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EXPRESSION OF FIBRONECTIN AND INTERSTITIAL COLLAGEN GENES IN SMOOTH MUSCLE CELLS: MODULATION BY LOW MOLECULAR WEIGHT HEPARIN FRAGMENTS AND SERUM*

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Abstract—The effect of low M_r heparin fragments (CY222) and fetal calf serum (FCS) on the level of fibronectin and fibrillar collagen mRNAs was investigated in smooth muscle cells (SMC) in culture. In the absence of FCS, addition of CY222 (100 $\mu g/10^6$ cells) to postconfluent early passage SMC resulted in a decrease in mRNA level of type III collagen. In contrast, mRNA levels coding for type I collagen, fibronectin and GAPDH (used as control of cellular activity) were not modified. Addition of 5% FCS (without CY222) to the culture medium did not affect mRNA levels of type I and type III collagens nor that of GAPDH. The level of fibronectin mRNA, however, increased in the presence of 5% FCS. In the presence of both 5% FCS and CY222, we observed a decrease in type III collagen mRNA and fibronectin mRNA levels (this level remained, however, above the control value without FCS and the level with CY222 alone). Our results demonstrate that low M_r heparin fragments can modulate the steady-state levels of type III collagen and fibronectin mRNAs.

Key words: fibronectin; collagens; biosynthesis; gene expression; messenger level; low molecular weight heparin fragments; modulation of phenotype; smooth muscle cells

Fibronectin, type I collagen, and type III collagen genes code for the three major proteins of the ECM§ [1, 2]. The regulation of the genes coding for ECM is an important part of many cellular functions such as adhesion, proliferation, migration and differentiation. Quantitative and qualitative modifications in ECM gene expression vary as a function of cell type, age and related pathological processes [3, 4]. SMC, the main cell type of the media of arteries, play an important role in the atherosclerotic process. They change from a contractile to a migratory and synthetic phenotype [5] and produce large quantities of ECM components, among them collagens and fibronectin, and decreased quantities of contractile proteins [6, 7]. Heparin and related compounds can inhibit cellular proliferation [8–12] by maintaining cells in the G1 phase [13]. Heparin can also modulate the biosynthetic phenotype of SMC, particularly the biosynthesis of fibrous collagens [14]. We were able to show that low M_r heparin fragments (CY222) [15], administered *in vivo* to diabetic mice modulated skin type III collagen and fibronectin biosyntheses [16]. Recently, we demonstrated that the biosynthetic phenotype of SMC could be modulated *in vitro* by CY222 [17]. These results illustrate the complex interactions between cells and ECM in the control of many cellular functions. In this study, we report that gene expression is modified differently in the presence of low M_r heparin fragments and/or in the presence of serum. Furthermore, serum components could interfere with heparin and related compounds such as CY222 [18]. We therefore studied the effect of CY222 with and without FCS in the culture medium. The results of these studies are described below.

MATERIALS AND METHODS

Cell culture. Pig aorta SMC were grown from media explants in Dulbecco's modified Eagle's medium supplemented with 20% FCS, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mM glutamine. At confluency, cells were dissociated with 0.5% trypsin in PBS. Cells were serially passaged with 10% FCS up to the third subculture, where they were used at overconfluency for RNA extraction. Cells were incubated at 37° in a 95% air, 5% CO_2 atmosphere. The day before the experiments, the culture medium was discarded, and the cells were incubated in the presence of

* This paper is dedicated to the memory of Dr Jean Choay who initiated the study of heparin fragments prepared in his laboratory.

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§ Abbreviations: DMEM, Dulbecco's modified Eagle's medium; SMC, smooth muscle cells; ECM, extracellular matrix; FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; DEPC, diethylpyrocarbonate; MOPS, 3-(*N*-morpholino)-propanesulfonic acid.

Table 1. Comparison of GAPDH, proα1(I) collagen, proα1(III) and fibronectin mRNA levels by SMC

Probes	Control	CY222	Control + 5% FCS	CY222 + 5% FCS
GAPDH	13,216 ± 5221	11,169 ± 3007	15,474 ± 4124	16,417 ± 5064
Coll. III/GAPDH	1.31 ± 0.12	0.84† ± 0.09	1.29 ± 0.03	0.62‡ ± 0.02
Coll. I/GAPDH	1.83 ± 0.14	2.02 ± 0.16	1.89 ± 0.08	2.10 ± 0.07
FN/GAPDH	0.57 ± 0.03	0.49 ± 0.06	0.92* ± 0.04	0.73§ ± 0.08

Control, with 5% FCS, or with 100 mg/mL of CY222, with or without 5% FCS. Relative amounts of the specific mRNA were quantified by densitometric analysis of slot-blots. The values represent the mean ± SD of eight independent determinations.

* Control 5% FCS vs control (DMEM alone), $P < 0.001$.

† CY222 vs control without CY222, $P < 0.001$.

‡ CY222 with 5% FCS vs control with 5% FCS, $P < 0.001$.

§ CY222 with 5% FCS vs CY222 alone, $P < 0.001$.

100 μg/10^6 cells heparin fragments CY222 (Institut Choay/Sanofi Recherches, Paris, France) with or without 5% FCS for 24 hr. These CY222 fragments (1500–8000 M_r) were obtained by chemical depolymerization of porcine mucosal heparin with HNO_2 and gel filtration as described by Barzu *et al.* [15]. They correspond to a mixture of hexamers and octamers.

RNA analysis. Total ribonucleic acids were extracted from confluent cultures washed free of media with 4 M guanidium isothiocyanate, according to the method of Chomczinsky and Sacchi [19]. Briefly, SMC were rapidly homogenized in a 4 M guanidium isothiocyanate, 25 mM sodium citrate, pH 7, 0.5% Sarcosyl, 0.1 M 2-mercaptoethanol buffer. Sequentially, 2 M sodium acetate, pH 4, phenol (water-saturated) and chloroform-isoamyl alcohol mixture (49:1) were added. RNAs were precipitated from the aqueous phase by isopropanol, and left overnight at −20°. After collection by centrifugation, pellets were rinsed with 1 mL of 75% ethanol and dissolved in 5 mM EDTA, pH 7.0, treated with 0.1% DEPC. Approximately 25 μg of total RNAs was extracted from 10^6 cells by this procedure.

For quantitative assessment, aliquots of 1, 1.5 and 2 μg of total RNA were heat-denatured at 65° for 15 min in a 50% formamide, 6% formaldehyde, 0.2 M MOPS, 0.05 M sodium acetate, pH 7.0, and 0.01 M EDTA buffer. Total RNA was then transferred to Nylon filters (Hybond N^+, Amersham, France), using a commercial "slot-blot" apparatus (Minifold II, Schleicher & Schull, Dassel, Germany). RNA was fixed to the filter by alkali treatment (5 min with 0.05 M NaOH, pH 5) and then washed with 2 × SSC (1×: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0).

DNA probes. The following cDNA probes were used: pHCAL1U specific for human proα1(I) collagen and pHFS3 specific for human proα1(III) collagen, provided by Vuorio and Mäkelä [20, 21]; a clone of rat fibronectin from the exon encoding the first extra type III region, given by R. O. Hynes and described in Paul *et al.* [22]; and the GAPDH (glyceraldehyde-3-phosphate-dehydrogenase) probe described by Fort *et al.* [23].

The specificity of the probes was tested by Northern blot analysis [20, 23]. Briefly, total RNA was separated by electrophoresis in a 1% agarose-formaldehyde gel. Resulting Northern blots were probed for α1(I) and α1(III) collagens and fibronectin with cDNA probes labelled as described below.

Slot-blot analysis. Prehybridization was carried out at 42° for 24 hr in a solution (A) containing 50% (v/v) formamide, 5× SSPE (1 × SSPE: 180 mM NaCl, 10 mM Na_2HPO_4, 1 mM EDTA, pH 7.4), 0.5% (w/v) SDS, 0.1% each of Ficoll, polyvinylpyrrolidone, and BSA and 200 μg/mL of heat-denatured sonicated salmon DNA. Hybridization was carried out in fresh buffer A, containing 3–5 × 10^8 cpm/mg of the appropriate ^{32}P-labelled probe for 24 hr at 42°. Labelling was performed using a nick translation kit (Bœhringer Mannheim, Germany) with [α-^{32}P]dCTP (111 TBq/mmol, Amersham, France). Labelled probes were purified by chromatography through an NACS Prepac column (Bethesda Research Labs, Bethesda, France). The filters were washed at 42° with 2 × SSPE, 1 × SSPE, 0.1 × SSPE, sequentially with 0.1% SDS. The dried filters were exposed to X-ray films at −80°. The autoradiograms were quantified by densitometric scanning with a laser densitometer (Ultrascan 2202 LKB, Integrator 2220 LKB).

Statistical analyses were performed using the Student's *t*-test.

RESULTS

Specificity of the probes was demonstrated by Northern blot analysis (Fig. 1). For α(I) mRNA and α1(III) mRNAs, the two spots arise from usage of alternative polyadenylation signals [20, 21].

Linearity of densitometric analysis from slot-blots

When specific mRNAs were quantified by densitometric analysis we obtain a linear relationship between the amounts of mRNA applied to the filter for hybridization. To illustrate this, Fig. 2 presents the calibration curve based upon the integrated intensity from autoradiograms of GAPDH mRNAs with a correlation coefficient $r = 0.97$, $P < 0.01$.

Effect of heparin fragment CY222 on extracellular matrix and GAPDH mRNAs levels

The aortic smooth muscle cells at postconfluence

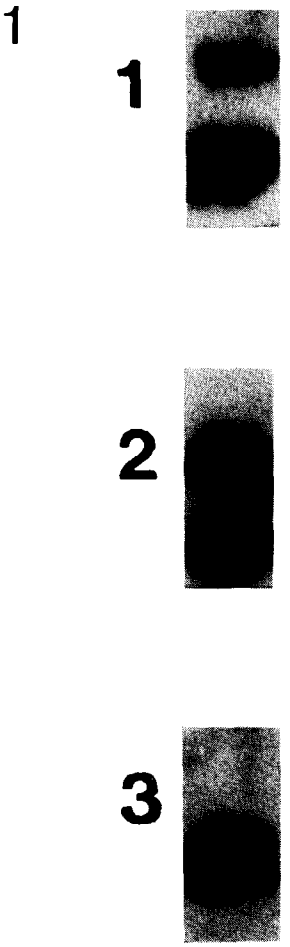

Fig. 1. Northern blot analysis of collagen and fibronectin mRNAs expressed in smooth muscle cells in culture. Total RNA was isolated from cultures of SMC and samples (10 μg/lane) were separated by electrophoresis in 1% agarose-formaldehyde gels. Resulting Northern blots were probed for α1(I) collagen mRNA (1), α1(III) collagen mRNA (2) and fibronectin mRNA (3). The two α(I) mRNA species as well as the two α1(III) mRNA species arise from usage of alternative polyadenylation signals [20, 21].

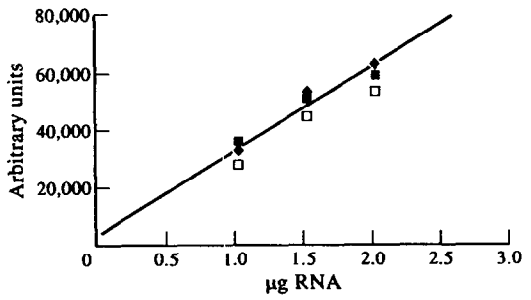

Fig. 2. Calibration curve based upon the integrated intensity from the autoradiogram of GAPDH RNA. Abscissa: amounts of total RNA applied to filters for hybridization with the GAPDH probe; ordinates: integrated autoradiography intensity in arbitrary units. Correlation coefficient $r = 0.97$.

incubated with 100 μg CY222/10^6 cells showed markedly reduced levels of proα1(III) mRNA, compared with cultures incubated with DMEM alone. In contrast, cultures incubated without heparin fragments (Fig. 3A) and those treated with CY222 alone (Fig. 3B) showed comparable levels of proα1(I) mRNAs. Likewise, the level of GAPDH mRNA remained relatively constant (Table 1).

These results are in agreement with the biosynthetic measurements previously obtained [16] which show that the addition of CY222 leads to inhibition of collagen III biosynthesis without change in collagen type I and total protein biosyntheses.

Effect of DMEM supplemented with 5% FCS or with a combination of CY222 and 5% FCS on extracellular matrix and GAPDH mRNAs levels

In these experiments, SMC were incubated in DMEM supplemented with 5% FCS. The levels of fibronectin mRNAs appeared to be increased by the presence of FCS, when compared with the levels seen with DMEM alone. Nevertheless, the addition of 5% FCS did not significantly affect GAPDH, proα1(I) collagen or proα1(III) collagen mRNA levels (Fig. 4).

The combination of 5% FCS and heparin fragments CY222 significantly down-regulated the mRNA levels of fibronectin and proα1(III) collagen without affecting GAPDH and proα1(I) collagen mRNA levels (Table 1), as compared with SMC incubated with DMEM supplemented with 5% FCS. The action of CY222 appeared to be greater in the presence of serum, since steady-state levels of collagen type III mRNA were decreased by 36% in the presence of CY222 alone. In the presence of CY222 and 5% FCS this decrease reached 52% ($P < 0.001$). Likewise, CY222 alone produced a slight decrease in fibronectin mRNA (14%, not significant). When SMC were incubated with a combination of CY222 and FCS, the mRNA fibronectin level was depressed by approximately 21% ($P < 0.001$).

DISCUSSION

The effect of polyanions on the phenotype of vascular cells has been studied by several authors [10, 14].

The proliferation of vascular smooth muscle cells (SMC) after endothelial injury and secretion of the extracellular matrix (ECM) components are postulated to be important early events in the pathogenesis of atherosclerosis [5–7].

Our results demonstrate that low M_r heparin fragments (CY222) can modulate ECM gene expression. Our results confirm and extend results from our and other laboratories on the action of heparin and derivatives on arterial SMCs [16] and diabetic skin explants of KK mice treated with CY222 [17]. SMC proliferation and biosynthesis *in vitro* were shown to be strongly influenced by heparin and related compounds [8–12, 14, 16, 17, 24–28]. Postconfluent SMCs and vascular endothelial cells both produce heparin-like substances exhibiting antiproliferative properties for smooth muscle cells [9, 28]. Binding and internalization of heparin by vascular SMCs and endothelial cells via specific sites

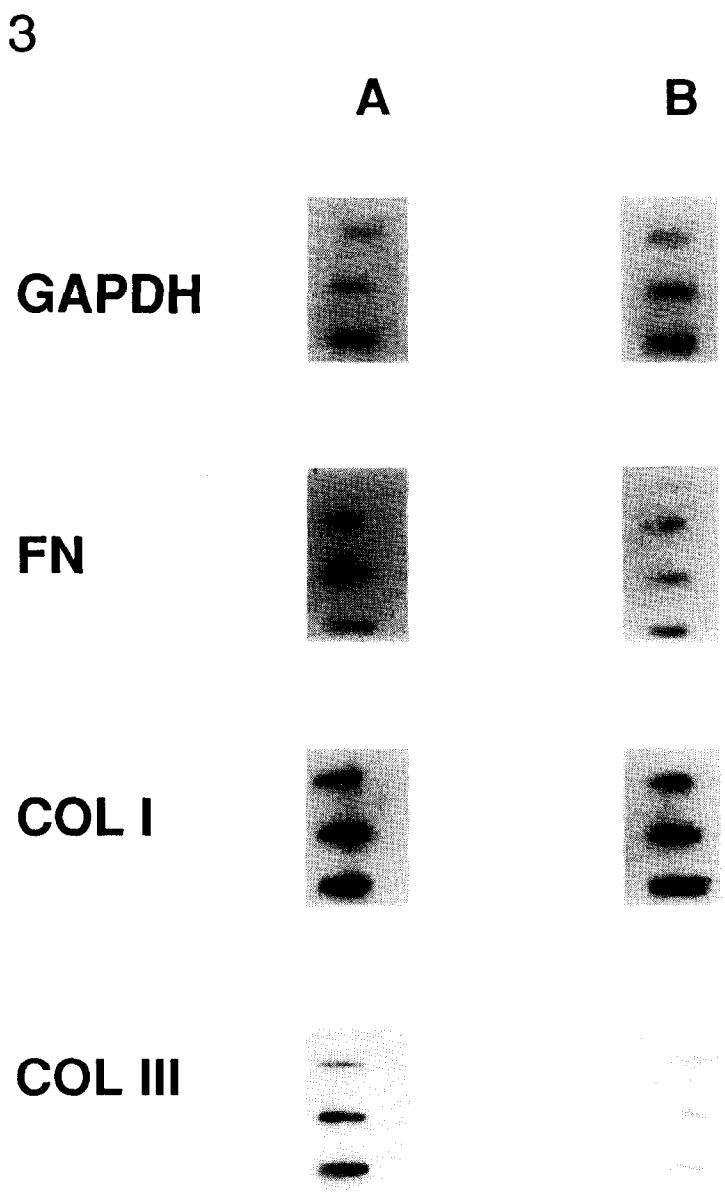

Fig. 3. Slot-blot analysis of GAPDH, fibronectin III A (FN), proα1(I) (Col I), and proα1(III) (Col III) collagen mRNA levels in pig aorta SMC cultures. Total RNA from SMC at the third passage was extracted with 4 M guanidium thiocyanate as described in Materials and Methods. Increasing quantities of total RNA (1, 1.5, and 2 mg) were applied to the filters. (A) Culture medium alone; (B) addition of 100 mg CY222/10^6 cells.

have been demonstrated [15, 25]. Moreover, the presence of heparan sulfate and other heparin derivatives on the cell surface [29], in cytoplasm [30], and in the nucleus [28] were also reported, and their size and degree of sulfation were shown to help determine their efficiency [15, 31]. Heparin can inhibit a PKC-dependent pathway for cellular proliferation by suppressing *c-fos* and *c-myc* expression [32].

We earlier observed a dose-dependent decrease in type III collagen biosynthesis in the presence of heparin fragments in SMC cultures [16]. In the present experiments, we have demonstrated that CY222 decreases the proα1(III) collagen mRNA level. These modifications seem to be relatively selective, since modifications in GAPDH, proα1(I) collagen and fibronectin mRNAs levels were not observed. Heparin and related compounds interfere with AP1 transactivating factor (*jun/fos* protein complex) [33]. These transacting factors are implicated in the regulation of both type I and type III collagen biosynthesis [34, 35]. However, the promoter and regulatory domains of type I and type III collagens are organized differently [35]. For

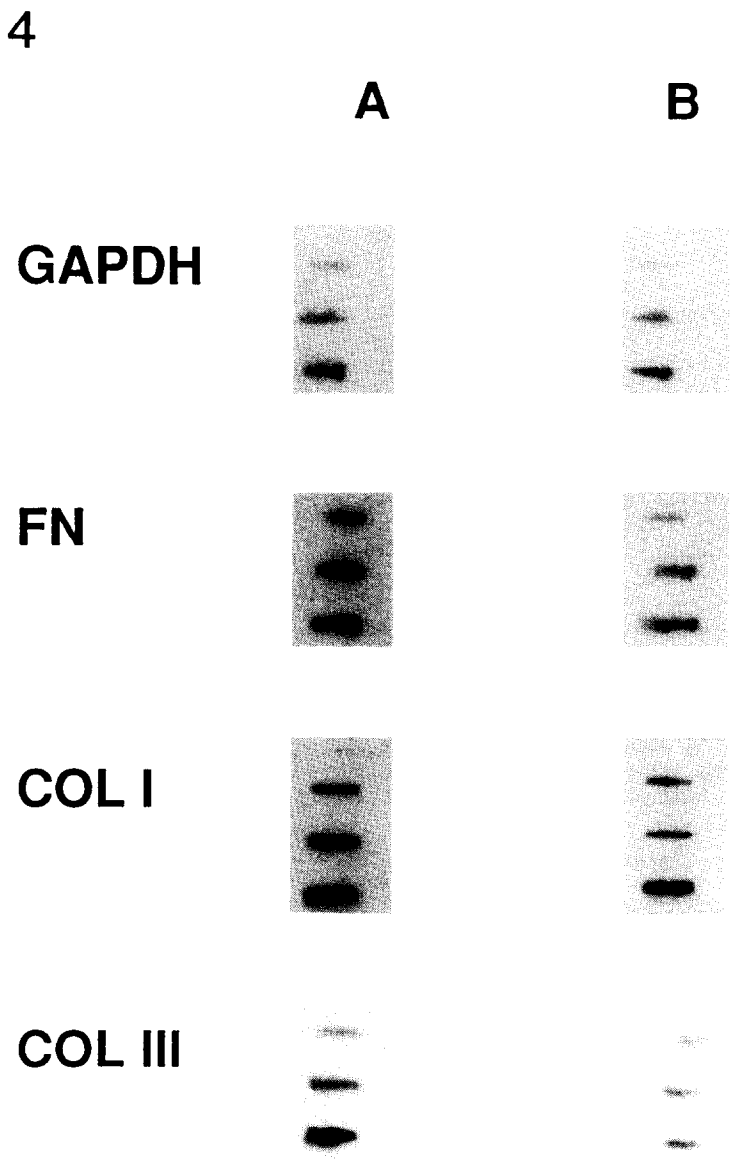

Fig. 4. Slot-blot analysis of GAPDH, fibronectin III A (FN), proα1(I) (Col I), and proα1(III) (Col III) collagen mRNA levels in pig aorta SMC, in the presence of 5% FCS in the culture medium. Total RNA was prepared and analysed as in Fig. 1. (A) Medium with 5% FCS; (B) medium plus FCS and 100 mg CY222/10^6 cells.

example, a heterodimeric CCAAT factor that activates type I collagen genes does not bind to this segment of collagen type III promoter [35]. Heparin fragments might well exhibit different effects on these regulatory processes. In the presence of 5% FCS in the culture medium (without CY222), the transcription of GAPDH, proα1(I) and proα1(III) collagens was not modified. Nevertheless, in the presence of FCS, the decrease in proα1(III) mRNA produced by CY222 was more substantial, suggesting a synergistic effect between serum components and heparin fragments.

In previous experiments, we observed that CY222 decreased fibronectin secretion without affecting fibronectin biosynthesis [16]. We have shown in the present study that CY222 alone did not modify the fibronectin mRNA level. However, the amount of fibronectin mRNA increased with FCS alone. This serum stimulation of fibronectin gene expression is mediated through a DNA sequence at position −170 bp of the fibronectin gene (cAMP element or CRE), resulting from a rapid serum-induced binding of nuclear protein to the CRE [38]. In the presence of both FCS and CY222, we observed a decrease in the fibronectin mRNA level vs serum alone. This decrease could be due to the complexing of serum

mitogens by heparin fragments [27, 38]. It has been demonstrated that TGFβ, EGF and PDGF can modify fibronectin gene expression [39, 40].

It appears that CY222 exhibits specific effects on the expression of matrix genes, especially on those of certain interstitial collagens and fibronectin. Their effect varies according to cell type and growth phase. Indeed, we demonstrated that CY222 could inhibit fibronectin biosynthesis in human dermal fibroblast cultures in the absence of serum (data not shown) and in skin explant cultures of diabetic KK mice [17]. Our results suggest that heparin and related compounds such as CY222 may be important physiologically and pharmacologically in the regulation of the phenotype of vascular SMC. Furthermore, heparin fragments, which inhibit proliferation and modulate extracellular matrix biosyntheses [41], appear to be able to suppress the modification in SMC phenotype during atherogenesis and diabetes where modifications in the expression of the genes of the fibrous collagens and fibronectin were demonstrated.

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REFERENCES

1. Hay ED, *Cell Biology of Extracellular Matrix*. Plenum Press, New York, 1981.
2. Hawkes S and Wang JL, *Extracellular Matrix*. Academic Press, New York, 1982.
3. Robert L, Labat-Robert J and Hornebeck W, Aging and atherosclerosis. In: *Atherosclerosis Review* (Eds. Gotto AM and Paoletti R), Vol. 14, pp. 143–170. Raven Press, New York, 1986.
4. Labat-Robert J, Structural glycoproteins and cell matrix interactions in normal and pathological conditions. In: *Front Matrix Biology* (Eds. Labat-Robert J, Timpl R and Robert L), Vol. 11, pp. 17–29. Karger, Basel, 1986.
5. Chamley-Campbell J, Campbell G and Ross R, The smooth muscle cell in culture. *Physiol Rev* **59**: 1–55, 1979.
6. Liau G and Chan L, Regulation of extracellular matrix RNA levels in cultured smooth muscle cells. Relationship to cellular quiescence. *J Biol Chem* **264**: 10315–10320, 1989.
7. Szendroi M, Labat-Robert J, Godeau G and Robert AM, Immunohistochemical detection of fibronectin using different fixatives in paraffin embedded sections. *Path Biol* **31**: 631–636, 1983.
8. Clowes A and Karnowsky M, Suppression by heparin of smooth muscle cell proliferation in injured arteries. *Nature* **265**: 625–626, 1977.
9. Castellot J, Addonizio M, Rosenberg R and Karnovsky M, Cultured endothelial cells produce a heparin-like inhibitor of smooth muscle growth. *J Cell Biol* **90**: 372–379, 1981.
10. Majack R and Bornstein P, Heparin alters the biosynthetic phenotype of vascular smooth muscle cells. Regulation of collagen biosynthesis. *Ann NY Acad Sci* **460**: 172–180, 1985.
11. Majack R, Cook SC and Bornstein P, Control of smooth muscle cell growth by components of the extracellular matrix: autocrine role for thrombospondin. *Proc Natl Acad Sci USA* **83**: 9050–9054, 1986.
12. Scott-Burden T and Bühler FR, Regulation of smooth muscle proliferative phenotype by heparinoid–matrix interactions. *Trends Pharmacol Sci* **9**: 94–98, 1988.
13. Reilly C, Kindy M, Brown K, Rosenberg R and Sonenshein G, Heparin prevents vascular smooth muscle cell progression through the D1 phase of the cell cycle. *J Biol Chem* **264**: 6990–6995, 1989.
14. Majack R and Bornstein P, Heparin regulates the collagen phenotype of vascular smooth muscle cells: induced synthesis of an M_r 60,000 collagen. *J Cell Biol* **100**: 613–619, 1985.
15. Bârzu T, Van Rijn J, Petitou M, Molho P, Tobelem G and Caen J, Endothelial binding sites for heparin. Specificity and role in heparin neutralization. *Biochem J* **238**: 847–854, 1986.
16. Asselot-Chapel C, Kern P and Labat-Robert J, Biosyntheses of interstitial collagens and fibronectin by porcine aorta smooth muscle cells. Modulation by low molecular weight heparin fragments. *Biochim Biophys Acta* **993**: 240–244, 1989.
17. Asselot C, Robert L, Labat-Robert J and Kern P, Heparin fragments regulate collagen phenotype and fibronectin synthesis in the skin of genetically diabetic mice. *Biochem Pharmacol* **38**: 895–899, 1989.
18. Reilly CF, Fritze LMS and Rosenberg RD, Heparin inhibition of smooth muscle cell proliferation: a cellular site of action. *J Cell Physiol* **129**: 11–19, 1986.
19. Chomczynski P and Sacchi N, Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162**: 156–159, 1987.
20. Mäkelä J, Raassina M, Virta A and Vuorio E, Human proα1(I) collagen: cDNA sequences for the C-propeptide domain. *Nucleic Acids Res* **16**: 349, 1988.
21. Sandberg M and Vuorio E, Localization of types I, II, III collagen mRNAs in developing human skeletal tissues by *in situ* hybridization. *J Cell Biol* **104**: 1077–1084, 1987.
22. Paul JI, Schwarzbauer J, Tamkun JW and Hynes RO, Cell-type-specific fibronectin subunits generated by alternative splicing. *J Biol Chem* **261**: 12258–12265, 1986.
23. Fort P, Marty L, Piechaczyk M, Sabrouty S, Dani C, Jeanteur P and Blanchard JM, Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate multigenic family. *Nucleic Acids Res* **13**: 1431–1442, 1985.
24. Moczar M and Robert L, Glycosaminoglycan synthesis by aortic smooth muscle cells is modified by heparan sulphate, heparin and heparin-derived oligosaccharides. *Biochem Soc Trans* **15**: 390–391, 1987.
25. Castellot J, Wong K, Herman B, Hoover R, Albertini D, Wrigus T, Caleb B and Karnovsky M, Binding and internalization of heparin by vascular smooth muscle cells. *J Cell Physiol* **124**: 13–20, 1985.
26. Tan E, Dodge G, Sorger T, Kovalszky I, Unger G, Yang L, Levine E and Iozzo R, Modulation of extracellular matrix gene expression by heparin and endothelial cell growth factor in human smooth muscle cells. *Lab Invest* **64**: 474–482, 1991.
27. Fritze LMSF and Rosenberg RD, An antiproliferative heparan sulfate species produced by postconfluent smooth muscle cells. *J Cell Biol* **100**: 1041–1049, 1985.
28. Furukawa K and Bhavanandan VP, Influence of glycosaminoglycans on endogenous DNA synthesis in isolated normal and cancer cell nuclei. Differential effect of heparin. *Biochim Biophys Acta* **697**: 344–352, 1982.
29. Kjellen L, Oldberg A and Hook M, Cell-surface

heparan sulfate. Mechanisms of proteoglycan-cell association. *J Biol Chem* **255**: 10407–10413, 1980.
30. Prinz R, Kelin U, Sudhakaran PR, Sinn W, Ulrich K and Von Figura K, Metabolism of sulfated glycosaminoglycans in rat hepatocytes. Synthesis of heparan sulfate and distribution into cellular and extracellular pools. *Biochim Biophys Acta* **630**: 402–413, 1980.
31. Furukawa K and Bhavanandan VP, Influences of anionic polysaccharides on DNA synthesis in isolated nuclei and by DNA polymerase α correlation of observed effects with properties of the polysaccharides. *Biochim Biophys Acta* **740**: 466–475, 1983.
32. Wright TC Jr, Pukac LA, Castellot JJ Jr, Karnovsky MJ, Levine RA, Kim-Park H-Y and Campisi J, Heparin suppresses the induction of c-fos and c-myc mRNA in murine fibroblasts by selective inhibition of a protein kinase C-dependent pathway. *Proc Natl Acad Sci USA* **86**: 3199–3203, 1989.
33. Busch SJ, Jackson RL and Sassone-Corti P, Heparin inhibits transcriptional activation by fos and jun oncoproteins. *Trans Assoc Am Physicians* **103**: 21–27, 1990.
34. Slack JL, Liska DJ and Bornstein P, Regulation of expression of the type I collagen genes. *Am J Med Genet* **45**: 140–151, 1993.
35. Crombrugghe B de, Karsenty G, Maity S, Vuorio T, Rossi P, Ruteshauser EC, McKinney SH and Lozano G, Transcriptional mechanisms controlling types I and III collagen genes. *Ann NY Acad Sci* **580**: 88–96, 1990.
36. Ulrich S, Lagente O, Choay J, Courtois Y and Lenfant M, Structure activity relationship in heparin: stimulation of non-vascular cells by a synthetic heparin pentasaccharide in cooperation with human acidic fibroblast growth factor. *Biochem Biophys Res Commun* **139**: 728–732, 1986.
37. Burgess WH and Maciag T, The heparin-binding (fibroblast) growth factor family of proteins. *Annu Rev Biochem* **8**: 575–606, 1989.
38. Dean D, McQuillan J and Weintraub S, Serum stimulation of fibronectin gene expression appears to result from rapid serum-induced binding of nuclear proteins to a cAMP response element. *J Biol Chem* **256**: 3522–3527, 1990.
39. Ignotz R, Endo T and Massagué J, Regulation of fibronectin and type I collagen mRNA levels by transforming growth factor β action. *J Biol Chem* **262**: 6443–6446, 1987.
40. Dean D, Expression of the fibronectin gene. *Am J Respir Cell Mol Biol* **1**: 5–10, 1989.
41. Ferrao AV and Mason RM, The effect of heparin on cell proliferation and type-I collagen synthesis by adult human dermal fibroblasts. *Biochim Biophys Acta* **1180**: 225–230, 1993.