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Different regulation of collagen I gene transcription in three-dimensional lattice cultures

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Human skin fibroblasts were cultivated in confluent monolayers, retracting collagen lattices, retracting fibrin lattices and non-retracting fibrin lattices and the expression of messenger RNA specific for the α_1 chain of type I procollagen comparatively studied by Northern blot and dot blot hybridization. Two factors contribute to the lower level of procollagen messenger RNA in collagen lattices: the retraction and the nature of the fibrillar protein that constitutes the lattices. Fibrin lattices, when they do not retract, make as much collagen and procollagen mRNA as monolayer confluent cells.

Collagen; Transcription; Cell shape; Fibroblast; Collagen lattice culture; Fibrin lattice culture

1. INTRODUCTION

The behavior of fibroblasts cultivated in 3-dimensional collagen lattices was found largely different from that of monolayer cultures [1,2]. These lattices appear to offer the conditions for a physiological environment comparable to dermis [3]. The most striking feature of the cells cultivated in these lattices is their quiescent status. They stop dividing or divide very slowly [4,5], they exhibit a low response to growth factors [6,7] and they synthesize far less proteins and principally collagen than fibroblasts in confluent monolayers [8,9]. It was already demonstrated that the inhibition of collagen synthesis observed in this condition depended in totality or for a large part on an inhibition of transcription [10].

The aim of the present work was to determine whether this inhibition of transcription was triggered by the presence of collagen in the lattice that surrounds the cells or by the property of collagen lattices to contract progressively. For this purpose, we compared the intensity of transcription in collagen lattices with that obtained in fibrin lattices. Fibrin lattices may be prepared under two varieties, the retracting and non-retracting ones. A comparison of transcription activity was made between confluent monolayer cultures (M), retracting collagen lattices (CL), retracting fibrin lattices (RFL) and non-retracting fibrin lattices (NRFL).

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2. MATERIALS AND METHODS

2.1. *Fibroblast cultures*

Human foreskin fibroblasts were explanted and grown according to routine techniques [11] and used between the 4th and 12th subcultures. The collagen and fibrin lattices were prepared as already described [9,12,13]. On the sixth day, the lattices had retracted up to a diameter of 1/10th the initial one.

2.2. *Protein synthesis studies*

Fifty mm diameter Petri dishes were seeded with 5×10^5 cells. The culture medium was removed after 5 days and replaced by a preincubation medium (DMEM solution containing 10% v/v dialyzed FCS) for 24 h. On day 6, this medium was replaced by the incubation medium (same medium containing in addition 10 μg/ml ascorbic acid, 74×10^3 Bq of [^{14}C]proline, diluted with 23 μg/ml of carrier proline). After 24 h incubation, the different fractions (supernatant, cells, lattices) were fractionated, intensively dialyzed and submitted to measurement of total radioactivity and collagenase-digestible radioactivity as previously described [9,13,14]. DNA content of the cells was evaluated by fluorometric assay [15].

2.3. *Study of the ribonucleic acids contained in cells*

For total RNA extraction, confluent monolayers or lattices were directly extracted in a 4 M guanidinium isothiocyanate/2.5 M sodium citrate buffer, pH 7, containing 0.5% sarcosyl and 0.1 M β-mercaptoethanol with turraxing for 20 s [16]. An equal volume of phenol/chloroform (10:2) was added. The mixture was stirred for 10 min, cooled on ice and centrifuged at $10{,}000 \times g$ for 20 min. The RNA contained in the aqueous phase was precipitated by addition of 2 vols. of ethanol and dissolved in water. Its purity and completedness were checked by measurement of A_{260nm}/A_{280nm} and by agarose gel electrophoresis.

Hybridization of dot-blot and Northern blots of the RNA samples were performed with a specific cDNA probe for pro-α_1(I)collagen mRNA [17]. Densitometric scans were performed with a Desaga (Heidelberg, Germany) CD 60 densitometer, and the results expressed according to a constant amount of mRNA specific for 36B4 that corresponds to an ubiquitously expressed gene [18].

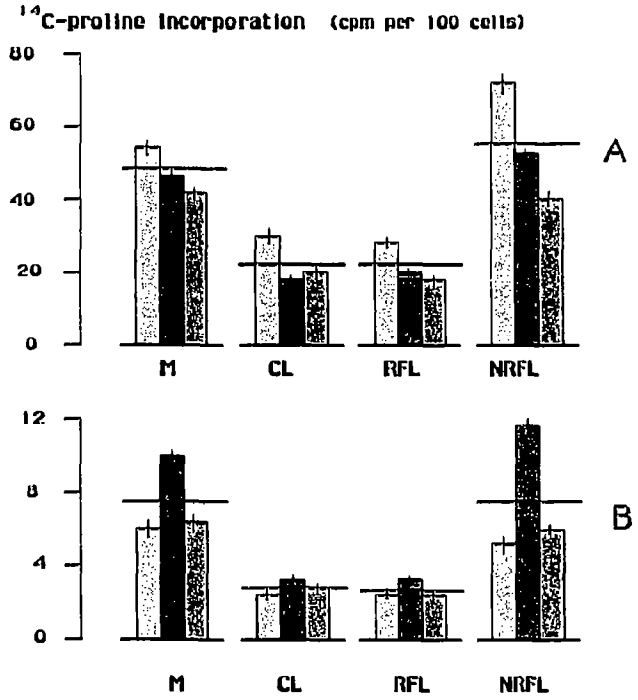

Fig. 1. Synthesis of total proteins (A) and collagen (B) by 3 different strains of skin fibroblasts cultivated in various systems. Every column corresponds to a different strain. The horizontal bars show the means of the 3 strains: M = monolayers; CL = collagen lattices; RFL = retracting fibrin lattices; NRFL = non-retracting fibrin lattices. Results represent the means of 4 determinations ± 1 S.E.M.

2.4. *Statistical calculations*

All the experiments were performed in quadruplicate and their significance evaluated by the Student's *t*-test.

3. RESULTS

The present work confirmed the marked differences in total protein and collagen syntheses between the several types of cultures studied, as shown in Fig. 1.

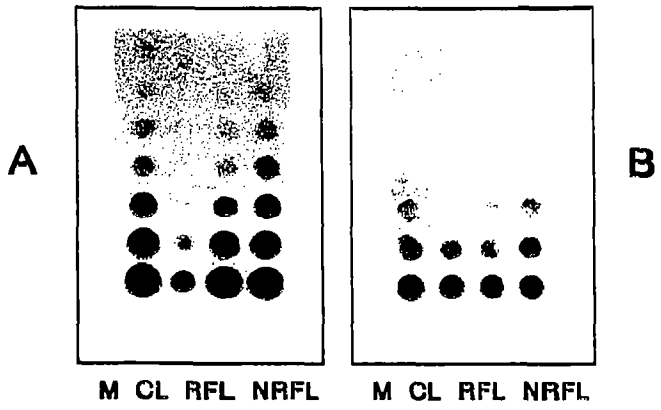

Fig. 2. Dot-blot analysis of pro-α_1(I)collagen and 36B4 mRNA extracted from various culture systems. Serial dilutions (10–0.15 μg) of total RNA extracted from monolayers (M), collagen lattices (CL), retracting fibrin lattices (RFL) and non-retracting fibrin lattices (NRFL) were deposited and hybridized with pro-α_1(I)collagen cDNA probe (A) and 36B4 cDNA probe (B).

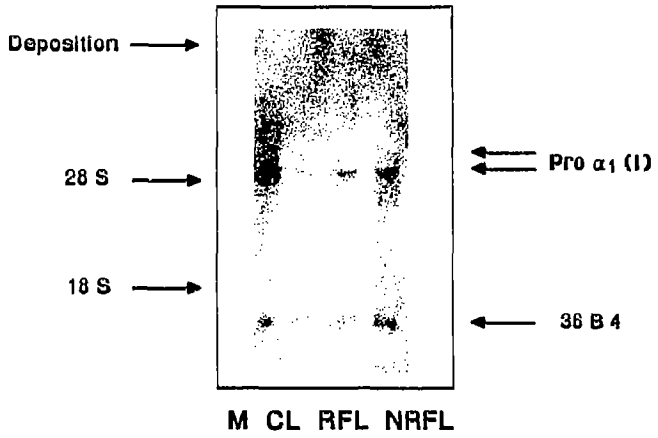

Fig. 3. Northern-blot analysis of pro-α_1(I)collagen and 36B4 mRNA extracted from various culture systems. Amount of 6.6 μg of total RNA extracted from monolayers (M), collagen lattices (CL), retracting fibrin lattices (RFL) and non-retracting fibrin lattices (NRFL) were deposited.

Inhibition evidently existed for the two types of retracting lattices in comparison to non-retracting lattices and monolayer confluent cells.

Dot-blot analysis showed that the amounts of pro-α_1(I)collagen messenger RNA expressed by fibroblasts in confluent cultures and in non-retracting lattices were practically identical (Fig. 2). The relative proportions on the basis of a constant expression of 36B4, were 100 for monolayer cells, 21 for collagen lattices, 75 for retracting fibrin lattices and 95 for non-retracting fibrin lattices. A Northern blot analysis of pro-α_1(I)collagen and 36B4 mRNAs is shown in Fig. 3.

4. DISCUSSION

The decrease of protein and collagen syntheses of fibroblasts embedded in retracting collagen lattices was already known [8,9] and the pretranslational step identified as the main stage of control [10] if not the only one.

In this paper, we demonstrate that fibrin lattices represent an environment somewhat less inhibitory than collagen. In a non-retracting fibrin lattice, type I procollagen mRNA is formed in amounts comparable to that of confluent monolayer cells. In retracting fibrin lattices, the amount of type I procollagen mRNA is intermediate between that of collagen retracting lattice culture and that of confluent culture.

However, cells grown in retracting lattices (RFL or CL) exhibit virtually identical levels of collagen production. On the other hand, no major difference in the types of collagen produced was previously noticed, either in collagen lattices [10] or in fibrin lattices [9]. This finding suggests that an additional post-transcriptional regulation of collagen production occurs.

These results indicate that the origin of the inhibition

of the pretranslational step of collagen synthesis in collagen lattice culture depends on two factors, the nature of the fibrils that surround cells and the degree of tension to which the cells are exposed. When the lattice is free to retract, the cells are exposed to little tension. On the other hand, when the lattice is prevented from retracting, stress fibres appear in the cells which become bipolar. These differences in shape are probably of prime importance for the induction of transcription of some genes such as those of collagen. In normal dermis also, there is no stress exerted on fibroblasts, and they are resting. When a wound affects the tissue, tensions appear, that trigger the translation of the collagen genes necessary for healing.

The use of fibrin lattices as culture substratum for fibroblasts provides a valuable model for investigating wound healing: it was shown that fibroblasts were able to infiltrate fibrin clots and to alter clot resistance to lysis by increasing collagen production [19,20]. Our results are in agreement with these data because they show that fibroblasts are stimulated to produce collagen when tractions are exerted on fibroblasts (like in the early steps of wound healing), thus participating in the organization of a newly synthesized extracellular matrix. The mechanisms of transmission of these stimulations from the molecules that constitute the fibres to the cells are presently under study.

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