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Elevated proα2(I) collagen mRNA levels in cultured scleroderma fibroblasts result from an increased transcription rate of the corresponding gene

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Fibroblasts cultured from affected and unaffected skin sites of three scleroderma patients were studied for the activation of type I collagen gene expression. Dot blot hybridizations with proα2(I) collagen specific cDNA probe revealed 2.9–4.8-fold increases in proα2(I) mRNA levels in the affected fibroblasts over the unaffected control cells. Transcription rate of the proα2(I) gene in the nuclei isolated from the same cells was 2.0–3.7-fold higher in the scleroderma fibroblasts than in the controls. The results show that scleroderma fibroblasts have undergone activation of collagen gene expression at the transcriptional level, which subsequently results in elevated procollagen mRNA levels, overproduction of collagen, and development of dermal fibrosis.

Collagen; Scleroderma; Transcription; (Fibroblast)

1. INTRODUCTION

Collagen is the main constituent of the extracellular matrix. Although the complicated biosynthesis of collagen provides many levels for possible control mechanisms [1], most of the current information suggests that collagen gene expression is regulated by modulating cellular mRNA levels. Correlation between collagen production and procollagen mRNA levels has been found in a number of developmental and experimental models [2–6]. On the other hand, translational control of collagen synthesis has also been reported [7,8]. In vitro transcription experiments have shown that cellular procollagen mRNA levels are mainly regulated at the transcriptional level [9,10], but modulation of mRNA degradation rates appears to be another regulatory step in collagen gene expression [11].

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Localized scleroderma is a fibrotic disorder characterized by excessive accumulation of collagen in skin. Cell culture studies have shown that fibroblasts cultured from scleroderma lesions produce increased amounts of type I and III collagen when compared to control fibroblasts [12,13]. We and others have shown that cultured scleroderma fibroblasts exhibit elevated cellular mRNA levels of type I and III collagen [14–17] to account for the increased collagen production. The purpose of this work was to extend these studies to measurement of the transcription rate of type I collagen genes in scleroderma fibroblasts. Our results show that collagen gene expression is activated at the level of transcription in scleroderma, suggesting an important role for factors involved in the regulation of the promoter regions of these genes.

2. MATERIALS AND METHODS

2.1. *Cell cultures*

Fibroblast cultures were started by explantation from affected and symmetrically located unaf-

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fected skin sites of three female patients, aged 9 (PAH), 13 (KLU) and 51 (JAL), with histologically confirmed localized scleroderma. For the analyses, early passage cultures were grown to confluence in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Hazleton, Denver, PA), and incubated with 50 μg/ml ascorbate for 24 h prior to isolation of nuclei.

2.2. *Nuclear run-off transcriptions*

Isolation of nuclei was carried out as described [18]. Aliquots were stained with trypan blue for determination of the number of nuclei. For transcription, aliquots of 1×10^6 nuclei were incubated in a 100-μl reaction containing 16% glycerol, 20 mM Tris-HCl, pH 8.0, 2 mM Mg acetate, 2.5 mM dithiothreitol, 5 mM $MgCl_2$, 100 mM KCl, 1 mM each ATP, GTP, UTP, 125 μCi [α-^{32}P]CTP (3000 Ci/mmol, Amersham, England) and 1 U/μl human placental RNase inhibitor, RNasin® (Promega Biotec, Madison, WI) for 45 min at 26°C. The overall incorporation of [^{32}P]CTP into RNA transcripts was estimated by precipitating 1-μl aliquots with cold 10% trichloroacetic acid and determining the radioactivity of the precipitates by liquid scintillometry. Transcription reactions were terminated by incubation with 10 μg/ml of DNase I (Boehringer Mannheim, FRG) for 5 min at 26°C. Thereafter 50 μg/ml of yeast tRNA was added for carrier.

Radiolabeled RNA was isolated as described [19], dissolved in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1% SDS and denatured by heating at 65°C for 15 min. Equal amounts of radioactivity were hybridized to 2-μg aliquots of non-radioactive plasmid DNA immobilized to nitrocellulose filters [20]. Hybridization and washing of the filters were carried out as described by Thomas [21]. The filters were exposed to Kodak X-Omat film at −70°C using intensifying screens. The intensity of hybridization was quantified by densitometry.

2.3. *Measurement of type I procollagen mRNA levels*

Cytoplasmic RNA was isolated from fibroblasts as described earlier [22]. Aliquots were applied to nitrocellulose filters, immobilized and prehybridized [21]. The filters were hybridized with plasmid pHCAL2, which contains an 1180 bp cDNA sequence specific for human proα2(I) collagen mRNA [17]. Plasmid DNA was nick-translated with [α-^{32}P]dCTP to a specific activity of approx. 5×10^7 cpm/μg, hybridized and washed as described above. Hybridization was detected and quantified as above.

3. RESULTS

Estimation of proα2(I) collagen mRNA levels by cytoplasmic dot blot hybridizations revealed 2.9–4.8-fold increases in proα2(I) collagen mRNA levels in the three affected scleroderma fibroblast lines compared to their unaffected controls (fig.1).

The incorporation of [^{32}P]CTP into nuclear run-off transcripts increased up to 45 min (fig.2). Total

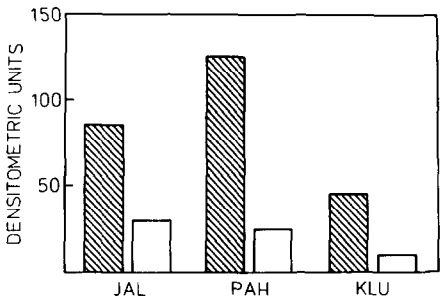

Fig.1. Cytoplasmic proα2(I) collagen mRNA levels in fibroblasts cultured from affected (hatched bar) and unaffected (open bar) skin sites of scleroderma patients JAL, PAH and KLU. Proα2(I) mRNA levels were measured by dot blot hybridizations and quantified densitometrically.

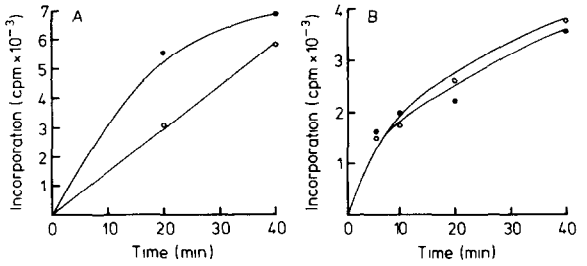

Fig.2. Incorporation of [^{32}P]CTP into the trichloroacetic acid precipitable nucleic acids in nuclei isolated from affected (●) and unaffected (○) skin fibroblasts of scleroderma patients JAL (A) and PAH (B). Nuclei were isolated from confluent fibroblast cultures and run-off transcriptions were carried out in the presence of [^{32}P]CTP. Aliquots taken at the time points indicated were precipitated with cold 10% trichloroacetic acid and the radioactivity of precipitates determined with liquid scintillometry.

[^{32}P]CTP incorporation was somewhat higher in nuclei isolated from affected fibroblasts of patient JAL compared to unaffected fibroblasts (fig.2A). Only slight differences were detected in [^{32}P]CTP incorporation between nuclei from scleroderma and control fibroblasts of patient PAH (fig.2B). Hybridization of the labeled nuclear RNA to plasmids pHCAL2 and pBR322 immobilized onto nitrocellulose filters (fig.3) revealed 2.0–3.7-fold increases in the transcription rate of the proα2(I) collagen gene in the nuclei from scleroderma fibroblasts compared to unaffected controls (fig.4). The results were reproducible in two independent transcription assays. No hybridization of the labeled transcripts to plasmid pBR322 could be detected.

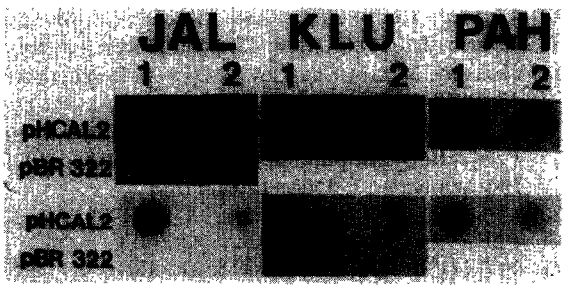

Fig.3. Transcription of proα2(I) collagen gene in 10^6 nuclei isolated from affected (1) and unaffected fibroblasts (2) of scleroderma patients JAL, PAH and KLU. Transcriptions were performed for 45 min in the presence of [^{32}P]CTP and the labeled RNAs were isolated. 2-μg aliquots of plasmid DNA containing an insert specific for human proα2(I) collagen mRNA (pHCAL2) and of plasmid pBR322 (negative control) were denatured and dotted onto nitrocellulose filters. After hybridization and washing, the filters were exposed to X-ray films. The results of two different exposures are shown for each of the three cell line pairs.

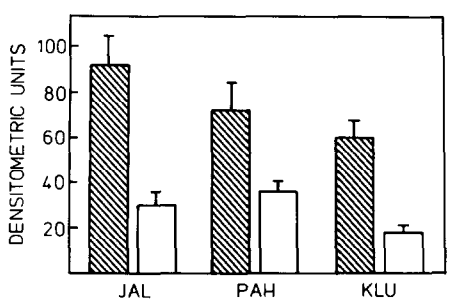

Fig.4. Transcription rate of proα2(I) collagen gene in nuclei isolated from affected (hatched bar) and unaffected (open bar) fibroblasts of three scleroderma patients (JAL, PAH and KLU). The mean and SE of 2–3 exposures from two transcriptions are indicated.

4. DISCUSSION

The capacity of fibroblasts cultured from progressing scleroderma lesions to produce increased amounts of collagen has been well documented [12,13]. A correlation between increased collagen synthesis and elevated procollagen mRNA levels in scleroderma fibroblasts has been reported [14–17]. In this study we show that fibroblasts cultured from affected skin sites of scleroderma patients exhibit increased transcription rates of the proα2(I) collagen gene to account for the elevated levels of the corresponding mRNA in these cells. Due to technical difficulties only the levels of proα2(I) collagen transcripts could be reliably determined, but all the information available suggests a strict control of the α1:α2 ratio in fibroblasts [17,23]. An alteration in the degradation rate of procollagen mRNAs is another way to regulate the cellular levels of these mRNAs [11]. Our results suggest, however, that the major mechanism responsible for increased procollagen mRNA levels in scleroderma is the increased transcription rate of the corresponding genes.

The fact that the activation of collagen synthesis in scleroderma fibroblasts disappears in culture within 5–10 subcultures, suggests that stimulatory factors present in situ, but not in cell culture, play an important role in the activation process [15,17,24]. In order to characterize these factors, it is important to determine the level of activation of collagen gene expression. Recently, the role of polypeptide growth factors and proto-oncogenes in the activation of connective tissue formation has been emphasized [25], although little is known of their exact role in the activation of collagen gene expression. The data available on transforming growth factor-β (TGF-β) indicate that this factor is a potent stimulator of collagen production both in vivo and in culture [26]. Transfection studies with NIH 3T3 cells have shown that certain viral oncogene products can regulate the promoters of proα1(III) collagen gene [27] and proα2(I) collagen gene [28]. We feel that scleroderma provides a good model for studying molecular mechanisms of collagen overproduction in human fibrotic condi-

tions. The elucidation of the underlying pathogenetic mechanisms in these disorders requires studies on the expression of various growth factors and proto-oncogene products, their effects on collagen production, and analyses of factors interacting with promoter regions of collagen genes.

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