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Decorin Interacts with Fibrillar Collagen of Embryonic and Adult Human Skin

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Biglycan (PG-I, BGN) and decorin (PG-II, DCN) are small proteoglycans that have been isolated in cartilage, skin, and bone. Although the function of biglycan is unknown, there is biochemical evidence that decorin interacts with fibrillar collagens (type I, type II). The purpose of this study was to perform immunofluorescence and immunoelectron microscopy and immunoblotting of human embryonic and adult skin with antibodies directed against biglycan and decorin. These antibodies were developed against synthetic peptides of the core proteins of biglycan (amino acid sequence 11–24) and decorin (amino acid sequence 5–17). Immunofluorescence microscopy showed that decorin stained embryonic and adult collagen fibrils. Biglycan did not stain collagen, but it appeared to stain the pericellular matrix of embryonic mesenchymal cells. Immunoelectron microscopy revealed labeling of all collagen fibrils with decorin antibodies regardless of their diameter, often at 60-nm periodicity. Positive stains suggest that most of the labeling was in the gap of the D-period (d and e bands) and also in one of the steps (c band). Decorin was identified by immunoblotting in fetal and adult skin. Also, significant amounts of core protein was identified lacking the dermatan sulfate chain. This study suggests that the core protein of decorin interacts with collagen fibrils although its specific function remains unknown. © 1991 Academic Press, Inc.

INTRODUCTION

Small proteoglycans are abundant in the extracellular matrix of various connective tissues (Heinegård and Uldbjerg, 1989). Their chemical structures have been characterized and consist of a protein core and one or two glycosaminoglycan side chains that may be either chondroitin sulfate or dermatan sulfate. Two small proteoglycans, biglycan and decorin, have been purified from bone, cartilage (Fisher *et al.*, 1983; Rosenberg *et al.*, 1985), and more recently from skin (Choi *et al.*, 1989). These small proteoglycans are now known to be the result of at least two different genes coding for proteins of approximately 38 000 Da (Krusius and Rouslahti, 1986; Fisher *et al.*, 1989b). However, there is considerable homology in the amino acid sequence of the core proteins to suggest that both proteins may have derived from gene duplication (Fisher *et al.*, 1989b). Both the human (Fisher *et al.*, 1989b) and the bovine (Neame *et al.*, 1989) forms of the larger of the two small proteoglycans, biglycan (BGN), have been sequenced. Because biglycan (also known as PG-I, PG-1, PG-s1, DSPG-I) has been localized to the human X chromosome and assigned the locus name BGN (McBride *et al.*, 1990), we will use the names biglycan or BGN for this report. Biglycan contains two glycosaminoglycan side chains and in the skin these side chains are dermatan sulfate. Biglycan has recently been shown to be a cell-associated or pericellular matrix component in a variety of fetal human tissues, including bone, skin, blood vessels, and cartilage but has not yet been seen associating with the classic fibrillar elements of connective tissues (Bianco *et al.*, 1990).

The smaller proteoglycan, called decorin (DCN; also known as PG-II, PG-2, PG-40, PG-s2, DSPG-2, DS-II and CSPG1), contains one dermatan sulfate side chain in skin. Human decorin has been sequenced (Krusius and Rouslahti, 1986) and has been shown to be on chromosome 12 (McBride *et al.*, 1990). It has been previously suggested that dermatan sulfate proteoglycans may interact with collagen (Scott and Orford, 1981). Vogel *et al.*, 1984 showed that decorin interacts with type I and type II collagens and may play a role in regulation of fibril diameter. Because decorin has been shown to bind to type I collagen *in vitro* (Brown and Vogel, 1989), it has become a logical but until now an unproven hypothesis that the electron-dense regions associated with collagen fibrils were, at least in part, decorin.

The purpose of this study was to perform immunofluorescence and immunoelectron microscopy of

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embryonic and adult human skin using antisera made against synthetic peptides that are monospecific to decorin or biglycan. This study suggests that biglycan may be associated with the pericellular matrix of mesenchymal cells while decorin interacts with all collagen fibrils, regardless of their diameter.

MATERIALS AND METHODS

Normal human embryonic skin about 15 and 23 weeks old was obtained from postabortions. Normal adult human skin was obtained following surgical procedures. Tissue specimens were stored at −170°C.

Preparation of Antibodies

Small bone proteoglycans were purified from prenatal and neonatal human calvaria as previously described (Fisher *et al.*, 1987, 1989b). Synthetic peptides corresponding to residues 11–24 of the secreted form of human bone BGN (Fisher *et al.*, 1987) and residues 5–17 of human fibroblasts DCN (Krusius and Rouslahti, 1986) were made on an Applied Biosystems Model 430 A peptide synthezizer as previously reported (Fisher *et al.*, 1989a). There appears to be considerable antigenic similarities between bone and skin small proteoglycans (Poole *et al.*, 1986). Antibodies were raised in rabbits against the synthetic peptides BGN (LF-15) and DCN (LF-30). The LF-15 was conjugated with bovine serum albumin while LF-30 was coupled to keyhole limpet hemocyanin. In addition, an antibody against the intact protein core of human DCN was also used (LF-4).

Immunofluorescence Microscopy

Indirect immunofluorescence microscopy was performed in 6- to 8-μm frozen sections. After fixation for 5 min in 100% acetone the sections were washed in phosphate-buffered saline (PBS) and predigested in chondroitinase ABC, protease free. (ICN Immunobiology, Lisle, IL). The specimens were incubated in 0.1 *M* Tris–HCl, pH 8.0, containing 0.03 *M* sodium acetate with 0.02 units of chondroitinase ABC at 37°C for 20 min. This step is required since the antibodies used against sequences of the protein core of BGN and DCN are near the glycosaminoglycan chains (Fisher *et al.*, 1987). After repeated washes with PBS, the primary antibody was placed for 40 min, washed in PBS, and followed by incubation with a fluorescein-conjugated goat antirabbit serum, 1 mg/ml, diluted 1:50 (Organon Teknika Corp., Westchester, PA) for 40 min. Controls consisted of IgG from nonimmunized rabbits. All specimens were counterstained with propidium iodide (0.02% in water; Sigma, St. Louis, MO) to outline the cell nucleus (Fleischmajer *et al.*, 1990).

Immunoelectron Microscopy

Skin specimens about 0.5 to 1 mm thick were obtained under a dissecting microscope and digested with chondroitinase ABC overnight at 37°C as previously described. Incubation with antibodies was carried out in a slow-speed rotator at a 45° angle. Following washings in PBS for about 16 hr, the specimens were incubated with the primary antibody for 24 hr, washed with PBS, and incubated for 24 hr with goat anti-rabbit IgG absorbed onto 5-nm collodial gold particles (Auroprobe Gold, Janssen Lab, Piscataway, NJ), diluted 1:5 in 20 m*M* tris HCl, pH 8.0, 0.9% NaCl, 20 m*M* sodium azide containing 0.1% bovine serum albumin. After extensive washings for 16 hr in a rotator at 4°C with the above buffer, some tissues were not fixed while others were fixed in 2% glutaraldehyde, 2.5% paraformaldehyde, 0.5 m*M* $CaCl_2$ in 0.1 *M* cacodylate buffer, pH 7.4, for 2 hr at 4°C. After dehydration in alcohols, specimens were embedded in Spur resin, cut, and stained with uranyl acetate–lead citrate or phosphotungstic acid. Some specimens were stained *en bloc* prior to the embedding step.

Polyacrylamide Gel Electrophoresis and Immunoblotting

The fresh frozen tissues were minced while thawing in 4 *M* guanidine HCl, 0.05 *M* tris, pH 7.4, containing several protease inhibitors (0.1 *M* 6-aminocaproic acid, 5 m*M* benzamidine HCl, and 1 m*M* phenylmethylsulfonyl fluoride). After extraction by vigorous stirring for 72 hr at 4°C, the filtered supernatant was exchanged into 0.1 *M* ammonium acetate on Trisacryl GF-05 (Reactifs IBF, France) (molecular weight exclusion limit of 2500 Da) and freeze-dried. Two portions of 100 mg each were dissolved in 20 ml of 0.05 *M* tris, 0.063 *M* NaCl, pH 8.0, with one portion also receiving 10 mUnits of chondroitinase ABC (ICN Biochemicals) in 2 ml. After incubating for 1 hr at 37°C, 20 ml of 2× reducing SDS sample buffer was added to each and the samples heated to 100°C for 2 min. Samples were electrophoresed on a 4–20% gradient polyacrylamide SDS gel, transferred to nitrocellulose, and processed for indirect immunodetection using peroxidase-

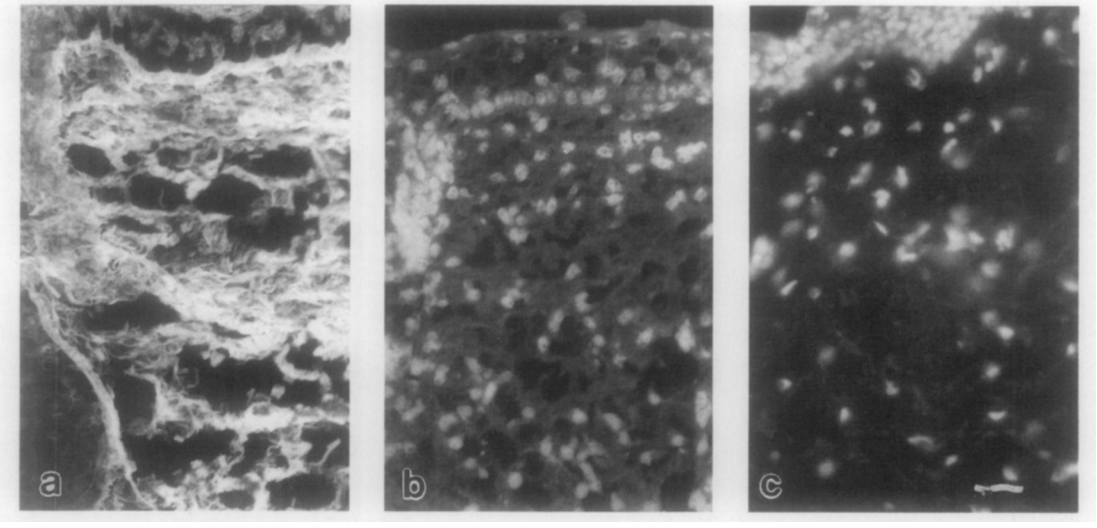

FIG. 1. Fetal human dermis. Indirect immunofluorescence microscopy. Bar = 20 μm. (a) DCN antibody stains the epidermodermal junction, around hair follicles and the surrounding collagen fibers. (b) BGN shows mild fluorescence. (c) Control stained with nonspecific rabbit IgG.

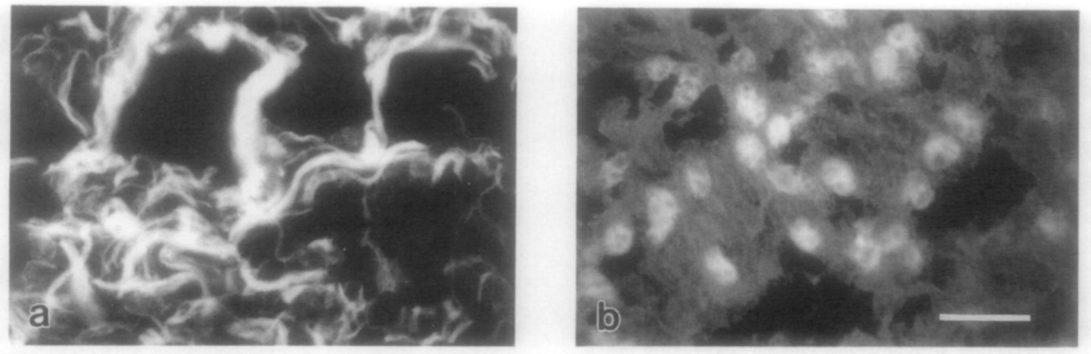

FIG. 2. Fetal human dermis. Indirect immunofluorescence microscopy. Bar = 20 μm (a) High magnification showing individual collagen bundles stained with DCN. (b) The pericellular matrix of mesenchymal cells but not the collagen stain with BGN.

conjugated second antisera (Kirkegaard and Perry Laboratories, Inc. Gaithersburg, MD) and 4-chloro-1-naphthol. The primary antiserum against human DCN synthetic peptide (Serum LF-30) was used at a 1:1000 dilution using procedures previously described (Fisher *et al.*, 1989b).

RESULTS

Immunofluorescence Microscopy

Fetal dermis stained with decorin revealed strong fluorescence throughout the entire thickness of the specimen. Decorin stained well the collagen and the epidermodermal junction of the follicles and interfollicular epidermis (Fig. 1a). There was mild staining of the dermis with biglycan (Fig. 1b) while controls, consisting of nonspecific IgG, were negative (Fig. 1c). At high magnification collagen fibrils were distinctly stained with decorin while mesenchymal cells did not stain (Fig. 2a). On the other hand, biglycan appears to stain the pericellular matrix of mesenchymal cells, presumably fibroblasts, but not the surrounding collagen (Fig. 2b). Adult dermis stained strongly with decorin, including the papillary and reticular dermis (Fig. 3a). Biglycan and controls were essentially negative. There was moderate staining of the epidermis with biglycan as previously reported (Bianco *et al.*, 1990).

Immunoelectron Microscopy

Fetal skin collagen revealed an average width of 32 nm with a range from 21 to 45 nm. Most collagen fibrils labeled with DCN with areas revealing a periodicity of about 60 nm. It was also possible to demonstrate labeling in the shape of ring or spiral-like structures around collagen fibrils. In these structures, gold particles were arranged in a linear fashion (Fig. 4a). In some areas labeling was sparse, suggesting that some DCN was released from the fibrils during the various incubation periods. Biglycan and nonspecific rabbit IgG stains were negative in embryonic skin (Figs. 4b, 4c). Collagen fibrils from adult human skin ranged in width from 60 to 120 nm, with a mean average of 92 nm. All collagen fibrils labeled with decorin, with segments revealing a 60-nm periodicity (Fig. 5a). Ring or spiral-like structures were noted in collagen fibrils cut tangen-

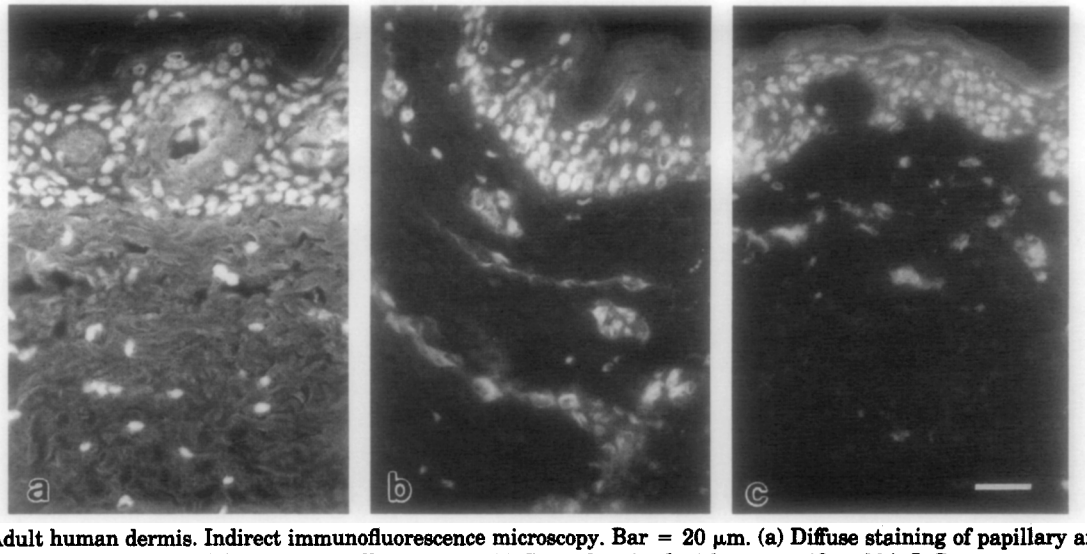

FIG. 3. Adult human dermis. Indirect immunofluorescence microscopy. Bar = 20 μm. (a) Diffuse staining of papillary and reticular dermis with DCN antibodies. (b) BGN is essentially negative. (c) Control stained with nonspecific rabbit IgG.

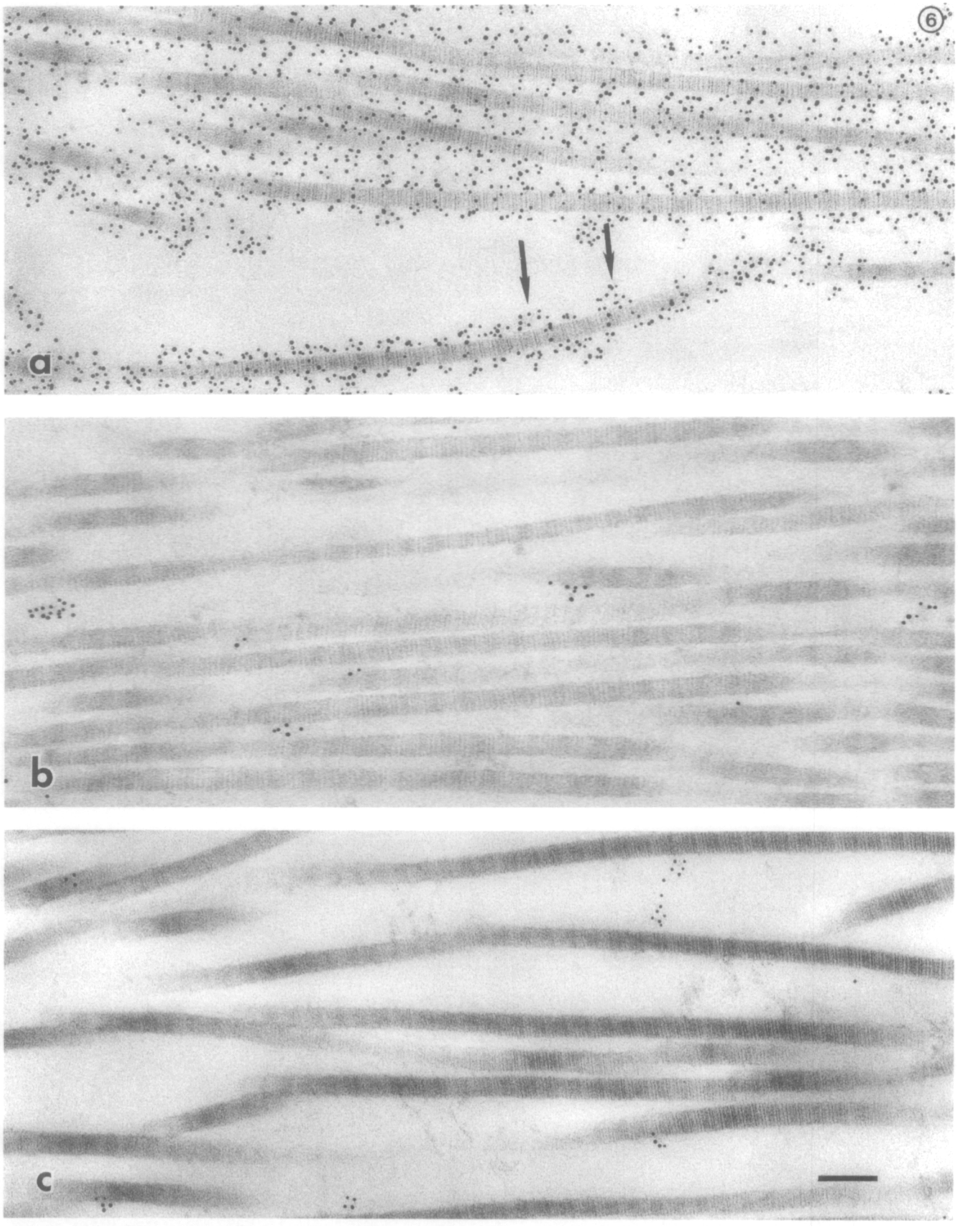

FIG. 4. Fetal human dermis. Indirect immunoelectron microscopy. Bar = 100 nm. Collagen fibrils about 30 nm thick, labeled with an antibody against DCN. (a) Note some ring or spiral-like decorations (arrows). (b) Collagen fibrils show negative labeling with BGN. (c) Control stained with nonspecific rabbit IgG.

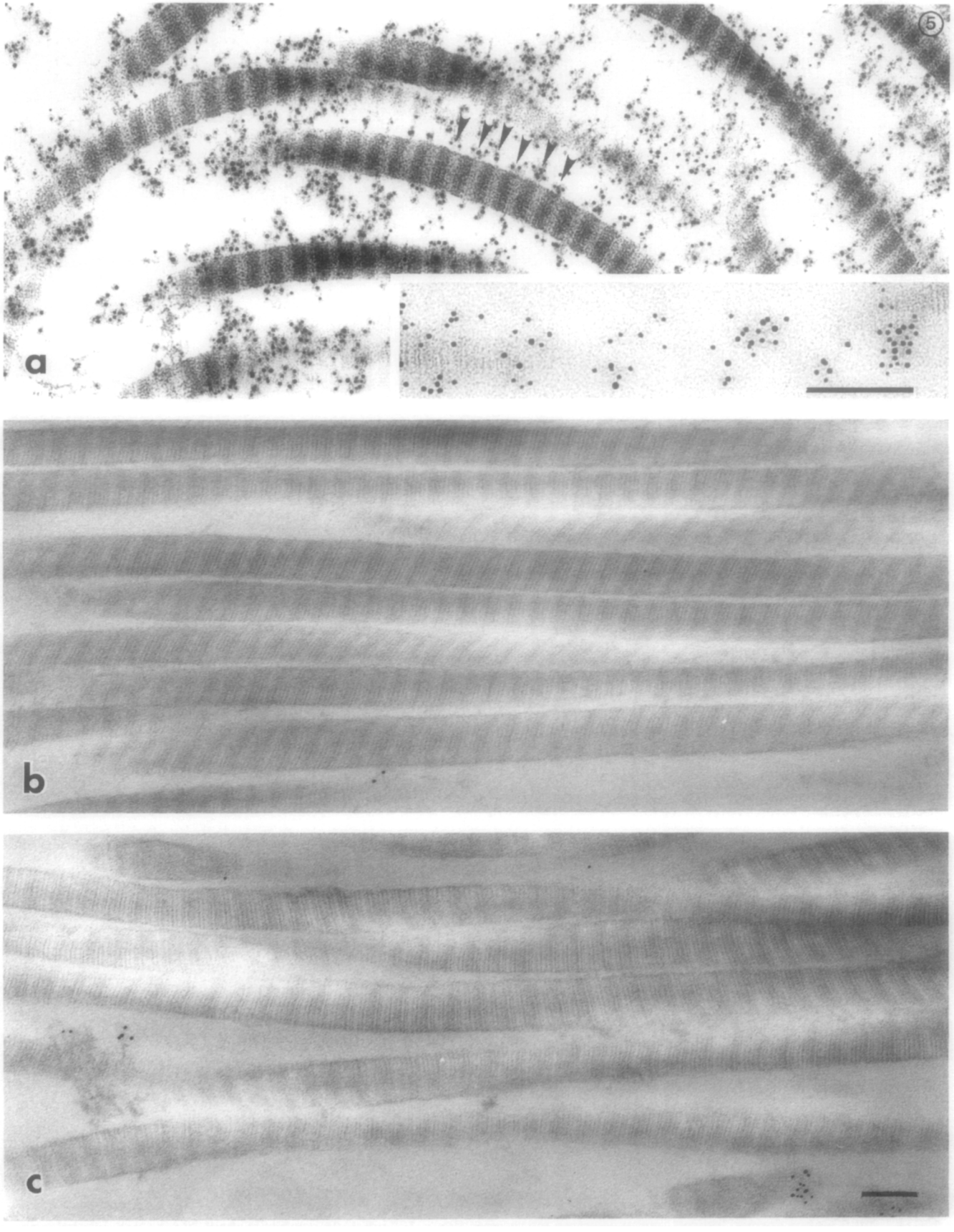

FIG. 5. Adult human dermis. Indirect immunoelectron microscopy. Bar = 100 nm. (a) Collagen fibrils, about 60–80 nm thick, labeled with DCN. Note areas with periodic labeling (arrows). Tangential section showing ring or spiral-like labeling (inset). (b) BGN does not label collagen fibrils. (c) Control stained with nonspecific rabbit IgG.

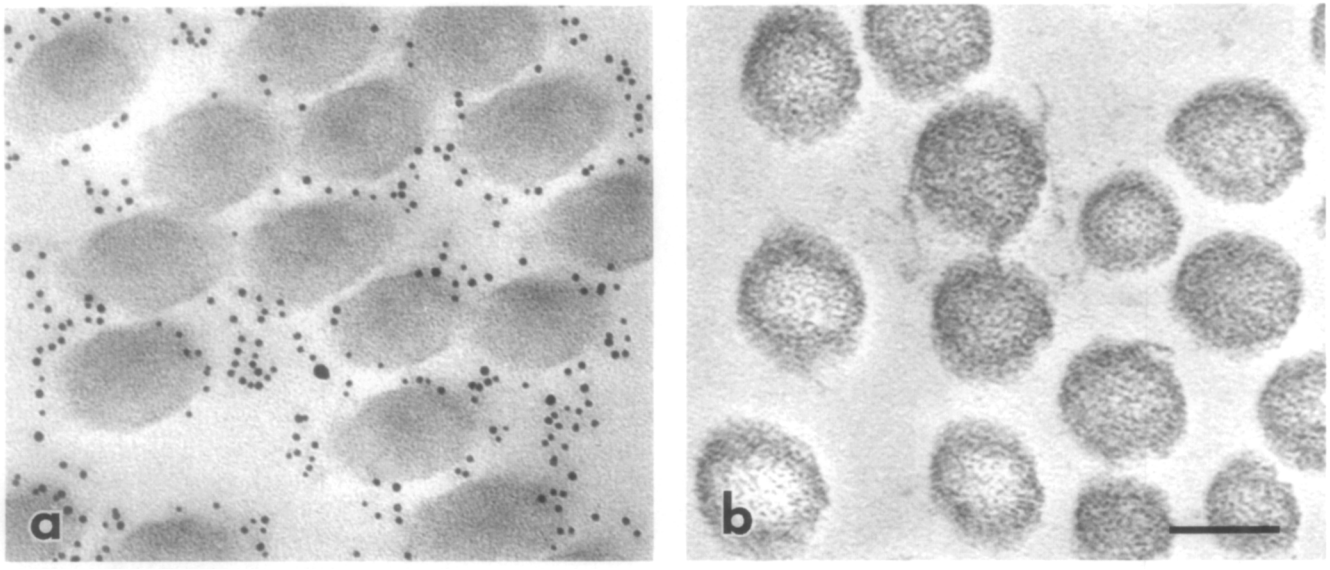

FIG. 6. Adult human dermis. Immunoelectron microscopy of cross sections of collagen fibrils. Bar = 100 nm. (a) Section stained with DCN. (b) Section stained with BGN.

tially (Fig. 5a, inset). Stains with biglycan and controls were negative (Figs. 5b, 5c). Cross sections of collagen fibrils also showed distinct labeling at the periphery of each fibril (Fig. 6). Elastic fibrils failed to stain with either decorin or biglycan (Fig. 7). Positive staining with uranyl acetate and or phosphotungtic acid revealed the classical banding patterns of collagen fibrils where the b band corresponds to the overlap area; the d and e bands correspond to the gap while the a and c bands are located in the steps or borderline zones between the gap and overlap areas (Table I). The most common labeling took place

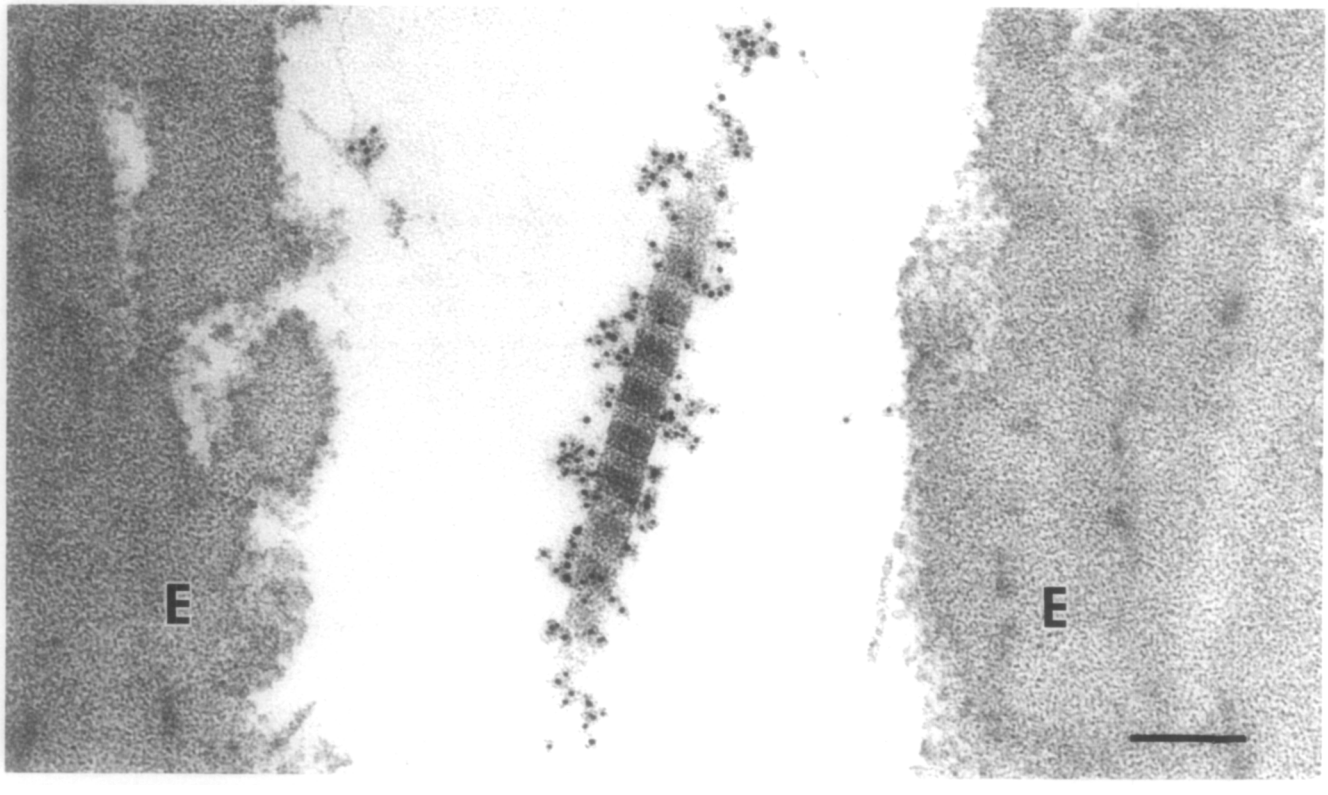

FIG. 7. Adult human dermis. Indirect immunoelectron microscopy showing a collagen fibril labeled with DCN. Note that adjacent elastic fibrils (E) do not label. Bar = 100 nm.

TABLE I

Number of D-periods counted	% Labeling				
	Step bands		Gap bands		Overlap
	a	c	d	e	b
100	16	54	79	49	5

Note. This table represents percentage distribution of labeling of each collagen band present in a D-period following positive staining. Gold particles counted were only those in contact with the fibril or at a distance no greater than 20 nm.

at the d and e bands (gap) although significant labeling was also noted at the c step (Fig. 8). No labeling was basically seen at the overlap areas (Table I). It should be stressed, however, that assessment of the exact location of the labeling was difficult due to clustering of gold particles. Furthermore, gold particles were often displaced from the collagen fibrils for up to 20 to 100 nm. This may be due to several factors: (a) the utilization of two antibodies, (b) dysruption of the collagen fibril and or proteoglycan, or (c) the presence of free proteoglycans.

Western Blot

Western blot and immunodetection analysis of adult human skin extracts showed the expected core protein after enzymic removal of the dermatan sulfate chain with chondrointinase ABC (Fig. 9, + lane). Interestingly, the electrophoresis lane containing skin extract without enzyme treatment (− lane) also showed significant activity at the same location. This result suggests that adult human skin contains detectable amounts of decorin "core protein" lacking the dermatan sulfate chain. A similar observation has been made in tendon (Koob and Horoschak, 1990). These data cannot determine whether this nonglycosaminoglycan containing protein was associated with collagen or was even in the extracellular environment at all. Because the antiserum used was specific for the portion of the decorin molecule immediately carboxy-terminal (amino acids 5–17) to the attachment site of the dermatan sulfate chain (at amino acid 4), it is unlikely that the "core protein" observed without enzyme digestion was a result of protease activity removing the glycosaminoglycan chain. Fetal skin extracts contained much smaller amounts of detectable decorin core protein (per 100 mg extract) after treatment with chondroitinase ABC (not shown).

DISCUSSION

The interaction between fibrillar collagens and proteoglycans and the possible role of these complex carbohydrates in fibril formation has been suggested in previous studies (Hoffman *et al.*, 1957; Toole and Lowther, 1968; Öbrink, 1973). *In vitro* polymerization of collagen molecules showed that the glycosaminoglycans may influence the kinetics of collagen precipitation but not their thermal stability, while proteoglycans could affect both, probably by modifying electrostatic interactions between collagen molecules (Fisher *et al.*, 1987). It has been shown that human skin fibroblasts synthesize an iduronic acid-rich dermatan sulfate that binds specifically to collagen (Gallagher *et al.*, 1983). Hyaluronic acid and keratan sulfate do not bind to collagen under physiological conditions (Greenwald *et al.*, 1975).

Scott *et al.* (Scott and Naigh, 1985; Scott and Orford, 1981) combined the use of a cationic dye and enzyme digestions and showed orthogonal arrangements of glycosaminoglycans in tendon, skin, and cornea. They showed that dermatan–sulfate proteo-

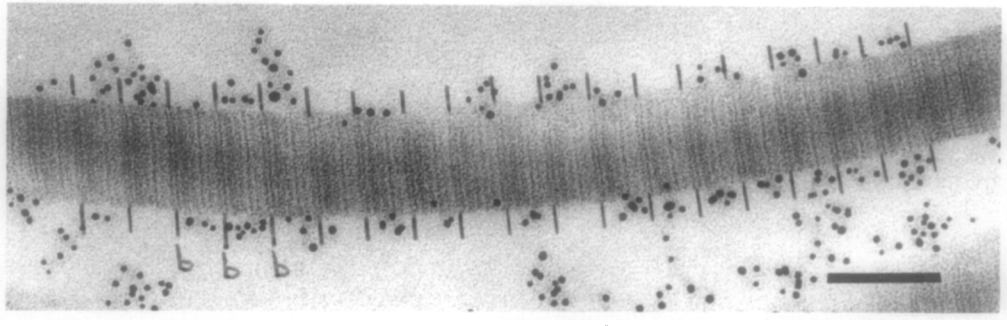

FIG. 8. Adult human dermis. Indirect immunoelectron microscopy. The bars along the collagen fibril point to the b band which corresponds to the overlap area. Note that most of the labeling take place in the gap area, usually next to the d and e bands.

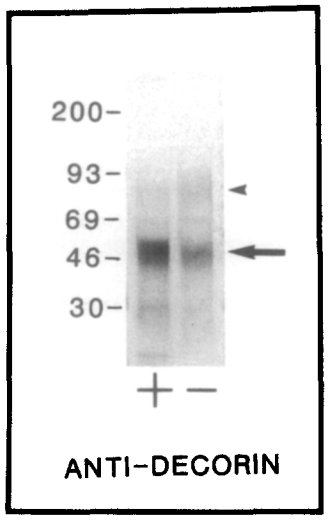

FIG. 9. Western-blot immunodetection of the core protein of decorin in adult skin. Blots were performed on samples treated with (+) or without (−) chondroitinase ABC digestion.

glycans were localized in the gap area (d and e bands) while keratan sulfate had predilection for the step zones (a and c bands). Small proteoglycans, biglycan and/or decorin, appear as ubiquitous components of the extracellular matrix of various fibrous connective tissues and may represent as much as 95% of the interstitial proteoglycans (Heinegård and Sommarin, 1987). Recently, localization studies of the protein core and messenger RNAs of biglycan and decorin showed that both are expressed and localized in a divergent and sometimes mutually exclusive fashion. Thus, decorin was found to be associated with type I-II collagens while biglycan was localized in connective tissue cells (skeletal myofibers, endothelial cells, keratinocytes and renal tubular epithelial cells) (Bianco *et al.*, in press). Preliminary immunoelectron microscopy studies revealed binding of dermatan sulfate proteoglycan to fibrillar collagen (Longas and Fleischmajer, 1985). There is also evidence that small proteoglycans may play a role in collagen fibrillogenesis. *In vitro* studies have shown that small dermatan sulfate proteoglycans from tendon inhibits fibril formation of type I and type II collagens (Vogel *et al.*, 1984). This effect was preserved following the removal of the dermatan sulfate, suggesting that the interference in fibrillogenesis was due to the protein core. Biglycan and large cartilage proteoglycans have no effect on fibril formation. In addition not all small dermatan sulfates proteoglycans affect fibrillogenesis. Those derived from aorta and cartilage are much less effective and probably contained large amounts of BGN (Vogel *et al.*, 1984) while those derived from human uterine cervix, although they bind to type I collagen, have no effect in the rate of fibrillogenesis and minimal effect in fibril diameter (Uldbjerg and Danielsen, 1988). The regulation of fibril formation is rather complex and may also involve collagen interactions, like type II and IX (Uldbjerg and Danielson, 1988), type I and type III (Keene *et al.*, 1987; Fleischmajer *et al.*, 1990), as well as other noncollagenous proteins of the extracellular matrix (Chandrasekhar *et al.*, 1986; Hedbom and Heinegård, 1989).

This study demonstrates the ultrastructural localization of the protein core of decorin along dermal collagen fibrils. The most common binding site appears to be the d and e bands within the gap area and the c band of one of the steps. It is attractive to speculate that binding of decorin to the gap area may interfere with further lateral accretion of collagen molecules and stop the growth of the fibril, and thus, be the regulatory mechanism for the final fibril diameter. However, the above hypothesis is contradicted by the presence of decorin along collagen fibrils during active fibrillogenesis. Recently, it has been shown that dermal fibrillar collagen consists of hybrids of type I and type III collagens. Preliminary data from our laboratories suggest that most dermal collagen fibrils consist of type I collagen coated with type III collagen (Fleischmajer *et al.*, in press). It would be of considerable interest to determine whether the epitope that interacts with decorin at the surface of fibrils corresponds to type I and or type III collagen molecules. Also it would be important to determine whether the core protein of decorin interacts with the N or C telopeptides or with the helical domain of the collagen molecule. Answers to the above questions will further improve our understanding of the interaction between decorin and collagen fibrils.

Note added in proof. Since this paper was accepted for publication, Pringle and Dodd (*J. Histochem. Cytochem.* 14, 1405–1411, 1990) reported the localization of decorin near the d and e bands of tendon collagen fibrils.

REFERENCES

BIANCO, P., FISHER, L. W., YOUNG, M. F., TERMINE, J. D., AND ROBEY, P. G. (1990) *J. Histochem. Cytochem.*, 38, 1549–1563.

BROWN, D. C., AND VOGEL, K. G. (1989) *Matrix* 9, 468–478.

CHANDRASEKHAR, S., LAURIE, G. W., CANNON, F. B., MARTIN, G. R., AND KLEINMAN, H. K. (1986) *Proc. Natl. Acad. Sci. USA* 83, 5126–5130.

CHOI, H. U., JOHNSON, T. L., SUBHASH, P., TANG, L. H., ROSENBERG, L. (1989). *J. Biol. Chem.* 264, 2876–2884.

FISHER, L. W., HAWKINS, G. R., TISROSS, N., AND TERMINE, J. D. (1987) *J. Biol. Chem.* 262, 9702–9708.

FISHER, L. W., LINDNER, W., AND TOUNG, M. F. (1989a) *Connect. Tissue Res.* 21, 373–380.

FISHER, L. W., TERMINE, J. D., DEJTER, S. W., JR., WHITSON, S. W., YANA-GISHITA, M., KUMURA, J. H., HASCALL, V. C., KLEINMAN, H. D., HASSELL, J. R., AND NILSSON, B. (1983) *J. Biol. Chem.* 258, 6588–6594.

FISHER, L. W., TERMINE, J. D., AND YOUNG, M. F. (1989b) *J. Biol. Chem.* 264, 4571–4576.

FLEISCHMAJER, R., PERLISH, J. S., BURGESON, R. E., SHAIKH-BAHAI, F., AND TIMPL, R. (1990) *Annals N.Y. Acad. Sci.* **580**, 161–175.

FLEISCHMAJER, R., MACDONALD, E. D., PERLISH, J. S., BURGERSON, R. E., FISHER, L. W. (1991) *J. Struct. Biology* in press.

GALLAGHER, J., GASIUNAS, T., AND SCHOR, S. L. (1983) *Biochem. J.* **215**, 107–116.

GREENWALD, R. A., SCHWARTZ, C. E., AND CANTOR, J. O, (1975) *Biochem. J.* **145**, 601–605.

HEDBOM, E., AND HEINEGÅRD, D. (1989) *J. Biol. Chem.* **264**, 6898–6905.

HEINEGÅRD, D., AND OLDBERG, A. (1989) *FASEB* **3**, 2042–2051.

HEINEGÅRD, D., AND SOMMARIN, Y. (1987) *in* CUNNINGHAM, L. W. (Ed.), Methods in Enzymology, Vol. 144, pp. 305–319, Academic Press, Orlando.

HOFFMAN, P., LINKER, A., AND MEYER, K. (1957) *Arch. Biochem. Biophys.* **69**, 435–440.

KEENE, D. R., SAKAI, L. Y., BACHINGER, H. P., AND BURGESON, R. E. (1987) *J. Cell. Biol.* **105**, 2393–2402.

KOOB, T. J., AND HOROSCHAK, S. A. (1990) *Trans. Annu. Meet. Orthop. Res. Soc.* **15**, 38.

KRUSIUS, T., AND ROUSLAHTI, E. (1986) *Proc. Nat. Acad. Sci. USA* **83**, 7683–7687.

LONGAS, M. O., AND FLEISCHMAJER, R. (1985) *Connect. Tissue Res.* **13**, 117–125.

MCBRIDE, O. W., FISHER, L. W., AND YOUNG, M. F. (1990) *Genomics* **6**, 219–225.

NEAME, P. J., CHOI, H. U., AND ROSENBERG, L. C. (1989) *J. Biol. Chem.* **264**, 8653–8661.

ÖBRINK, B. (1973) *Eur. J. Biochem.* **33**, 387–400.

OEGEMA, T. R., LAIDLAW, J., HASCALL, V. C., AND DZIEWIATKOWSKI, D. D. (1975) *Arch. Biochem. Biophys.* **170**, 698–709.

POOLE, A. R., WEBER, C., PIDOUX, I., CHOI, H., AND ROSENBERG, L. (1986) *J. Histochem. Cytochem.* **34**, 619–627.

ROSENBERG, L. C., CHOI, H. U., TANG, L. H., JOHNSON, T. T., PALS, S., WEBBER, C., REINER, A., AND POOLE, A. R. (1985) *J. Biol. Chem.* **260**, 6304–6313.

SCOTT, J. E., AND NAIGH, M. (1985) *Biosci. Rep.* **5**, 765–774.

SCOTT, J. E., AND ORFORD, C. R. (1981) *Biochem. J.* **197**, 213–216.

SNOWDEN, J. MCK, AND SWANN, D. A. (1980) *Biopolymers* **19**, 767–780.

TOOLE, B. P., AND LOWTHER, D. A. (1968) *Biochem. J.* **109**, 857–866.

ULDBJERG, N., AND DANIELSEN, C. C. (1988) *Biochem. J.* **251**, 643–648.

VAUGHAN, L. M., MENDLER, S., HUBER, S., BRUCKNER, P., AND WINTERHALTER, H. H. (1988) *J. Cell. Biol.* **106**, 991–997.

VOGEL, K. G., PAULSSON, M., AND HEINEGÅRD, D. (1984) *Biochem. J.* **223**, 587–597.