Collagen Telopeptides (Cross-Linking Sites) Play a Role in Collagen Gel Lattice Contraction

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Solubilized interstitial collagens will form a fibrillar, gel-like lattice when brought to physiologic conditions. In the presence of human dermal fibroblasts the collagen lattice will contract. The rate of contraction can be determined by computer-assisted planimetry. The mechanisms involved in contraction are as yet unknown. Using this system it was found that the rate of contraction was markedly decreased when collagen lacking telopeptides was substituted for native collagen. Histidinohydroxylysinonorleucine (HHL) is a major stable trifunctional collagen cross-link in mature skin that involves a carboxyl terminal, telopeptide site 16^c, the sixteenth amino acid residue from the carboxy terminal of the telopeptide region of $\alpha_1(I)$ in type I collagen. Little, if any, HHL was present in native, purified, reconstituted, soluble collagen fibrils from 1% acetic acid-extracted 2-year-old bovine skin. In contrast, HHL cross-links were present (0.22 moles of cross-link per mole of collagen) in lattices of the same collagen contracted by fibroblasts. However, rat tail

tendon does not contain HHL cross-links, and collagen lattices made of rat tail tendon collagen are capable of contraction. This suggests that telopeptide sites, and not mature HHL cross-links per se, are essential for fibroblasts to contract collagen lattices. Beta-aminopropionitrile fumarate (BAPN), a potent lathyrogen that perturbs collagen cross-linking by inhibition of lysyl oxidase, also inhibited the rate of lattice cell contraction in lattices composed of native collagen. However, the concentrations of BAPN that were necessary to inhibit the contraction of collagen lattices also inhibited fibroblast growth suggestive of cellular toxicity. In accordance with other studies, we found no inhibition of the rate of lattice contraction when fibronectin-depleted serum was used. Electron microscopy of contracted gels revealed typical collagen fibers with a characteristic axial periodicity. The data provide evidence that collagen telopeptide sites play a role in collagen gel lattice contraction. *J Invest Dermatol* 97:580-585, 1991

The healing of cutaneous wounds is a complex process that includes platelet activation, the formation of a provisional matrix, inflammation, neovascularization, re-epithelialization, tissue remodeling, and scarring. One central element of healing is contraction of the wound, a process that probably involves an interaction between dermal fibroblasts and dermal interstitial collagens. In vivo, this interaction occurs in the context of the multiple processes of healing mentioned above, and it is difficult to explore this interaction using in vivo models. However, an in vitro system has been established by which the contraction of a collagen gel lattice by the presence of dermal fibroblasts can be studied [1]. When acid solubilized collagen is brought to physiologic salt and pH and warmed to 37°C, a solidified, gel-like mass of reconstituted fibrils is formed. When this procedure is performed in the presence of human, dermal fibroblasts, the collagen gel begins to contract from all sides into a resultant round or oval button that is a fraction of the original size of the

gel [1-5]. This phenomenon depends upon several variables, such as the passage number and number of the cells [1], the presence of serum [1,2,5-7], and the concentration of the collagen [1]. For example, increasing the number of cells in the system or decreasing the concentration of the soluble collagen will increase the contraction rate. It also is known that fibroblasts within a collagen gel lattice do not proliferate and that cell proliferation is not necessary for contraction to occur [8]. In fact, fibroblasts in the presence of cytosine arabinonucleoside (Ara-C), which blocks DNA synthesis, contract collagen lattices as well as those without the addition of this inhibitor [1]. The addition of cytochalasin B, which blocks microfilament function [1], the presence of certain mediators of inflammation [9], heparin [10], or corticosteroids [11] will inhibit collagen lattice contraction. Guidry and Grinnell [6] have shown that when the synthesis and secretion of fibroblast proteins are blocked that the organization of collagen lattices by fibroblasts is inhibited.

Although the rates of contraction can be altered by such variables, the actual mechanism by which collagen gel lattices contract is unknown. Most studies have been done using native collagen [1-3,5,7,8,11-14]. However, a few studies have also been performed with commercially available bovine skin collagen (Vitrogen, Collagen Corporation of America, Palo Alto, California) [4,6,10] and pepsinized human collagen obtained from hypertrophic scars [9,15]. While reviewing these studies, it was noted that in studies in which pepsinized collagen was used, the level and rate of lattice contraction was less than those studies in which native collagen was used. In a paper in which calf skin collagen was used, the authors stated "Any pepsinized collagen should be avoided since cleavage of the telopeptides results in lack of contraction of the dermal equivalent

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Abbreviations:

BAPN: beta-aminopropionitrile fumarate

DMEM: Dulbecco's modified Eagle's medium

HHL: histidinohydroxylysinonorleucine

lent" [14]. Although it has been suggested that pepsinized collagen does not contract well, no data were presented to support this notion. Delvoye et al [16] showed that dermatosparactic fibroblasts do not have the capacity to contract collagen lattices containing pepsinized type I collagen. To our knowledge, a systematic examination of the role of telopeptides in the mechanism of collagen lattice contraction has not been done before. The purpose of the experiments reported here was to examine the influence of collagen telopeptide ends on collagen gel lattice contraction.

METHODS

Collagen Lattice Contraction Assay A modification of the collagen gel lattice contraction assay previously described by Bell et al [1] was established. In our assay, we used three different types of collagen (see below) solubilized in 0.0125–0.036 N HCl at a concentration of 2 mg per ml. Two milliliters of solubilized collagen were placed in a 60-mm petri dish in addition to 60 μ l of 0.4 M NaOH, 800 μ l of 3 $\times$ concentrated Dulbecco's modified Eagle's medium (DMEM) containing 4500 mg of D-glucose per liter, 400 μ l of fetal calf serum, and 250,000 or 500,000 human dermal fibroblasts suspended in 1 $\times$ DMEM-H. These items were kept separate on the bottom of the dish until the beginning of the assay, at which time the dish was swirled and the additions mixed. Under these conditions the mixture maintained a neutral pH, which is critical for consistent contraction rates. In selected experiments, assays were performed in the presence of beta-aminopropionitrile fumarate (BAPN), a potent lathyrogen, and with fibroblasts that had been exposed to the lathyrogen for 48 h prior to the assay (vide infra). The dishes were placed in a water-jacketed, humidified incubator containing 5% CO₂ at 37°C. Contraction of the lattice gel was assessed at 2, 4, 6, 8, 12, 24, and in some experiments, at 48 h, by the use of a computer-assisted planimetry program that measured the area [17] of the contracted lattice gel in the dish after it was traced on a digitizing tablet.

In order to assess cell viability within the collagen lattices, fibroblasts were isolated from fully contracted lattices by the method of Nakagawa et al [18]. Briefly, contracted lattices were floated in 0.05% trypsin, 0.53 mM ethylenediamine tetraacetate (EDTA) (Gibco-BRL, Grand Island, New York) for 10 min at 37°C. Collagenase (type I, Sigma, St. Louis, Missouri) was dissolved in 130 mM NaCl, 20 mM HEPES buffer, 10 mM calcium acetate at a concentration of 3 mg per ml (Collagenase Solution). For each 1 ml of trypsin-EDTA solution containing the contracted lattice, 300 μ l of collagenase solution was added and the mixture incubated for 45–60 min at 37°C. After the collagen lattice had dissolved, the reaction was terminated by the addition of 50 μ l of fetal calf serum per 1.3 ml of solution. Isolated cells were pelleted by centrifugation at 1000 rpm for 10 min, washed, and resuspended in DMEM containing 20 mM HEPES buffer, pH 7.2. An aliquot of the isolated cells were suspended and mixed 1:1 with trypan blue and mounted on a slide and covered with a coverslip. One hundred or more cells were counted from the cell suspension isolated from each lattice. Viability was determined by the percentage of cells counted that excluded the trypan blue dye and appeared white. From each suspension of cells, 10,000 cells were placed into duplicate 60-mm dishes and cultured in DMEM with 10% fetal calf serum. The cultured cells were counted after trypsinization and suspension at days 5 and 10 after the initiation of the culture.

Cell Cultures Human dermal fibroblasts derived from neonatal foreskin or surgical specimens were cultured in 1 $\times$ DMEM-H + 10% fetal calf serum in 100-mm petri dishes in a water jacketed humidified incubator containing 5% CO₂ at 37°C. The cells were maintained by changing the culture medium twice a week until the cells became confluent. In certain experiments, confluent dishes of cells were treated with 25 or 100 μ g per ml of BAPN for 48 h prior to use in the contraction assay (vide supra).

Collagen Preparations Three sources of collagen were used. Native bovine type I collagen was extracted with dilute acetic acid (1%) from two-year-old bovine skin and purified by sequential salt

precipitation according to Miller [19]. In certain experiments, the native bovine skin type I collagen was equilibrated in 0.5% acetic acid precooled to 4°C and then half of the sample was incubated with 5 or 10% (w/w) pepsin (Worthington Biochemical, Freehold, New Jersey) overnight with gentle stirring. In the morning, the preparation was dialyzed against 0.5% acetic acid precooled to 4°C and neutralized in the contraction assay. This procedure provided native and pepsinized collagen from an identical source. In other experiments, partially pepsinized bovine skin collagen was used (Vitrogen, Collagen Corporation of America, Palo Alto, California) or native rat tail tendon collagen (Collaborative Research, Bedford, Massachusetts). In some experiments, commercially available collagens were treated as above with the pepsinization procedure. All of the collagen preparations before and after pepsinization were subjected to electrophoresis on 5% or 7.5% polyacrylamide slab gels in 0.1% sodium dodecyl sulfate that were stained for protein with Coomassie blue [20].

Cross-Link Analysis Collagen molecules *in vivo* are known to form intermolecular cross-links. Recently, Yamauchi et al [21] have identified a non-helical trifunctional cross-link in skin type I collagen that is formed between hydroxylysine on one alpha 1 chain at amino acid residue 87, lysine aldehyde on another alpha 1 chain at residue 16^c (the sixteenth amino acid residue from the carboxyl terminus of the telopeptide region) and histidine on an alpha 2 chain at position 92. This collagen cross-link has been formally termed histidinohydroxylysinonorleucine (HHL) and is the major collagen cross-link in mature and aged skin [21,22]. In order to assess the possible *in vitro* formation of HHL cross-links in contracted collagen lattices (native type I as well as pepsin treated type I), lattices in various states of contraction were removed from the petri dishes, washed with cold phosphate buffered saline, and frozen at –70°C. The gels were then cut into small pieces, washed with 0.015 M TES buffer (pH 7.4) 4–5 times, then with distilled water, and lyophilized. Aliquots of the dried materials (2–3 mg dry weight) were hydrolyzed with constant boiling 6 N HCl under N₂ for 24 h at 115°C. The hydrolysates were then evaporated and the residues were dissolved in 1 ml of distilled water and filtered. Contents of hydroxyproline and HHL in each hydrolysate were determined by the method described previously [21,22].

Electron Microscopy In order to determine if collagen molecules *in vitro* would form fibrillar structures, contracted collagen lattices were removed from petri dishes, washed extensively with phosphate-buffered saline and fixed in half-strength Karnovsky's glutaraldehyde fixative at 4°C overnight, post-fixed in osmium tetroxide, embedded in epoxy resin, and stained with uranyl acetate and lead citrate. The specimens were viewed and photographed with a JEM 100B electron microscope.

RESULTS

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the collagen preparations revealed characteristic gamma, beta, and alpha chains. Figure 1 is a photograph of a Coomassie blue stained, 5% polyacrylamide SDS-slab gel of native (lanes 2 and 4) or pepsinized (lane 3) bovine skin type I collagen. The samples were electrophoresed under reducing conditions in the presence of 0.1 M dithiothreitol. It is noteworthy that the pepsinized collagen alpha chains are 1000–2000 daltons smaller than the native collagen alpha chains. In addition, a dimer of approximately 240 kDa is seen in the native collagen preparation that is missing after pepsinization.

In the collagen lattice contraction assay, native collagen contracted at a faster rate and to a greater degree than pepsinized collagen at each time point evaluated. Figure 2 is a 24-h contraction assay containing 500,000 fibroblasts and 4 mg of native or pepsinized bovine skin type I collagen. Each time point represents the mean area derived from identical triplicate dishes. When native collagen is used, lattice contraction is evident by 4 h. At 12 h, the lattice has contracted to 69% of the original area of the petri dish. In contrast, when the same collagen is pepsinized and used in the assay, lattice

Figure 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of native calf skin collagen (lanes 2 and 4) or pepsinized calf skin collagen (lane 3). Lane 1 contains globular high-molecular-weight standards. The 5% gel is stained with Coomassie brilliant blue. Note that the $\alpha_1(I)$ and $\alpha_2(I)$ chains of the pepsinized collagen migrate slightly lower than those of native collagen. (NC, native collagen; PC, pepsinized collagen; MS, molecular weight standard.)

contraction is not evident at all by 12 h, and by 24 h only minimal contraction (18%) is seen.

Vitrogen is commercially available bovine skin collagen that is partially pepsinized. Figure 3 is a 48-h contraction assay in which vitrogen collagen untreated or after pepsinization (5% and 10% pepsin w/w) was substituted for native bovine skin collagen. At 24 h, untreated vitrogen collagen contracted 35% of the original area, whereas pepsinized vitrogen collagen had not contracted. At 48 h, untreated vitrogen collagen contracted 54% of the original area, whereas the pepsinized vitrogen contracted substantially less. The onset of lattice contraction was apparent after 5 h of culture in experiments in which non-pepsinized vitrogen was used. In contrast, even as late as 29 h in culture no lattice contraction was evident in experiments in which pepsinized vitrogen was used. (Fig 3).

BAPN is a potent lathyrogen that when fed to animals causes bone and joint deformations and aortic aneurysms due to its prop-

Figure 2. A collagen gel lattice contraction assay comparing native calf skin collagen (▲) with pepsinized calf skin collagen (Δ) from the same donor. Point, identical triplicate petri dishes in which the areas of the contracted lattices are measured by computer-assisted planimetry, the mean of the three areas calculated and the SE of the mean determined.

Figure 3. A collagen gel lattice contraction assay with unaltered Vitrogen calf collagen (Δ) or vitrogen calf collagen that has been treated with 5% (○) or 10% (□) pepsin. The onset of lattice contraction was evident at 5 h in experiments in which the lattices were composed of non-pepsinized vitrogen, whereas no contraction was evident after 29 h of culture in experiments with pepsinized vitrogen. In the latter situation, only minimal contraction occurred even after 48 h.

erty of perturbing collagen cross-links via inhibition of the enzyme lysyl oxidase [23]. In order to assess the effect of BAPN on collagen lattice contraction, daughter dishes of fibroblasts were cultured for 48 h in the presence of 25 or 100 $\mu\text{g}/\text{ml}$ of BAPN. Figure 4 is a contraction assay in which identical numbers (250,000) of untreated or BAPN-treated human dermal fibroblasts were added to triplicate dishes in addition to the presence of BAPN and 4 mg of native collagen. In the presence of BAPN, lattice contraction was both delayed and decreased. In experiments without BAPN, significant lattice contraction occurred after 4 h of culture. In the presence of BAPN, lattice contraction was not apparent after 16 h in culture.

Figure 4. A lattice contraction assay using native, calf skin collagen with or without the presence of the lathyrogen, beta-aminopropionitrile (BAPN). In experiments without BAPN, significant lattice contraction occurred after 4 h of culture. In the presence of BAPN no contraction of the lattices was apparent after 16 h of culture. After 24 h, significant lattice contraction was evident in dishes containing 25 $\mu\text{g}/\text{ml}$ of BAPN but not in those containing 100 $\mu\text{g}/\text{ml}$. At the same time point, the lattices had contracted over 50% in dishes without BAPN. After 48 h of culture, the lattices in dishes without BAPN had contracted over 90%, whereas those with BAPN had contracted approximately 50%.

Table I. Viability of Fibroblasts in Lattices After Contraction

	Experiment 1 (%)	Experiment 2 (%)
I. Trypan blue exclusion		
No additive	94	96
Pepsin	90	92
BAPN	88	90
	5 d	10 d
II. Cell Number in Culture After Release from Lattice		
No additive/dish 1	1.2×10^6	3.0×10^6
No additive/dish 2	1.4×10^6	
Pepsin/dish 1	1.6×10^6	3.0×10^6
Pepsin/dish 2	1.1×10^6	
BAPN/dish 1	8.4×10^5	3.2×10^6
BAPN/dish 2	9.5×10^5	
Fibroblasts/dish 1	1.5×10^6	3.3×10^6
Fibroblasts/dish 2	1.5×10^6	

At 24 h, the control lattices contracted 58% of the original area, whereas lattices in the presence of 25 $\mu\text{g/ml}$ of BAPN only contracted 12% and those in the presence of 100 $\mu\text{g/ml}$ of BAPN had not contracted. At 48 h, there is little difference between the lattices in the presence of BAPN, which have contracted to 53% and 49%, respectively, whereas the control lattice contracted to 92% of the original area.

In order to assess the affect of the experimental conditions upon the cells within the contracting lattices, fibroblasts were isolated from contracted lattices [18] and their viability assessed by trypan blue exclusion. As shown in Table I, approximately 90% of the fibroblasts isolated from contracted lattices were viable regardless of the experimental condition. There were small differences between the viability of cells isolated from lattices made from pepsinized and unpepsinized collagen. The fibroblasts isolated from contracted lattices in experiments performed in the presence of BAPN demonstrated a small decrease in viability compared with the viability of cells from assays without BAPN (Table I).

In order to examine the growth potential of the fibroblasts isolated from contracted lattices, equal number of cells were placed into culture, and the cells were counted after 5 and 10 d. As shown in part II of Table I, human fibroblasts (from the same donor) that had not experienced the contraction assay but were simply cultured under the same conditions as the cells isolated from lattices expanded from 1.5×10^6 cells at day 5 to 3.3×10^6 cells at day 10 in culture. The rate of cell expansion in fibroblasts isolated from contracted lattices composed of unpepsinized or pepsinized collagen was very similar to that generated by fibroblasts that had been kept in routine culture. Fibroblasts isolated from contracted lattices in assays with BAPN generated fewer cells in the dishes after 5 d of culture compared with routinely cultured fibroblasts or those isolated from contracted gels in assays without BAPN (approximately 9×10^5 versus 1.2×10^6). However, after 10 d of culture the fibroblasts derived from experiments with BAPN had expanded to approximately the same level as the fibroblasts derived from other conditions (Table I).

Minoxidil has been used for hypertension and to induce hair growth in humans and monkeys [24]. Recently, minoxidil at a 1 mM concentration has been shown to inhibit fibroblast proliferation as well as the enzyme lysyl hydroxylase which is necessary for the hydroxylation of lysine [25]. In order to assess the effect of minoxidil on collagen lattice contraction, assays were performed with either native bovine collagen or vitrogen with or without the presence of 50, 100, 200, or 400 $\mu\text{g/ml}$ of minoxidil [25]. At concentrations that did not inhibit fibroblast proliferation in culture (50 and 100 $\mu\text{g/ml}$), minoxidil had minimal effect upon the rate of lattice contraction compared with controls. At concentrations of minoxidil above 200 $\mu\text{g/ml}$, the rate and degree of lattice contrac-

tion was reduced by approximately 30% compared with controls (data not shown). However, parallel fibroblast culture experiments performed with and without minoxidil demonstrated that minoxidil concentrations above 200 $\mu\text{g/ml}$ dramatically inhibited cell growth. Therefore, the reduced rate of lattice contraction in the presence of minoxidil at concentrations of 200 $\mu\text{g/ml}$ or greater was thought to be due to a toxic effect of the drug upon the cells rather than a direct effect upon the collagen lattices.

Other maneuvers were attempted to see if the rate or degree of collagen lattice contraction could be altered. We depleted fibronectin from the fetal calf serum used in our culture medium with a gelatin column and then used medium containing fibronectin depleted serum for several days in our fibroblast cultures as well as in the assay. No observable change in collagen lattice contraction was observed when assays were performed using fibronectin depleted serum (data not shown). Phorbol esters are known to have profound effects upon cell shape, collagen synthesis, and collagenase expression. Although we performed our contraction assay in the presence of 1, 2, 5, 10, or 20 ng/ml of 12-O-tetradecanoyl-phorbol 13-acetate, there were minimal changes in the rate or degree of lattice contraction compared with control lattices (data not shown). Collagen gel lattices without cells did not contract at all, and those incubated with human keratinocytes rather than fibroblasts contracted minimally.

HHL cross-links were found to be present in contracted gel lattices composed of native skin collagen and increased with the degree of contraction. Prior to the onset of the contraction assay, HHL content was about 0.06 moles/mole of collagen in both the native and pepsinized collagen. At 24 h, when the native collagen lattice had contracted 80% of the initial area and the pepsinized collagen lattice only 16%, the HHL content was 0.22 moles/mole of collagen in the native lattice compared with about 0.06 in the pepsinized collagen lattice. In well-contracted lattices (80%) composed of rat tail tendon collagen, the HHL content was below the level of detection.

Electron microscopy of contracted gels revealed collagen fibrils with characteristic axial periodicity (Fig 5) that is due to fibril formation in the typical stagger arrangement. Collagen fibers, collagen fibrils, cells, and dense amorphous material were present in the contracted lattices, but these elements were surprisingly sparse considering the firm tissue-like quality of the contracted gels.

DISCUSSION

The mechanisms by which collagen gel lattices contract in the presence of human skin dermal fibroblasts are not known. The number of cells, the passage number of the cultured cells, the presence of serum, and the concentration of collagen all influence the rate and amount of contraction [1]. Fibroblasts within collagen gel lattices do not proliferate, but the addition of cytochalasin B or

Figure 5. An electron micrograph of a contracted lattice gel at 24 h composed of native calf skin collagen. Small arrows point to the 640-nm cross banding that is characteristic of collagen fibers.

colchicine will inhibit contraction [1,8]. The experiments reported here show that when all other conditions are held constant, contraction can be profoundly influenced depending upon whether the collagen is in a native or pepsinized state. Pepsinization removes the small non-triple helical telopeptide portions of the collagen molecule. These telopeptide domains are involved in the collagen cross-linking process. Contracted collagen gel lattices composed of reconstituted soluble native collagen fibrils had detectable amounts of HHL cross-link and the amount of the cross-link increased with contraction. Moreover, when the native collagen was treated with pepsin and used to form the lattices, the level of HHL cross-link remained very low. BAPN, an inhibitor of lysyl oxidase, and minoxidil, an inhibitor of lysyl hydroxylase, are both compounds that are capable of inhibiting the formation of collagen cross-links. We found that collagen lattice contraction was decreased in the presence of these two agents. However, in order to appreciate a significant inhibition of lattice contraction, it was necessary to use concentrations of BAPN and minoxidil that inhibited the growth of fibroblasts in culture and suggested that these agents are somewhat cytotoxic to the cells. Given this effect upon the fibroblasts in the system, we cannot conclude that these agents inhibit collagen lattice contraction purely by a perturbation of the formation of collagen cross-links. Although it has been shown that fibroblast proliferation does not occur to any significant degree within collagen lattices that readily contract [6,26,27], it is possible that BAPN and minoxidil are inhibiting the contraction of the collagen lattices in our system by their inhibitory effects upon the fibroblasts within the lattices.

Taken together, it appears that telopeptides have a role in the mechanism by which collagen gel lattices contract. It may be that human dermal fibroblasts attach and pull on the collagen cables in the lattices at or near the telopeptide sites on the collagen molecule. Although HHL cross-links were increased in contracted lattices composed of native skin collagen compared with those composed of pepsinized skin collagen, the HHL cross-link does not appear to be a necessary requirement for contraction to occur because native rat tail tendon collagen, which does not contain HHL cross-links, can be used to form well-contracted (60–80% at 24 h) lattices. However, other types of cross-links that are also dependent upon telopeptide sites may be necessary for lattice contraction. Nevertheless, the lack of HHL cross-links in the lattices composed of pepsinized collagen (but not native collagen) and the higher R_f migration of the pepsinized collagen alpha chains on SDS-polyacrylamide gels provide evidence that pepsinization removed telopeptides.

Although pepsinized collagen contracts much slower than native collagen, the poor contraction capability is not absolute because at late time points some contraction of the lattices does occur. Although cell division is markedly depressed within the lattices, it has been shown that the fibroblasts are metabolizing and synthesizing a variety of proteins including collagen [6,26]. Collagen synthesis by fibroblasts in lattices, however, is only about 5% of the values in monolayer culture [26]. Nevertheless, it is possible that the synthesis of collagen and perhaps other proteins by the fibroblasts cultured in collagen lattices may contribute in some unknown manner to the modest contraction observed in late cultures. Collagen fibrils are a population of collagen molecules and perhaps newly synthesized collagens by the fibroblasts in culture are capable of interacting with exogenous collagens forming the lattice in such a manner that allows for contraction to occur at a minimal level. It may be that our pepsinized collagen preparations contained at least some unpepsinized or partially pepsinized collagen molecules that would be available for some contraction to occur.

Fibroblasts are known to attach to type I collagen, and this attachment may be mediated by fibronectin because this molecule has a 40,000-dalton collagen-binding domain near the amino terminus and is on the surface of fibroblasts. The collagen-binding domain of fibronectin is known to interact with the $\alpha_1(I)$ chain of type I collagen at a site near the collagenase cleavage site between residues 775 and 776 [28,29]. Although the use of fibronectin-depleted serum did not influence lattice contraction, we cannot definitively say that fibronectin does not play a role because the fibroblasts

themselves are capable of rapidly synthesizing cell-surface fibronectin in culture. Our results, however, are in accordance with those of Guidry and Grinnell [6] who also found no alteration in lattice contraction when fibronectin was excluded from the assay and with other studies [7,10] in which fibronectin added to the system had no effect upon collagen lattice contraction. It is also known that fibroblasts have an integrin receptor that allows the cells to bind directly to type I collagen without mediation through fibronectin by recognizing a peptide sequence of arginine, glycine, aspartic acid, and serine (RGDS). However, the RGDS sequence is not present in the telopeptides at the amino or carboxy termini of either $\alpha_1(I)$, or $\alpha_2(I)$ chains. Regardless of the mechanism or mechanisms by which fibroblasts bind to collagen in the gel lattice, the data reported here suggest that telopeptide sites within the collagen molecule play a role in the fibroblast-collagen interaction that influences the rate of collagen lattice contraction.

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