c-*myb* Proto-Oncogene Is Expressed by Quiescent Scleroderma Fibroblasts and, Unlike B-*myb* Gene, Does Not Correlate With Proliferation

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Systemic sclerosis (scleroderma) is characterized by excessive deposition of extracellular matrix constituents. Although it has been proposed that tissue fibrosis is due to increased fibroblast synthesis of various collagen polypeptides, there is some experimental evidence that patients with systemic sclerosis have a defect in the control of fibroblast growth. The *myb* family of genes includes, among others, the c-*myb* proto-oncogene and the structurally related gene, B-*myb*, which are both implicated in the regulation of differentiation and/or proliferation of hematopoietic and nonhematopoietic cells. To elucidate the molecular basis responsible for scleroderma fibroblast proliferation, we therefore elected to investigate the expression of c-*myb* and B-*myb* genes in scleroderma and control cells. Using the reverse transcriptase polymerase chain reaction technique, we detected c-*myb* transcripts in scleroderma skin fibroblasts rendered quiescent by serum deprivation. Under the same experimental conditions, c-*myb* message was not found in normal skin fibroblasts, but, after serum stimulation, c-*myb* RNA was clearly evident from 3 to 72 h in both normal and pathologic cells. Treatment of these cells with c-*myb* antisense oligonucleotides caused downregulation of c-*myb* expression, and the inhibition of scleroderma fibroblast proliferation was 42%, whereas in normal fibroblasts the inhibition was weaker (22%). In contrast to c-*myb*, in normal and scleroderma fibroblasts the level of expression of B-*myb* correlated with cell proliferation assessed by cell count, and densitometric analysis showed that B-*myb* message was 1.5–5 times higher in most of pathologic cells studied. The antisense B-*myb* oligonucleotides had a weaker antiproliferative effect compared with antisense c-*myb*, inhibiting scleroderma and normal fibroblasts by 23% and 13%, respectively. These data suggest that the B-*myb* and c-*myb* genes may play a role in scleroderma fibroblast proliferation and function. *J Invest Dermatol 106:1281–1286, 1996*

Systemic sclerosis (scleroderma) is characterized by extensive fibrosis of the skin and internal organs (Krieg and Meuer, 1988; LeRoy, 1992). Although the initial pathogenic mechanisms are ill defined, functional derangement of affected organs has been ascribed to obliterative microvascular lesions and to excessive deposition of extracellular matrix (Krieg and Meuer, 1988; LeRoy, 1992). *In vitro* studies have demonstrated that scleroderma skin fibroblasts show enhanced synthesis of connective tissue proteins (LeRoy, 1972; Uitto *et al*, 1979) associated with elevated levels of corresponding messenger RNA (Vuorio *et al*, 1985; Jimenez *et al*, 1986; Kahari *et al*, 1987). Furthermore, scleroderma fibroblasts exhibit abnormal cell growth, as manifested by persistent proliferation in serum-free medium and by the increased expression of c-*myc* proto-oncogene (Trojanowska *et al*, 1988). Nevertheless, the molecular mechanisms responsible for scleroderma fibroblast proliferation remain insufficiently understood, and for this reason we elected to investigate the possible role of other genes known to be involved in cell proliferation.

The *myb* family includes the virally encoded v-*myb* oncogene, which is able to induce neoplastic transformation of hematopoietic cells influencing cell growth and differentiation (Moscovici and Gazzolo, 1982; Radke *et al*, 1982). Its normal cellular counterpart, the proto-oncogene c-*myb*, codifies for a transcription factor of 73 kDa that seems to be involved in the regulation of differentiation and/or proliferation of hematopoietic cells (Gewirtz and Calabretta, 1988; Caracciolo *et al*, 1990; Mucensky *et al*, 1991; Valtieri *et al*, 1991) and nonhematopoietic cells (Melani *et al*, 1991; Raschella *et al*, 1992; Simons *et al*, 1992; Olson *et al*, 1993).

Recently, two *myb*-related genes, B-*myb* and A-*myb*, have been cloned (Nomura *et al*, 1988), and although their function is not yet clear, their homology to c-*myb* suggests that they may act as transcription factors (for review, see Introna *et al*, 1994). B-*myb* protein seems to play a key role in the regulation of hematopoietic cell proliferation (Arsura *et al*, 1992; Foos *et al*, 1992) and has been found expressed in hematopoietic cells (Arsura *et al*, 1992), in epithelial cells (Nomura *et al*, 1988), in Balb/c-3T3 cells, and in WI-38 human diploid fibroblasts (Nomura *et al*, 1988; Reiss *et al*, 1991).

Little is available on the expression of A-*myb*, which has been

Manuscript received April 4, 1995; revised December 22, 1995; accepted for publication February 1, 1996.

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Abbreviation: EMEM, Eagle's minimum essential medium.

0022-202X/96/$10.50

Table I. Demographic Features of the Systemic Sclerosis (SSc) Patients Included in the Study

Patient Number	Sex/Age (y)	Disease Duration (y)	SSc Subset[a]	Visceral Involvement[b]
1	M/66	13	dSSc	L, E, H
2	F/56	12	dSSc	L, E, GI
3	F/69	2	dSSc	L, E, H
4	F/68	11	dSSc	E
5	F/52	13	dSSc	L, E

[a] dSSc, diffuse cutaneous SSc.
[b] L, lung; E, esophagus; H, heart; GI, gastrointestinal tract.

observed only in a few cell lines of different origins (Nomura *et al*, 1988).

In view of these considerations, we tried to assess the potential role of c-*myb* and B-*myb* in the pathogenesis of systemic sclerosis by investigating their expression in resting and proliferating normal and scleroderma fibroblasts.

MATERIALS AND METHODS

Cells Human skin fibroblasts were obtained from punch biopsies taken from the involved skin of the forearms of five patients who fulfilled the preliminary criteria of the American Rheumatism Association for the diagnosis of systemic sclerosis (Masi *et al*, 1980). All patients had diffuse cutaneous systemic sclerosis; their clinical data are presented in **Table I**. Control fibroblasts were grown from skin biopsy specimens of five age- and sex-matched healthy subjects. Primary explant cultures were established in 25-cm^2 culture flasks in Eagle's minimum essential medium (EMEM) containing 10% fetal bovine serum (FBS), 2 mM glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml). EMEM with these supplements is hereafter referred to as "culture medium." Monolayer cultures were maintained at 37°C in 5% CO_2.

Experimental Fibroblast Cultures Fibroblasts at the fourth subpassage were used for all experiments. Briefly, fibroblasts were harvested from confluent cultures with trypsin and plated in culture medium at the concentration reported below. After attachment for 72 h, the culture medium was discarded and changed to EMEM containing 0.2% FBS to induce cell quiescence. After a further 48 h of incubation, the medium was again changed to EMEM supplemented with 10% FBS, and cells were collected before and at 3, 12, 24, 48, and 72 h after serum stimulation.

Probes The probe used for detection of the B-*myb* transcript was an insert of 2,300 bp, containing the entire coding region of the human B-*myb* gene, obtained after restriction digestion with *Bam*HI of the plasmid pAT-B18-B*myb*. The probe used for detection of the c-*myb* transcript was a fragment containing the first 668 bp of the human c-*myb* cDNA obtained after restriction digestion with *Eco*RI of the plasmid pCM8. The probes were labeled with [^{32}P]dCTP by the random priming standard procedure (Sambrook *et al*, 1989) to specific activities of 5×10^8 cpm/μg DNA.

RNA Isolation and Analysis Total cellular RNA was extracted according to the guanidine isothiocyanate–cesium chloride method, as described elsewhere (Sambrook *et al*, 1989). Fifteen micrograms of total RNA was loaded in each lane of a 1% agarose-formaldehyde gel and transferred to nylon membranes (Du Pont, Boston, MA) by standard blotting procedures. The gels as well as the membranes were photographed under ultraviolet light to check for the quantity and even transfer of RNA. Blots were baked at 80°C for 2 h. Prehybridizations were performed for 7 h at 60°C in 1 M NaCl, 1% sodium dodecyl sulfate, 10% dextran sulfate, and 100 μg denatured salmon sperm DNA per ml. Hybridizations were performed overnight at 60°C in the same buffer by adding 1.2×10^6 cpm/ml of the labeled probe. The filters were washed twice for 10 min at 60°C with 2× standard saline citrate/1% sodium dodecyl sulfate, 15 min with 0.5× standard saline citrate/0.5% sodium dodecyl sulfate, and finally twice at room temperature with a large volume of 0.5× standard saline citrate. The blots were dried and exposed to Kodak X Omat x-ray film with two intensifying screens. Northern blots were quantified by laser densitometric scanning.

Oligonucleotides The oligonucleotides used for the experiments were all 18-mer two antisense and two sense oligonucleotides used as a control. The sequence of each oligonucleotide was as follows (5′-3′): B-*myb* antisense, dGCAGCGCGTCCGCCGAGA; B-*myb* sense, dTCTCGGCGGACGCGCTGC; c-*myb* antisense, dGTGCCGGGGTCTTCGGGC; and c-*myb* sense, dGCCCGAAGACCCCGGCAC. These oligonucleotides have been shown to inhibit the proliferation of hematopoietic cells (Introna *et al*, 1994) and of bone marrow fibroblasts (Szczylik *et al*, 1993).

Oligomer Treatment of the Cells Fibroblasts were seeded in triplicate (1×10^3 cells/0.1 ml culture medium/well) in flat-bottom 96-well plates. After attachment, subconfluent fibroblasts were washed three times with phosphate-buffered saline and then induced to quiescence in EMEM containing 0.2% FBS for 48 h. After this incubation, the medium was changed to EMEM containing 0.2% FBS and the oligonucleotides at 30, 60, and 100 μg/ml. After a 3-h incubation at 37°C, FBS was added to reach 10%. After 24 and 48 h culture, the same oligonucleotides were added to each well to a final concentration equivalent to half of the initial dose. The following day, the medium was discarded and the cells were washed twice with phosphate-buffered saline, fixed with 0.25% glutaraldehyde for 10 min, air dried, stained with 0.1% crystal violet for 25 min, lysed with 0.2% Triton X, and counted in a spectrophotometer at 550 nm.

Semiquantitative Analysis of RNA by Polymerase Chain Reaction (PCR) Total RNA was extracted from relatively few cells for PCR analysis by a modification of the method of Belyavsky *et al* (1989). Fibroblasts were lysed in 150 μl guanidine thiocyanate solution, and 1 μg of yeast tRNA, 4.5 μl of 2 M sodium acetate, pH 4, 180 μl water-saturated phenol, and 35 μl chloroform were added sequentially. The samples were vortexed, placed on ice for 10 min, and then centrifuged at 12,000*g* for 10 min at 4°C.

The water phase was collected, and the RNA was precipitated with 2.7 vol ethanol and centrifuged at 12,000*g* for 20 min at 4°C. The pellet was washed in 70% ethanol and resuspended in 3 μl of distilled water. The RNA was directly reverse transcribed in 5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2.5 mM random hexamers, 1 mM dNTP, 1 U/μl RNase inhibitor, and 2.5 U/μl M-MLV reverse transcriptase. A master mix of all reagents was prepared on ice, and 17 μl were added to each RNA sample. The samples were incubated for 10 min at room temperature and then for 45 min at 42°C. Aliquots of the reverse transcription reactions were amplified by PCR using sets of primers specific for c-*myb* or β-actin in the following reaction conditions: 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, 0.2 mM each dNTP, 25 U *Taq* DNA polymerase per ml, and 5 ng/ml of each primer. The sequence of each primer was as follows: c-*myb* (forward), dCGCCGCGCCATGGCCCGAAGACCC; c-*myb* (reverse), dTTCTGGAAGCTTGTGGCCACTGCTG; β-actin (forward), dCCTTCCTGGGCATGGAGTCCTG; β-actin (reverse), dGGAGCAATGATCTTGATCTTC.

PCR was performed in an automated DNA Thermal Cycler (Perkin-Elmer, Norwalk, CT) for 22 cycles. The PCR products were run in a 1.5% ultrapure agarose gel (BRL, Bethesda, MD) and transferred to nylon membranes by standard Southern blotting procedures. The probes and hybridization conditions were identical to those described above for Northern blots.

Statistical Analysis Data are expressed as mean ± 1 SD. Mean values regarding inhibition of cell proliferation by oligonucleotides were compared using the bilateral Mann-Whitney U test. A p value of less than 0.05 was considered to indicate significance. All values are two-tailed.

RESULTS

B-*myb* Gene Is Not Expressed in Quiescent Fibroblasts and Correlates With Proliferation Subconfluent monolayers of skin fibroblasts from normal donors and scleroderma patients were obtained and made quiescent by incubation in 0.2% FBS for 48 h, as described previously (Trojanowska *et al*, 1988). The cell cultures were then stimulated by exposure to 10% FBS, and cell count and B-*myb* expression were evaluated at several intervals. The experiment reported in **Fig 1*a*** is representative of five and shows that the scleroderma and normal fibroblasts responded in a comparable way, with a cell count peak after approximately 48 h. In agreement with previous observations (Reiss *et al*, 1991; Lam *et al*, 1992), there was a dramatic induction of B-*myb* RNA **(Fig 1*b*,*c*)** that was apparent 24 h after FBS addition.

In all the experiments, the peak of the B-*myb* message preceded the peak of actual cell number, and after 48 h of stimulation, in keeping with the findings of Lam *et al* (1992), there was a decrement in its level concomitant with the reduction in cell count **(Fig 1*a*,*b*)**. The B-*myb* message disappeared completely after 72 h in normal fibroblasts, however, whereas it remained relatively high for scleroderma fibroblasts **(Fig 1*c*)**.

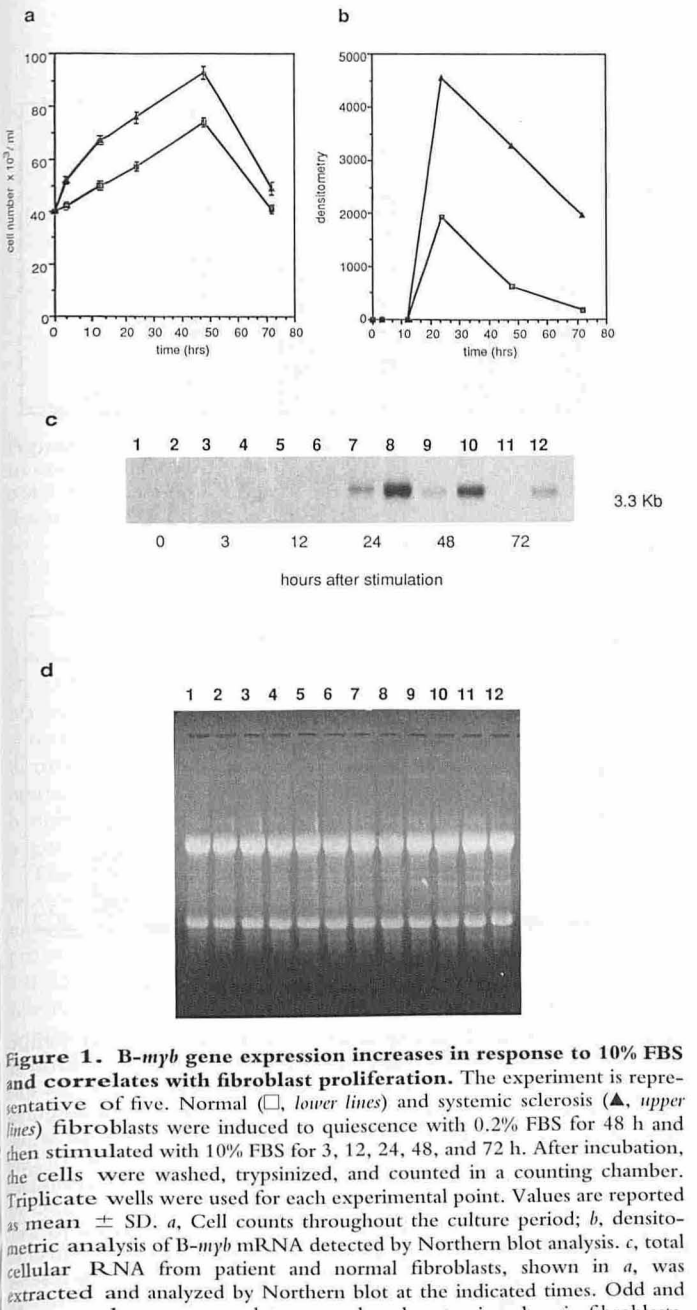

Figure 1. B-*myb* gene expression increases in response to 10% FBS and correlates with fibroblast proliferation. The experiment is representative of five. Normal (□, *lower lines*) and systemic sclerosis (▲, *upper lines*) fibroblasts were induced to quiescence with 0.2% FBS for 48 h and then stimulated with 10% FBS for 3, 12, 24, 48, and 72 h. After incubation, the cells were washed, trypsinized, and counted in a counting chamber. Triplicate wells were used for each experimental point. Values are reported as mean ± SD. *a*, Cell counts throughout the culture period; *b*, densitometric analysis of B-*myb* mRNA detected by Northern blot analysis. *c*, total cellular RNA from patient and normal fibroblasts, shown in *a*, was extracted and analyzed by Northern blot at the indicated times. Odd and even numbers correspond to normal and systemic sclerosis fibroblasts, respectively. *d*, photograph of the ethidium bromide-stained 1% formaldehyde agarose gel.

Finally, the feasibility of the comparison between normal and scleroderma B-*myb* expression is witnessed by the data in **Fig 1*d***, which reflect the amount of total RNA per each lane that had been loaded on the gel and transferred to the membranes. When all the proliferative experiments were analyzed, the time course of one normal fibroblast fell above that of the least proliferating scleroderma fibroblasts, and this behavior was reflected by a higher expression of B-*myb* (data not shown).

Expression of the c-*myb* Gene Is Elevated in Quiescent and FBS-Stimulated Fibroblasts When the blots used to study B-*myb* expression were reprobed with a human c-*myb* probe, the signal was extremely weak (data not shown), perhaps reflecting the low levels of c-*myb* expression in these cells. To study c-*myb* expression, we therefore used a semiquantitative reverse transcriptase (RT)-PCR approach, detailed previously (Arsura *et al*, 1992). Identical aliquots of total RNA were taken from the same RNA preparations used to investigate B-*myb* transcripts. In all cases, the c-*myb* message was clearly augmented after 12–24 h from FBS addition **(Fig 2)**, preceding the highest level in cell count by approximately 24 h **(Fig 1)**, and subsequently declined, although it maintained at much higher levels in scleroderma fibroblasts **(Fig 2)**, as was observed for the B-*myb* gene.

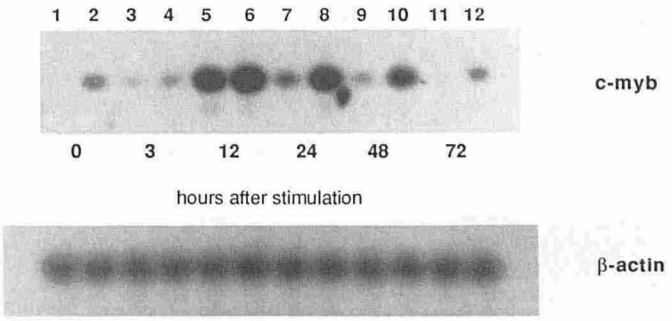

Figure 2. c-*myb* transcripts increase upon fibroblast stimulation with FBS. Normal and scleroderma fibroblasts were induced to quiescence for 48 h with 0.2% FBS and then stimulated with 10% FBS for 3, 12, 24, 48, and 72 h. Total cellular RNA was extracted and c-*myb* transcripts were analyzed by RT-PCR. As control, β-actin mRNA expression was analyzed in all samples. One experiment representative of five is shown. Odd and even numbers correspond to control and scleroderma fibroblasts, respectively.

In addition, although no c-*myb* message could be observed in the quiescent normal fibroblasts **(Fig 3*a*)**, a clear signal for the c-*myb* gene was evident in scleroderma cells in similar conditions as shown in **Fig 3*b*** and with the densitometric analysis in **Fig 3*c***. In all scleroderma fibroblasts studied, c-*myb* transcripts were more highly expressed than in cells from normal subjects.

Exposure to B-*myb* and c-*myb* Antisense Oligonucleotides Lowers Fibroblast Proliferation To explore further the functional role of B-*myb* and c-*myb* expression in scleroderma and normal fibroblasts, we decided to use the antisense oligonucleotide approach, which had been used previously for both genes (Arsura *et al*, 1992; Szczylik *et al*, 1993). Microcultures of fibroblasts were first rendered quiescent and subsequently stimulated with FBS in the presence of antisense oligonucleotides. Although the addition of the antisense oligonucleotides is never sufficient to arrest the proliferation completely, as reported previously (Arsura *et al*, 1992; Szczylik *et al*, 1993), nevertheless, both genes seem to be necessary for the full proliferation to occur **(Fig 4)**.

Inhibition of scleroderma fibroblast proliferation by c-*myb* antisense oligonucleotides was statistically significant at all doses tested ($p < 0.05$; **Fig 4*a***), ranging from 20% (30 μg/ml) to 35% (60 μg/ml) and to 42% (100 μg/ml). On the other hand, the addition of anti B-*myb* oligonucleotides did not show a significant inhibition of proliferation. When normal fibroblasts were tested, significant inhibition was present after the addition of 60 μg/ml (23%) or 100 μg/ml (13%) of anti c-*myb* or of 100 μg/ml (13%) anti B-*myb* ($p < 0.05$; **Fig 4*b***). Interestingly, at the doses of 60 μg/ml and 100 μg/ml, the inhibitory effect by anti c-*myb* antisense oligonucleotides was more pronounced on scleroderma fibroblasts than on normal fibroblasts ($p < 0.05$ by Mann-Whitney U test; **Fig 4*a***). In all the experimental conditions, the addition of the controls (sense oligonucleotides) did not significantly affect the cellular response **(Fig 4)**. To determine the molecular mechanism involved in the described biologic effect of the antisense oligonucleotides, we performed RT-PCR analysis on treated cells. As reported previously, this analysis could be performed only on the antisense c-*myb*–exposed cells, because the expected mechanism of action of the antisense anti–B-*myb* oligonucleotides is at the translational level (Arsura *et al*, 1992). The exposure to anti c-*myb* antisense oligonucleotides was indeed able to dramatically reduce the amount

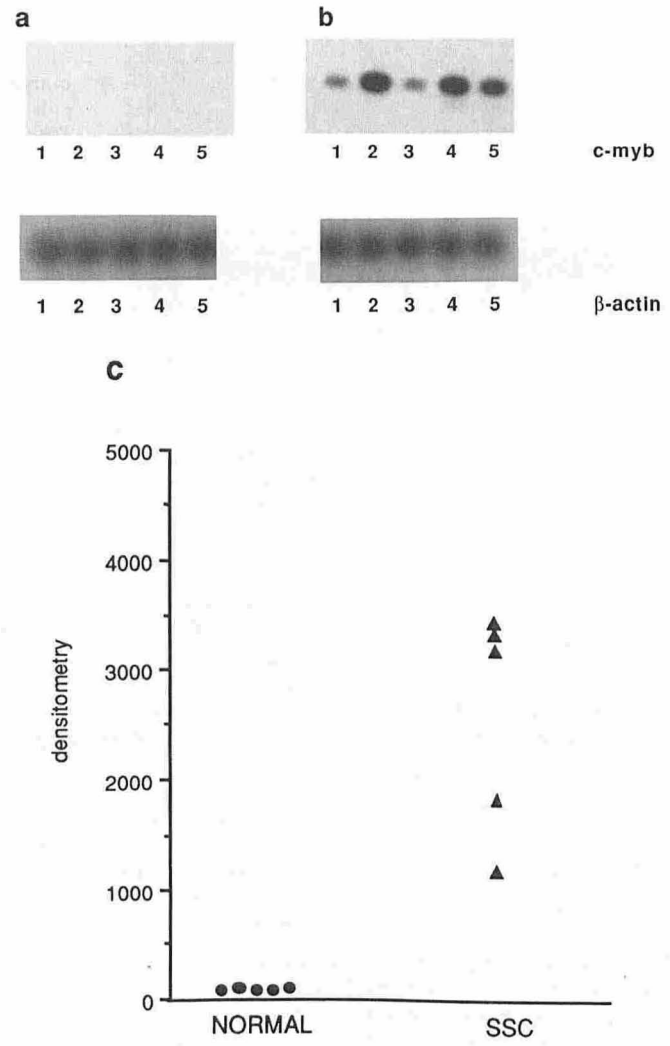

Figure 3. Elevated expression of c-*myb* in quiescent scleroderma fibroblasts. Fibroblasts from five scleroderma patients and from five normal subjects were induced to quiescence for 48 h with 0.2% FBS. Total cellular RNA was extracted, and c-*myb* transcripts were analyzed by RT-PCR (*a,b*). *c*, Densitometric analysis of c-*myb* RNA detected by RT-PCR. SSC, systemic scleroderma.

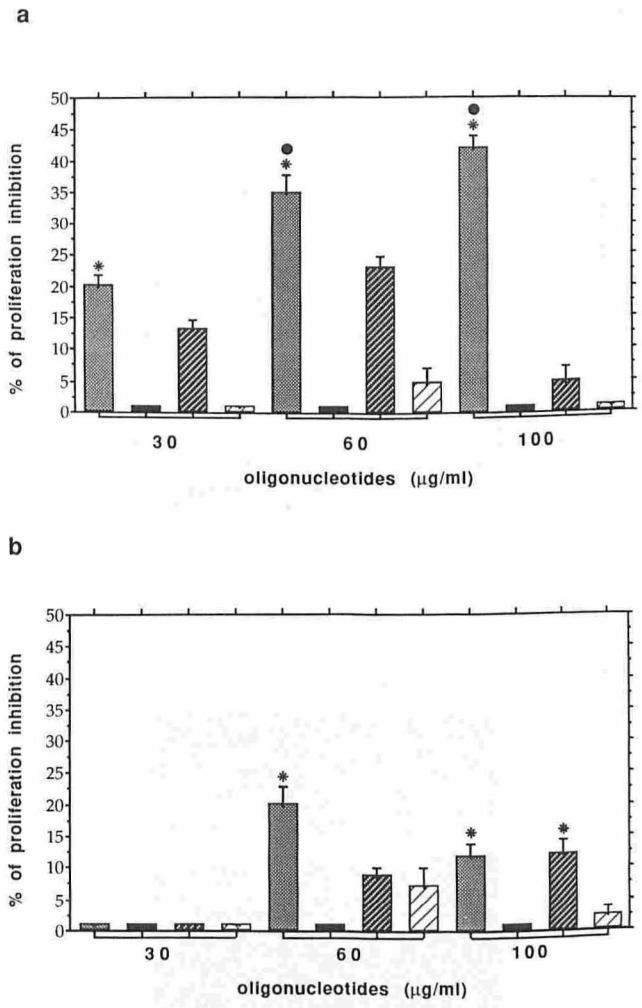

Figure 4. c-*myb* antisense oligonucleotides lower fibroblast proliferation. Fibroblasts were seeded (1×10^3 cells/0.1 ml medium) in flat-bottom 96-well plates. Cells were induced to quiescence with 0.2% FBS for 48 h, treated with medium containing 10% FBS (control) or oligonucleotides, and then counted as reported in *Materials and Methods*. The results are expressed as percentage of inhibition of growth relative to control cells treated with FBS; values are means ± SD for three different experiments with the same fibroblast cell lines, each done in triplicate. *$p < 0.05$ by Mann-Whitney U test when compared with c-*myb* sense **(Fig 4*a*)** and with c-*myb* and B-*myb* sense **(Fig 4*b*)**. ●$p < 0.05$ when compared with normal fibroblasts. *a*, Scleroderma fibroblasts; *b*, normal fibroblasts. c-*myb* antisense (▨); c-*myb* sense (■); B-*myb* antisense (▨); B-*myb* sense (▨).

of c-*myb* message, whereas no effect was found for the fibroblasts exposed to the control oligonucleotides **(Fig 5)**.

DISCUSSION

In this study, the use of RT-PCR allowed the detection of c-*myb* transcripts in scleroderma fibroblasts when their growth had been arrested by serum deprivation and the cells had entered a quiescent state. In the same experimental conditions, c-*myb* RNA was not found in normal skin fibroblasts.

The relevance of these findings to pathogenic mechanisms of systemic sclerosis remains unclear, although after the demonstration that the product of c-*myb* is necessary for hematopoietic (Gewirtz and Calabretta, 1988; Caracciolo *et al*, 1990; Mucensky *et al*, 1991; Valtieri *et al*, 1991) and nonhematopoietic cell cycle progression (Melani *et al*, 1991; Raschella *et al*, 1992; Simons *et al*, 1992; Olson *et al*, 1993), it is possible that they reflect a defect in the regulation of scleroderma fibroblast function. In fact, first, c-*myb* has been detected in immature cells, but its expression decreases and disappears as differentiation takes place (Westin *et al*, 1982; Craig and Bloch, 1984; Gonda and Metcalf, 1984); second, constitutive overexpression of c-*myb* prevents cellular differentiation (Clarke *et al*, 1988; McMahon *et al*, 1988; Selvakumaran *et al*, 1992); and finally, inhibition of c-*myb* expression by antisense c-*myb* oligonucleotides induces growth arrest of several hematopoietic (Gewirtz and Calabretta, 1988; Anfossi *et al*, 1989; Gewirtz *et al*, 1989; Valtieri *et al*, 1991; Arsura *et al*, 1992) and nonhematopoietic cell lines (Melani *et al*, 1991; Raschella *et al*, 1992; Simons *et al*, 1992; Simons and Rosenberg, 1992).

Furthermore, a pivotal role for c-*myb* in the control of differentiation has been postulated because of the lack of correlation between the proliferative state and c-*myb* levels in different bone marrow subpopulations (Kastan *et al*, 1989); on the basis of data showing that c-*myb* is required for SCF and granulocyte macrophage colony-stimulating factor synthesis by bone marrow fibroblasts (Szczylik *et al*, 1993); by the dissociation between B-*myb* and c-*myb* expression during the proliferation and differentiation of human myeloid cell lines (Arsura *et al*, 1994); and, finally, by the recent report showing that the loss of carboxyl-terminal but not amino-terminal sequences can convert *myb* into an *in vivo* trans-

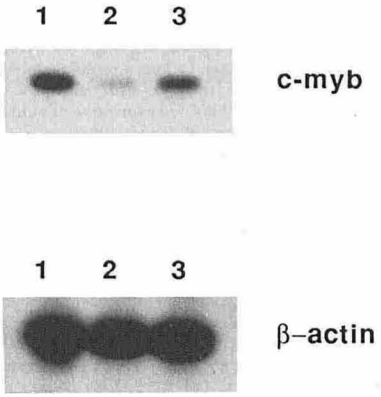

Figure 5. c-*myb* antisense oligonucleotides lower c-*myb* RNA in treated cells. *Lane 1*, cells treated with 10% FBS (untreated cells); *lane 2*, c-*myb* antisense treated cells; *lane 3*, c-*myb* sense treated cells. As control, β-actin mRNA expression was analyzed in all samples.

forming protein for nonhematopoietic mesenchymal cells (Press *et al*, 1994). No data are available, however, regarding skin fibroblasts, particularly from patients with disorders characterized by abnormal fibroblast function such as scleroderma or other fibrotic disorders. Nevertheless, the findings reported here reinforce the opinion that c-*myb* is not restricted to hematopoietic cells and operates in a broader spectrum of cell types than initially acknowledged (Szczylik *et al*, 1993).

The mechanism of fibrosis in systemic sclerosis is unknown, but recent reports suggest that, in addition to enhanced extracellular matrix production and deposition, an abnormality of fibroblast growth may contribute to the fibrotic lesions (Trojanowska *et al*, 1988). In this respect, particular emphasis has been placed upon fibroblast insensitivity to the induction of quiescence by serum deprivation and upon persistence of c-*myc* expression (Trojanowska *et al*, 1988).

Our data, showing that both normal and scleroderma fibroblasts are sensitive to the addition of c-*myb* antisense oligonucleotides, with a reduced proliferative capacity upon stimulation with FBS, imply that the c-*myb* gene is involved in the proliferation of these cells. As stated above, however, its presence in quiescent fibroblasts leads to the conclusion that this is not its only function. Quite surprisingly, scleroderma fibroblasts show an even higher sensitivity to the addition of anti c-*myb* oligonucleotides with respect to normal cells; however, we can only speculate on this difference. Perhaps this can be ascribed to the constant expression of the c-*myb* gene observed even in the quiescent state or to a peculiar sensitivity of scleroderma fibroblasts to extracellular signals. The extent of the inhibition of proliferation obtained with single antisense oligonucleotides may be limited because of the supplementary influence of other genes.

The expression of B-*myb* correlates with cell proliferation in both normal and pathologic fibroblasts, although its peak slightly precedes that of cell count. If B-*myb* is somehow involved in cell proliferation, it is not surprising that the molecular events anticipate cell division. These findings are in agreement, however, with other reports that have demonstrated that B-*myb* is more strictly bound to proliferation than is c-*myb* (Golay *et al*, 1991; Arsura *et al*, 1992). Nevertheless, our experiments showing that B-*myb* is more expressed than c-*myb* imply that B-*myb* has a different role in skin fibroblast proliferation, although it cannot be ruled out that its overexpression is not the cause, but the effect of an abnormal proliferative state. Further studies are needed to clarify the effective role of *myb* genes in this novel cellular target and in systemic sclerosis.

This work was partly supported by Grants MPI 40% 1993 and 1994, by the Italian Association for Cancer Research (AIRC), and by the finalized CNR project ACRO. We are indebted to Mrs. Gabriella Riganelli for assistance in preparation of the manuscript.

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