [42] suggests that the 3′ UTR of COL1A1 is important, but not sufficient, for regulating transcript stability, and they propose that parts of the coding region, in particular those corresponding to propeptides, might be critical.

Alterations in pro-α1(I) collagen transcript stability may provide a possible explanation for the more pronounced down-regulation seen with the longer (5.8 kb) of the two transcript species in lattice cultures. In contrast to fibronectin, these two mRNA species do not arise by alternative splicing [43,44], but by the utilization of two different polyadenylation sites which are separated by 1113 nucleotides [45], which explains the size difference of 4.8 and 5.8 kb. Therefore the 3′ UTR of the longer transcript may contain sequence elements downstream of its AAUAAA signal which are unique to this transcript and are not contained in the shorter one, and which might render this 5.8 kb transcript less stable in the ECM environment. Alternatively, the use of the two polyadenylation signals could differ in monolayer and lattice culture, with preferred use of the first AAUAAA signal in lattice culture.

Increased fibronectin levels have been detected in extracts of scleroderma skin [3] and most frequently in cultures of SSc fibroblasts [10], including three-dimensional lattice culture [46], in cultures of alveolar macrophages from SSc patients with pulmonary fibrosis [11], and in cells of patients with related diseases such as morphea [47] and eosinophilic fasciitis [48]. In addition, one group reported point mutations in the fibronectin gene in SSc patients [49] which were suggested as a possible marker of scleroderma [50].

In scleroderma there is no information available regarding the mechanisms leading to increased fibronectin mRNA and protein levels. However, there are some indications for the co-ordinate regulation of fibronectin and collagens, mainly collagen I. TGF-β, a potent fibrogenic cytokine, induces mRNA levels for both matrix proteins in SSc fibroblasts [51]. Dean et al. [52] reported that in HT1080 cells this increase is due to increased gene transcription. In contrast, using human foreskin fibroblasts, Raghow et al. [53] observed no effect of TGF-β on either α1(I) collagen or fibronectin transcription, but suggested that the increase in transcripts was due to their preferential stabilization. In human gingival fibroblasts, increases have been ascribed to both elevated transcription and increased stability for α1(I) collagen and fibronectin [54,55]. Co-ordinate transcriptional increases could be mediated by the NF-1 binding site, which is present in the fibronectin promoter [56] and which has been demonstrated to be involved in TGF-β-mediated transcriptional activation in the murine α2(I) collagen promoter [57]. Furthermore, stabilization of pro-α1(I) collagen mRNA by TGF-β has also been described by Penttinen et al. in 3T3 fibroblasts [58]. In addition to providing an explanation for the up-regulation of matrix proteins, transcript stability has also been implicated in down-regulation. For example, transformation of cells, which is often accompanied by severely decreased fibronectin levels, could be shown to be correlated with destabilization of fibronectin transcripts in N-*ras*-transformed HT1080 fibrosarcoma cells [59]. The same group later described altered processing of primary fibronectin transcripts in the nucleus in the absence of changes in mRNA stability for an Ha-*ras*-transfected osteosarcoma cell line [60]. Down-regulation of both fibronectin and collagens I and III by prostaglandin E_2 in a co-ordinated fashion has been ascribed to transcriptional control, since no changes at the level of transcript stability were observed [61].

These examples strongly suggest the presence of cis elements/trans-acting proteins in genes encoding matrix proteins, such as collagens I, III and fibronectin, which in many cases seem to be up- or down-regulated in a co-ordinated manner. The function of such elements/factors is easily conceivable at a transcriptional level of control by means of DNA–protein interactions. Post-transcriptional control operating at the level of (de)stabilization of mRNA may, in addition to RNA–protein interactions, involve structural elements arising from partial base complementarity and recognition of such structures by RNases or stabilizing proteins. Such co-ordinating elements could be responsible for the up-regulation of collagens and fibronectin in scleroderma and other activated states which are characterized by increased matrix deposition.

We thank Stephan Sollberg and Roswitha Nischt (Cologne) for valuable discussion and criticism, and Peter Angel and Ingrid Herr (Kernforschungszentrum Karlsruhe) for helpful discussions regarding the run-on transcription assays. This work was supported by the Deutsche Forschungsgemeinschaft (DFG Kr 558/6). C.M. is a fellow of the German Cancer Research Center (Deutsches Krebsforschungszentrum, Heidelberg).

REFERENCES

1 LeRoy, E. C. (1992) Ann. Rheum. Dis. **51**, 286–288
2 Krieg, T., Langer, I., Gerstmeier, H., Keller, J., Mensing, H., Goerz, G. and Timpl, R. (1986) J. Invest. Dermatol. **87**, 788–791
3 Perlish, J. S., Lemlich, G. and Fleischmajer, R. (1988) J. Invest. Dermatol. **90**, 48–54
4 Uitto, J., Halme, J., Hannuksela, M., Petrokallio, P. and Kivirikko, K. I. (1969) Scand. J. Clin. Lab. Invest. **23**, 241–247
5 Peltonen, L., Palotie, A., Myllylä, R., Krieg, T. and Oikarinen, A. (1985) J. Invest. Dermatol. **48**, 14–18
6 LeRoy, E. C. (1979) J. Clin. Invest. **54**, 880–889
7 Uitto, J., Bauer, E. A. and Eisen, A. Z. (1979) J. Clin. Invest. **64**, 921–930
8 Jimenez, S. A., Feldman, G., Bashey, R. I., Bienkowski, R. and Rosenbloom, J. (1986) Biochem. J. **237**, 837–843
9 Peltonen, J., Kähäri, L., Uitto, J. and Jimenez, S. A. (1990) Arthritis Rheum. **33**, 1829–1835
10 Fleischmajer, R., Perlish, J. S. and Krieg, T. (1981) J. Invest. Dermatol. **76**, 400–403
11 Kinsella, M. B., Smith, E. A., Miller, K. S., LeRoy, E. C. and Silver, R. M. (1989) Arthritis Rheum. **32**, 577–583
12 Buckingham, R. B., Prince, R. K. and Rodnan, G. P. (1983) J. Lab. Clin. Med. **101**, 659–669
13 Kulozik, M., Hogg, A., Lankat-Buttgereit, B. and Krieg, T. (1990) J. Clin. Invest. **86**, 917–922
14 Kikuchi, K., Hartl, C. W., Smith, E. A., LeRoy, E. C. and Trojanowska, M. (1992) Biochem. Biophys. Res. Commun. **187**, 45–50
15 Klareskog, L., Gustafsson, R., Scheynius, A. and Hällgren, R. (1990) Arthritis Rheum. **33**, 1534–1541
16 Yamakage, A., Kikuchi, K., Smith, E. A., LeRoy, E. C. and Trojanowska, M. (1992) J. Exp. Med. **175**, 1227–1234
17 Sollberg, S., Peltonen, J., Uitto, J. and Jimenez, S. A. (1992) Arthritis Rheum. **35**, 290–298
18 Ivarsson, M., McWhirter, A., Black, C. M. and Rubin, K. (1993) J. Invest. Dermatol. **101**, 216–221
19 Graves, P. N., Weiss, I. K., Perlish, J. S. and Fleischmajer, R. (1983) J. Invest. Dermatol. **80**, 130–132
20 Vuorio, T., Mäkelä, J. K. and Vuorio, E. (1985) J. Cell. Biochem. **28**, 105–113
21 Kähäri, V. M., Multimäki, P. and Vuorio, E. (1987) FEBS Lett. **215**, 331–334
22 Mauch, C., Hatamochi, A., Scharffetter, K. and Krieg, T. (1988) Exp. Cell Res. **178**, 493–503
23 Mauch, C., Kozlowska, E., Eckes, B. and Krieg, T. (1992) Exp. Dermatol. **1**, 185–190
24 Eckes, B., Mauch, C., Hüppe, G. and Krieg, T. (1993) FEBS Lett. **318**, 129–133
25 Olsen, A. E. and Prockop, D. J. (1989) Matrix **9**, 73–81
26 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor University Press, Cold Spring Harbor, NY
27 Feinberg, A.P and Vogelstein, B. (1983) Anal. Biochem. **132**, 6–13
28 Chu, M. L., Myers, J. C., Bernard, M. P., Ding, J. F. and Ramirez, F. (1982) Nucleic Acids Res. **10**, 5925–5934
29 Bernard, M., Kolbe, M., Weil, D. and Chu, M.-L. (1985) Biochemistry **24**, 2698–2704
30 Marzluff, W. F. and Huang, R. C. C. (1984) in Transcription and Translation (Hames, B. D. and Higgins, S. J., eds.), pp. 89–130, IRL Press, Oxford
31 Hämäläinen, L., Oikarinen, J. and Kivirikko, K. I. (1985) J. Biol. Chem. **260**, 720–725
32 Wisdom, R. and Lee, W. (1991) Genes Dev. **5**, 232–243
33 Speth, C. and Oberbäumer, I. (1993) Exp. Cell Res. **204**, 302–310
34 Botstein, G. R., Sherer, G. K. and LeRoy, E. C. (1982) Arthritis Rheum. **25**, 189–195
35 Krieg, T., Perlish, J. S., Fleischmajer, R. and Timpl, R. (1985) Arch. Dermatol. Res. **277**, 373–378
36 Fries, K. M., Blieden, T., Looney, R. J., Sempowski, G. D., Silvera, M. R., Willis, R. A. and Phipps, R. A. (1994) Clin. Immunol. Immunopathol. **72**, 283–292
37 Jimenez, S. A., Varga, J., Olsen, A., Li, L., Diaz, A., Herhal, J. and Koch, J. (1994) J. Biol. Chem. **269**, 12684–12691
38 Karsenty, G., Ravazzolo, R. and de Crombrugghe, B. (1991) J. Biol. Chem. **266**, 24842–24848
39 Bornstein, P., McKay, J., Morishima, J. K., Devarayalu, S. and Gelinas, R. E. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 8869–8873
40 Määttä, A. and Penttinen, R. P. K. (1993) Biochem. J. **295**, 691–698
41 Määttä, A. and Penttinen, R. P. K. (1994) FEBS Lett. **340**, 71–77
42 Määttä, A. (1994) Ph.D. Thesis, University of Turku, Finland
43 Kornblihtt, A. R., Vibe-Pedersen, K. and Baralle, F. E. (1984) Nucleic Acids Res. **12**, 5853–5868
44 Ffrench-Constant, C., van de Water, L., Dvorak, H. F. and Hynes, R. O. (1989) J. Cell Biol. **109**, 903–914
45 Määttä, A., Bornstein, P. and Penttinen, R. P. K. (1991) FEBS Lett. **279**, 9–13
46 Gillery, P., Serpier, H., Polette, M., Bellon, G., Clavel, C., Wegrowski, Y., Birembaut, P., Kalis, B., Cariou, R. and Maquart, F. X. (1992) Eur. J. Cell Biol. **57**, 244–253
47 Stefanato, C. M., Gorkiewicz-Petkow, A., Jarzabek-Chorzelska, M. and Jablonska, S. (1992) Int. J. Dermatol. **31**, 190–192
48 Kähäri, V. M., Heino, J., Niskanen, L., Fraki, J. and Uitto, J. (1990) Arch. Dermatol. **126**, 613–617
49 Deguchi, Y., Negoro, S. and Kishimoto, S. (1990) Arthritis Rheum. **33**, 1868–1870
50 Welsh, K. I. and Briggs, D. C. (1991) Curr. Opin. Rheumatol. **3**, 944–946
51 Xu, W. D., LeRoy, E. C. and Smith, E. A. (1991) J. Rheumatol. **18**, 241–246
52 Dean, D. C., Newby, R. F. and Bourgeois, S. (1988) J. Cell Biol. **106**, 2159–2170
53 Raghow, R., Postlethwaite, A. E., Keski-Oja, J., Moses, H. L. and Kang, A. H. (1987) J. Clin. Invest. **79**, 1285–1288
54 Overall, C. M., Wrana, J. L. and Sodek, J. (1991) J. Biol. Chem. **266**, 14064–14071
55 Wrana, J. L., Overall, C. M. and Sodek, J. (1991) Eur. J. Biochem. **197**, 519–528
56 Dean, D. C., McQuillan, J. J. and Weintraub, S. (1990) J. Biol. Chem. **265**, 3522–3527
57 Rossi, P., Karsenty, G., Roberts, A. B., Roche, N. S., Sporn, M. B. and de Crombrugghe, B. (1988) Cell **52**, 405–414
58 Penttinen, R. P., Kobayashi, S. and Bornstein, P. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 1105–1108
59 Chandler, L. A. and Bourgeois, S. (1991) Cell Growth Differ. **2**, 379–384
60 Chandler, L. A., Ehretsmann, C. P. and Bourgeois, S. (1994) Mol. Cell. Biol. **14**, 3085–3093
61 Varga, J., Diaz-Perez, A., Rosenbloom, J. and Jimenez, S. A. (1987) Biochem. Biophys. Res. Commun. **147**, 1282–1288

Received 20 September 1995; accepted 12 December 1995