Proteoglycan Biosynthesis in Chondrocytes: Protein A–Gold Localization of Proteoglycan Protein Core and Chondroitin Sulfate within Golgi Subcompartments

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ABSTRACT The intracellular pathway of cartilage proteoglycan biosynthesis was investigated in isolated chondrocytes using a protein A–gold electron microscopy immunolocalization procedure. Proteoglycans contain a protein core to which chondroitin sulfate and keratan sulfate chains and oligosaccharides are added in posttranslational processing. Specific antibodies have been used in this study to determine separately the distribution of the protein core and chondroitin sulfate components. In normal chondrocytes, proteoglycan protein core was readily localized only in smooth-membraned vesicles which co-labeled with ricin, indicating them to be galactose-rich medial/*trans*-Golgi cisternae, whereas there was only a low level of labeling in the rough endoplasmic reticulum. Chondroitin sulfate was also localized in medial/*trans*-Golgi cisternae of control chondrocytes but was not detected in other cellular compartments. In cells treated with monensin (up to 1.0 μM), which strongly inhibits proteoglycan secretion (Burditt, L. J., A. Ratcliffe, P. R. Fryer, and T. Hardingham, 1985, *Biochim. Biophys. Acta.*, 844:247–255), there was greatly increased intracellular localization of proteoglycan protein core in both ricin-positive vesicles, and in ricin-negative vesicles (derived from *cis*-Golgi stacks) and in the distended rough endoplasmic reticulum. Chondroitin sulfate also increased in abundance after monensin treatment, but continued to be localized only in ricin-positive vesicles. The results suggested that the synthesis of chondroitin sulfate on proteoglycan only occurs in medial/*trans*-Golgi cisternae as a late event in proteoglycan biosynthesis. This also suggests that glycosaminoglycan synthesis on proteoglycans takes place in a compartment in common with events in the biosynthesis of both O-linked and N-linked oligosaccharides on other secretory glycoproteins.

Proteoglycans are a specialized group of glycoproteins containing glycosaminoglycan chains. The aggregating proteoglycan found abundantly in cartilage is of particularly high molecular weight (M_r 1×10^6–4×10^6) (22). It consists of a protein core which, when newly synthesized, has a molecular weight of 300,000–350,000 (34, 40, 68, 70, 74) and to which are attached many chondroitin sulfate and keratan sulfate chains, and both O-linked and N-linked oligosaccharides (16, 21, 23). The protein core contains three main regions: a folded globular binding region, which forms a specific site for interaction with hyaluronate; an adjacent region rich in keratan sulfate chains; and a major extended region bearing the chondroitin sulfate chains (16, 20, 23) (see Fig. 1). Proteoglycans are present in the extracellular matrix of cartilage as large aggregates in which many proteoglycans are bound to a chain of hyaluronate (19), with each proteoglycan–hyaluronate bond being stabilized by a globular link protein (M_r 40,000) (4, 15, 32, 43, 61, 67).

Proteoglycan biosynthesis involves the synthesis of a protein core and the addition to this of more than 10 times its weight of polysaccharide components in posttranslational processing (17, 18, 24, 54). Investigations of the kinetics of proteoglycan

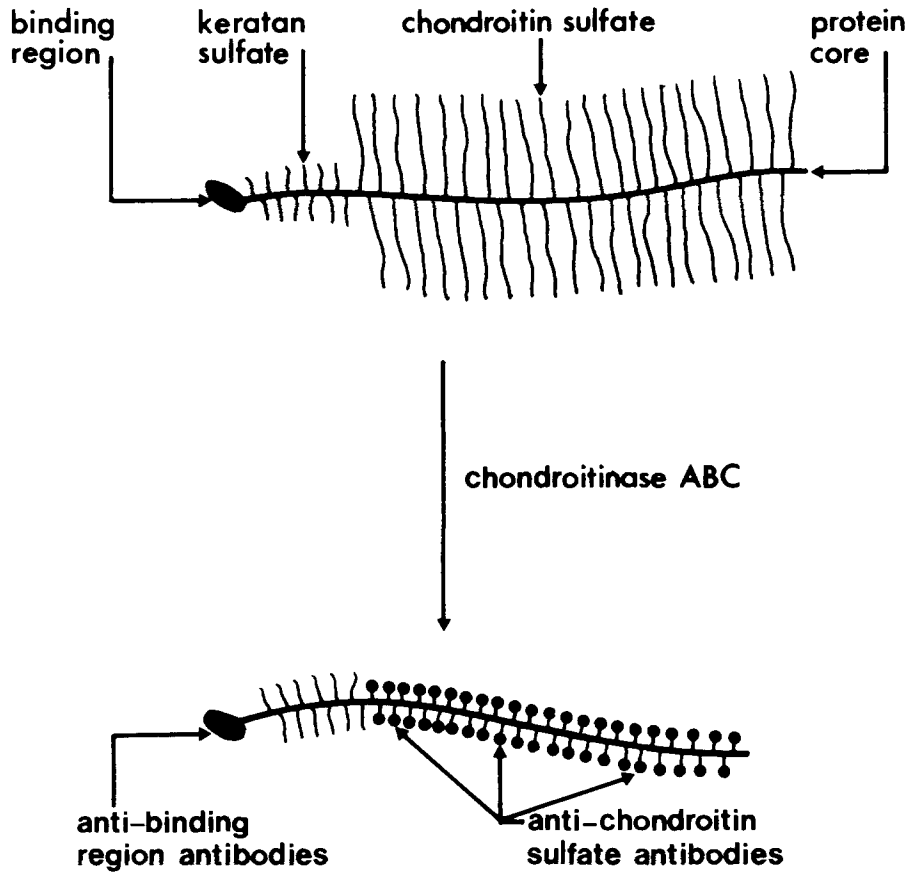


FIGURE 1 Antibody binding sites on proteoglycan. Diagram of a cartilage proteoglycan and the sites of antibody binding after chondroitinase ABC digestion. Digestion of the chondroitin sulfate chains generate epitopes of 4, 6, and non-sulfated chondroitin sulfate, which remain attached to the protein core and are recognized by the monoclonal antibodies 9-A-2, 3-B-3, and 1-B-5, respectively. The binding of antibodies to binding region is unaltered by the chondroitinase ABC digestion.

biosynthesis in rat chondrosarcoma cells showed that protein core synthesis was followed by a considerable delay (60–90 min) before the addition of chondroitin sulfate chains, and that chondroitin sulfate synthesis was rapid and was quickly followed (<10 min) by secretion (9, 30, 31, 36). This was corroborated by the identification of an intracellular pool of protein core devoid of chondroitin sulfate chains (5, 9, 34, 40). Biosynthetic labeling experiments showed that only the N-linked oligosaccharides were attached to the protein core soon after protein synthesis within the rough endoplasmic reticulum (RER)[1] (11, 33). The glycosaminoglycans and O-linked oligosaccharides were added in smooth membrane systems (11) which appeared to be part of the Golgi complex (33). This supported previous evidence from autoradiography and immunofluorescence which showed that the perinuclear (Golgi) region in chondrocytes was the major site of [^{35}S]-sulfate incorporation (12, 29, 45) and of proteoglycan and glycosaminoglycan localization (41, 71–73). The evidence thus suggested the pathway of proteoglycan biosynthesis to be similar to that of other secretory glycoproteins.

In studies of glycoprotein and proteoglycan biosynthesis, monensin has been used to interfere with intracellular translocation of secretory proteins in many different cell types. At low concentration it inhibits transport within the Golgi complex, but does not inhibit protein synthesis (62–65). In chondrocytes, it potently inhibits chondroitin sulfate synthesis on proteoglycans (27, 37, 39, 60) and leads to an intracellular accumulation of the proteoglycan protein core (5, 39, 40).

In the present study using electron microscopic immunolocalization (44, 50), the intracellular localization of proteoglycan protein core and chondroitin sulfate has been determined in chondrocytes. Using *Ricinus communis* agglutinin I (ricin) (28) and anti-ricin antibodies (13), it has been possible to identify the intracellular site of chondroitin sulfate synthesis on cartilage proteoglycan within Golgi subcompartments.

[1] *Abbreviation used in this paper:* RER, rough endoplasmic reticulum.

MATERIALS AND METHODS

Chondrocyte Cultures: Chondrocytes were isolated from fresh pig laryngeal cartilage, as described previously (5). The cartilage was digested with 0.05% hyaluronidase (Sigma Chemical Co., Poole, England) for 30 min at 37°C, for 2 h in 0.25% collagenase (Flow Laboratories, Inc., Irvine, England) at 37°C, and finally overnight in 0.1% collagenase at 37°C. The released cells were washed and plated at a density of 2×10^6 cells/35-mm tissue culture dish. The chondrocytes were cultured for 24 h in medium containing 10% fetal calf serum and for an additional 24 h in medium containing 2% fetal calf serum. The cells were then removed from the dishes by gentle agitation, resuspended in medium containing 2% fetal calf serum and the appropriate concentration of monensin (Sigma Chemical Co.). After a 4-h incubation, the cells were pelleted by centrifugation and fixed and embedded for electron microscopy.

Antisera and Monoclonal Antibodies: The localization of proteoglycan protein core was with a polyclonal antiserum directed against the binding region domain (43). There is one such domain in each aggregating proteoglycan, and the detection of binding region therefore reflects the distribution of proteoglycan protein core, irrespective of structural variations in other regions of the molecule (for example, changes in carbohydrate composition). These antibodies have previously been used for quantitative determination of proteoglycan distribution in cartilage sections by radioimmunoassay and electron microscopic immunolocalization (44), and the antiserum was used at a 1:5 dilution. The antiserum to ricin was a gift from Drs. Graham Warren and Daniel Louvard (European Molecular Biological Laboratories, Heidelberg, Federal Republic of Germany) and was as previously described and was used at a dilution of 1:100 (13).

The monoclonal antibodies to chondroitin sulfate (9-A-2, 3-B-3, and 1-B-5) were a gift from Dr. Bruce Caterson (University of West Virginia, Morgantown, VA) and were as previously described (6). They recognize unsaturated determinants on 4, 6, and non-sulfated chondroitin sulfate, respectively, and these epitopes are generated by chondroitinase ABC digestion and remain attached to proteoglycan, one on each chondroitin sulfate chain residue (Fig. 1). A mixture of all three antibodies was used in order to maximize the detection of

chondroitin sulfate and also to preclude any influence of changes in sulfation on the results. The final dilution of each monoclonal antibody was 1:50.

Electron Microscopy and Immunolocalization: For standard morphological studies, chondrocytes were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, for 2 h at room temperature, postfixed in 1% osmium tetroxide in the same buffer, dehydrated in graded acetone, and embedded in Spurr resin. Sections were cut using a diamond knife, mounted on copper grids, and stained with aqueous uranyl acetate and Reynold's lead citrate.

The electron microscopic immunolocalization used a post-embedding technique (44) with protein A–gold as the immunolabel. The cells were fixed in 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 2 h at room temperature, and embedded in Lowicryl K4M embedding resin (Agar Aids, Stanstead, England) using modifications described by Fryer et al. (10) of the method of Roth et al. (50). The immunolocalization procedure was carried out on the surface of ultrathin sections mounted on parlodion-coated nickel grids. Colloidal gold of 18-nm diameter was prepared using trisodium citrate (1) and of 4-nm diameter using white phosphorus (56). Complexes of protein A (Pharmacia Fine Chemicals, Sweden) with gold were prepared as described by Slot and Geuze (56).

The single immunolabeling procedure was as described previously (44). Nonspecific background labeling was minimized by preincubating the sections in 1% bovine serum albumin/phosphate-buffered saline (BSA/PBS) for 30 min. The sections were then digested with chondroitinase ABC at 0.5 U/ml^{-1} (Worthington Biochemical Corp., Millipore, England) for 45 min at 37°C (44), washed (three times) with 1% BSA/PBS for 5 min, incubated with an antibody dilution for 2 h, and then washed as before and incubated with protein A–gold for 1 h. The sections were finally washed three times with PBS and then rinsed in a stream of distilled water, lightly stained with uranyl acetate and lead citrate, and viewed in a JEOL 1200 EX electron microscope. With the anti–chondroitin sulfate mouse monoclonal antibodies (1-B-5 and 3-B-3), a second 1-h antibody incubation with rabbit anti-mouse IgG was included before incubation with protein A–gold since they bind only weakly to protein A. Immunolabeling with ricin (Sigma Chemical Co.) and anti-ricin antiserum was carried out as described by Griffiths et al. (13). After the sections had been incubated with 1% BSA/PBS and digested with chondroitinase ABC, they were incubated with ricin (50 μg/ml^{-1}) for 30 min, stream washed with PBS, and then washed with 1% BSA/PBS, and incubated for 30 min with the anti-ricin antiserum. Double-immunolabeling experiments using protein A–gold of two different sizes were performed essentially as described by Roth (48). The first immunolabeling procedure was as described above but using 4-nm diameter protein A–gold particles. This procedure was then repeated on the same section to localize the second antigen but this time using the 18-nm protein A–gold particles. The antibody dilutions used were selected by titration of the antibodies in an immunolocalization experiment. The concentration of antibody selected was that which gave high specific labeling with low background labeling. Small variations in the concentrations used did not affect the level of labeling achieved.

The density of labeling over different cellular compartments (nucleus, mitochondria, Golgi region, RER, and cytoplasm) was determined from four experiments in which a minimum of 30 cells per concentration of monensin was examined. The distribution of each antigen amongst smooth membrane (Golgi) vesicles was determined by counting the number of smooth-membraned vesicles per cell, the proportion of these vesicles that contained protein A–gold, and the number of gold particles per vesicle. The labeling of Golgi vesicles was assessed including all labeling clearly associated with the vesicle. Where labeling was present over the more electron-dense margins of the vesicles, this was included in the area assessed. These results were obtained from a minimum of four experiments in which 50–120 cells were examined. The quantitative data shown for single label experiments were obtained using 18-nm diameter protein A–gold. The distribution of labeling in both the single- and double-labeled experiments was independent of gold particle size, although in general more of the smaller gold particles were bound per unit area.

Control experiments using (*a*) non-immune rabbit serum or (*b*) 1% BSA/PBS showed no localized labeling in untreated or monensin-treated chondrocytes and the average density of protein A–gold over the cells was <2 gold particles/μm^2 (Fig. 2).

The density of label within each compartment for each treatment of the cells was analyzed by Wilcoxon Paired Rank Sum Tests on all available pairs. *P* values larger than 0.005 were considered as not significant. The density of label in each compartment was compared between normal, and 0.1 μM and 1.0 μM monensin–treated cells using one-way analysis of variance.

RESULTS

Normal Chondrocytes

The chondrocytes in culture showed a normal general morphology (Fig. 3, *a* and *b*). Sections embedded in Spurr resin showed abundant RER and Golgi stacks composed of flattened cisternae closely apposed to each other. Planimetric determinations showed the RER to occupy more than nine times the volume of the smooth membrane Golgi components. Previous experiments showed the cells to synthesize and secrete into the medium the aggregating chondroitin sulphate proteoglycan typical of cartilage (5).

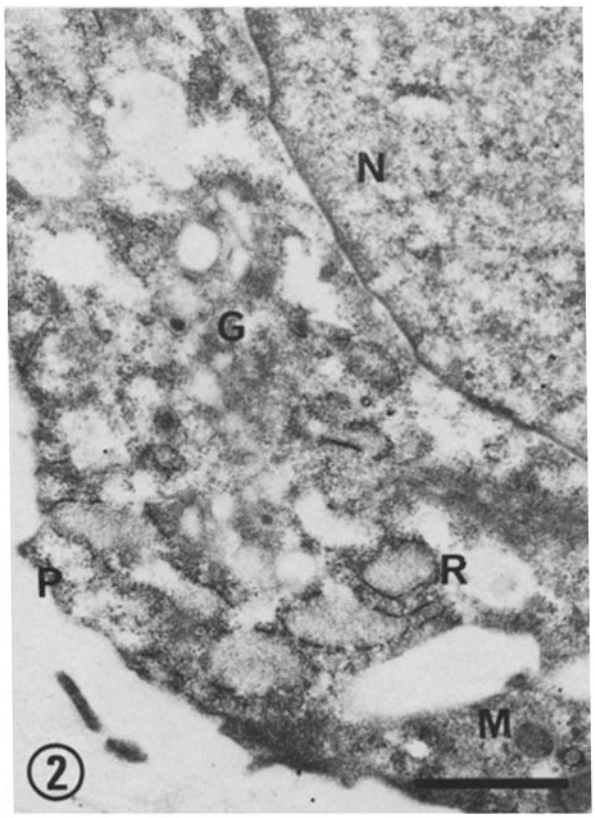


FIGURE 2 Chondrocyte embedded in Lowicryl k4M resin, non-immune serum control. A chondrocyte section incubated with non-immune rabbit serum followed by protein A–gold (18-nm diameter) as an immunolabeling control. This section shows Golgi region (*G*), RER (*R*), mitochondria (*M*), nucleus (*N*), and plasma membrane (*P*). There is no localized labeling and the density of protein A–gold over the cells is low (<2 gold particles/μm^2). Bar, 1.0 μm. × 22,200.

To identify the intracellular compartments containing proteoglycan at different stages of biosynthesis, Lowicryl-embedded sections of chondrocytes were incubated with antibodies to specific proteoglycan components, followed by localization with protein A–gold. Preliminary experiments showed localization in Spurr-embedded non-osmicated tissue to be unsuccessful and low-temperature Lowicryl K4M resin was thus chosen as it gave good ultrastructural preservation together with efficient immunocytochemical labeling (2, 3, 44, 50) which was also appropriate for quantitative analysis (2, 44).

IMMUNOLOCALIZATION OF PROTEOGLYCAN PROTEIN CORE: When antiserum to the binding region of proteoglycan was used to localize the protein core, the labeling was not abundant, but occurred mainly over the Golgi region and in membrane-bound secretory vesicles (Fig. 4) (5). The distribution was similar to that we had previously observed in chondrocytes within articular cartilage (44). Quantitative evaluation of the distribution of the protein A–gold (Table I)

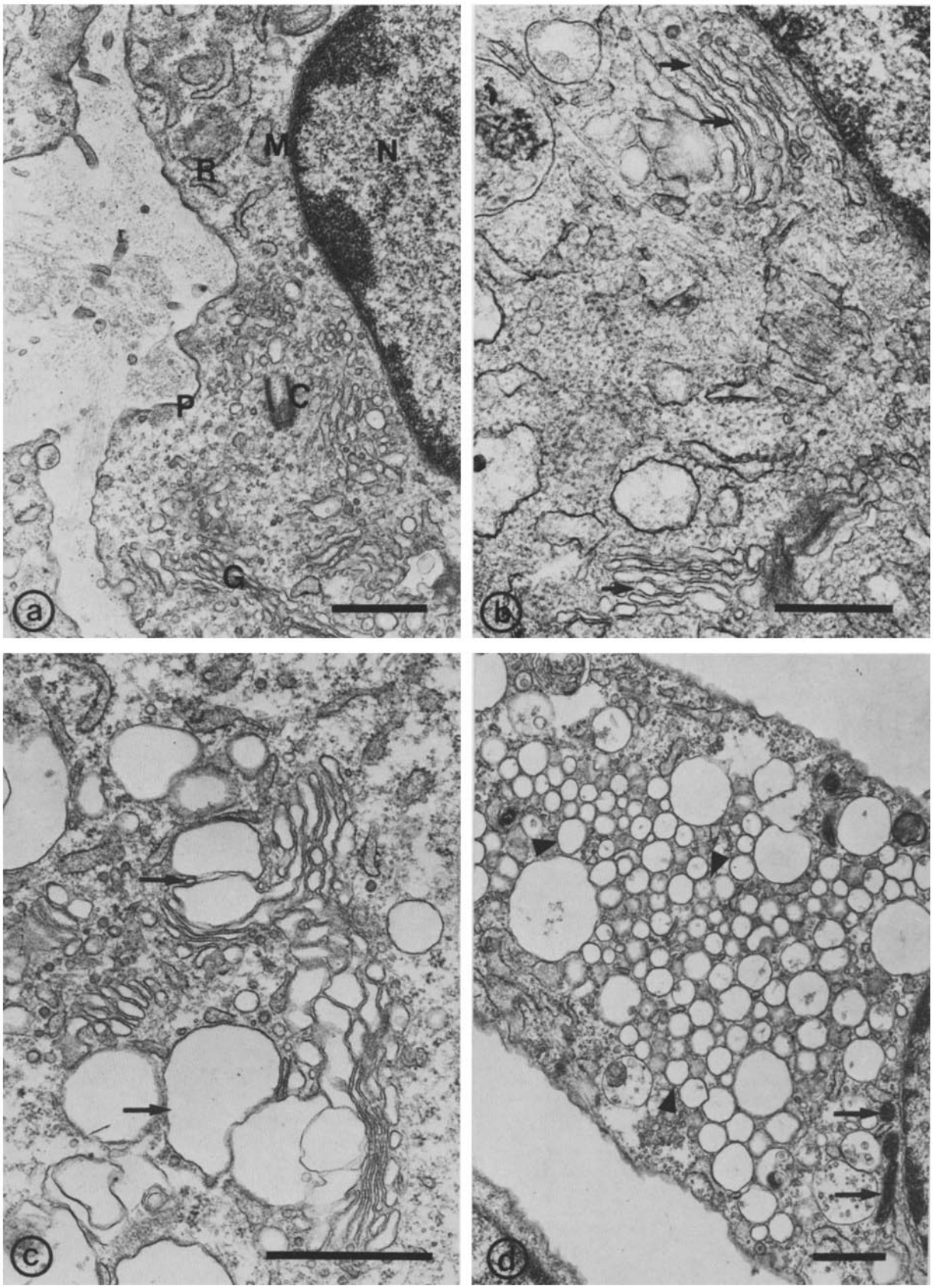
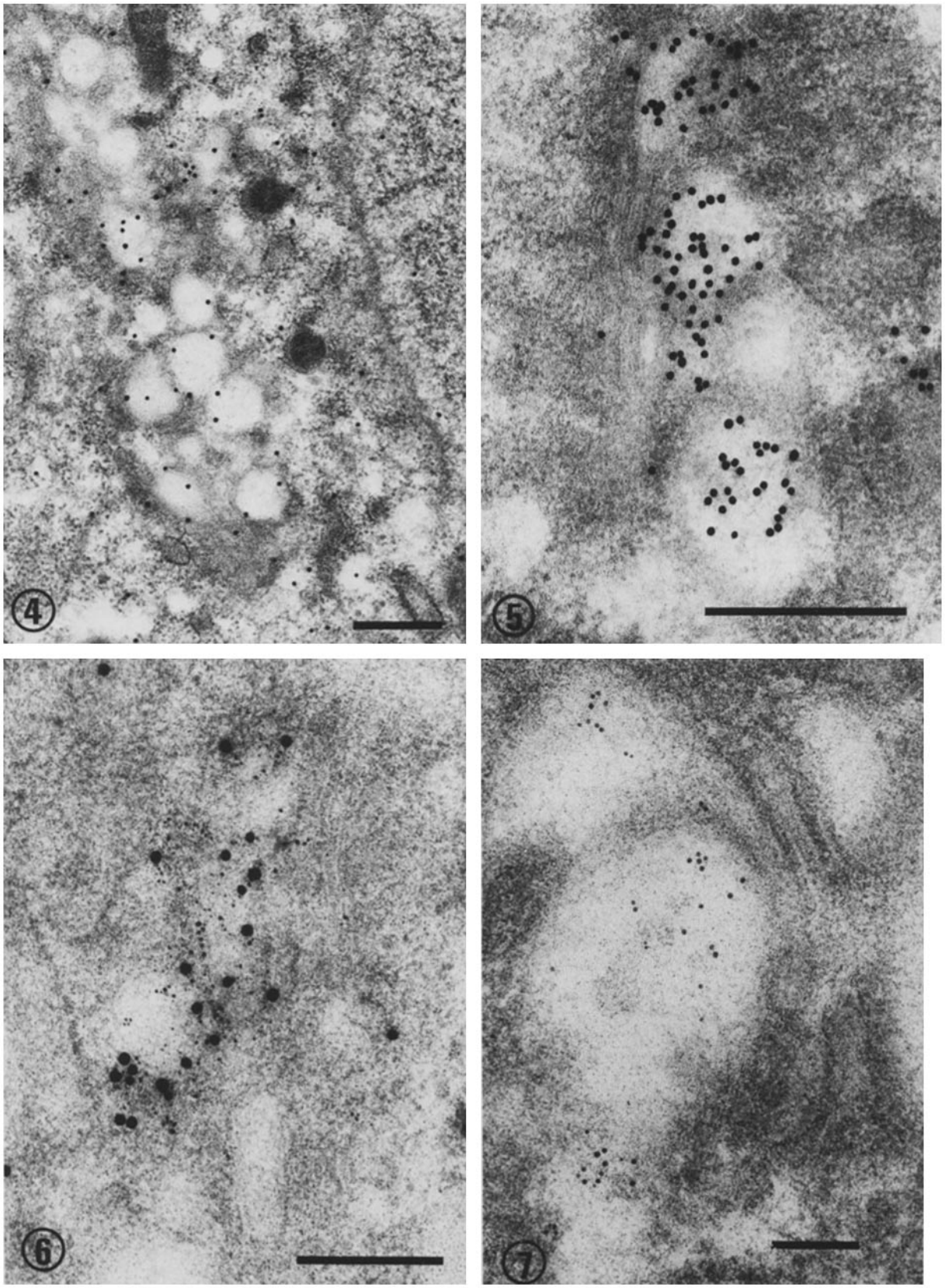


FIGURE 3 Chondrocytes treated with various concentrations of monensin and embedded in Spurr resin. (*a*) Control chondrocyte showing Golgi stacks (*G*), RER (*R*), mitochondria (*M*), nucleus (*N*), plasma membrane (*P*), and centriole (*C*). × 17,000. (*b*) Control chondrocyte at higher magnification (× 39,000) showing typical Golgi stacks (arrows). (*c*) Chondrocyte treated with 0.1 μM monensin showing some distended cisternae of the Golgi stacks (arrows). × 29,000. (*d*) Chondrocyte treated with 1.0 μM monensin showing a large number of distended vesicles (arrowheads), and condensed mitochondria (arrows). × 13,000. Bars, (*a*, *c*, and *d*) 1 μm and (*b*) 0.5 μm.



FIGURES 4–7 Chondrocytes embedded in Lowicryl k4M resin, and proteoglycan protein core, medial/*trans*-Golgi, and chondroitin sulfate localized with protein A–gold. (Fig. 4) Chondrocytes showing a low density of labeling of protein core in the Golgi complex (18-nm diameter protein A–gold). (Fig. 5) Golgi stack in a chondrocyte labeled with ricin and anti-ricin antibodies followed by protein A–gold (18-nm diameter). The immunolabel binds to cisternae and vesicles only on one side of the stack, indicating these to be the medial/*trans*-Golgi compartments, with the unlabeled cisternae being on the *cis* side of the Golgi stack. (Fig. 6) Golgi stack in a chondrocyte double-labeled for ricin (4-nm diameter protein A–gold) and protein core (18-nm diameter protein A–gold). The immunolabel for ricin and protein core co-localize, and are present only on one side of the Golgi stack. (Fig. 7) Golgi stack in a chondrocyte labeled for chondroitin sulfate (4-nm diameter protein A–gold). The label is seen only on one side of the Golgi stack. Bars, 5, 0.5, 0.25, and 0.125 μm, respectively. × 25,000, 67,500, 100,000, and 136,000, respectively.

TABLE I. *Density of Protein A-Gold Labeling of Proteoglycan Protein Core Over Cellular Compartments (Gold Particles/μm^2 ± SD).*

	Golgi region*	RER	Nucleus	Mitochondria	Cytoplasm
Control	35.0 ± 18.8	7.2 ± 3.7	2.4 ± 1.5	5.1 ± 4.1	2.3 ± 1.1
Monensin (0.1 μM)	48.2 ± 28.0	9.8 ± 3.6	2.0 ± 0.8	2.0 ± 1.9	1.8 ± 0.8
Monensin (1.0 μM)	60.1 ± 26.0	13.8 ± 8.3	2.4 ± 1.5	2.5 ± 4.1	2.0 ± 0.9

The density of label for binding region in the untreated and monensin-treated cells over the Golgi region and RER was significantly different from each other and from all other compartments ($P < 0.0001$) except RER versus mitochondria in untreated cells ($P > 0.05$). The density of label in the nucleus, cytoplasm, and mitochondria was not significantly different from each other ($P > 0.05$). Comparison of each subcellular compartment in normal, and in 0.1 μM and 1.0 μM monensin-treated cells showed a significant increase in the density of label in the Golgi region ($P = 0.0006$) and RER ($P = 0.0007$) of treated cells, but there was no significant difference in the other compartments ($P > 0.1$).

* All smooth-membraned vesicles.

TABLE II. *Single-Label Experiments*

	Labeled vesicles (% of total)		
	Binding region	Ricin	Chondroitin sulfate
Control	50	50	50
Monensin (0.1 μM)	78	46	48
Monensin (1.0 μM)	90	47	48

Chondrocyte sections were labeled with specific antibodies and protein A-gold, and the proportion of smooth membrane-bound vesicles containing immunolabel was determined.

TABLE III. *Double-Label Experiments for Binding Region and Ricin**

	Labeled vesicles (% of total)		
	$BR^+/ricin^-$	$BR^+/ricin^+$	$BR^-/ricin^+$
Control	7	86	7
Monensin (0.1 μM)	47	51	2
Monensin (1.0 μM)	54	46	0

* BR, binding region of proteoglycan protein core.

confirmed this specificity of labeling, the Golgi region was the most densely labeled cellular compartment, and 50% of the vesicles were labeled (Table II). The labeling of other cellular compartments was low, but showed a slight increase over the cisternal space of the RER, which was significantly higher than the background labeling of nuclear and cytoplasmic compartments, but was not above that of the mitochondria. The general level of background labeling was similar to that previously reported using similar techniques for detecting other intracellular antigens (2).

IMMUNOLOCALIZATION OF MEDIAL AND *TRANS*-GOLGI ELEMENTS: To determine the sites of protein core localization within the Golgi complex, ricin was used as a selective marker of galactose-rich membranes that are characteristic of medial and *trans*-Golgi elements (13). When sections of chondrocytes were labeled with ricin and anti-ricin antibodies, followed by protein A-gold, the immunolabel bound to the cisternae only on one side of the stack (Fig. 5) which suggested these to be the medial/*trans*-subcompartments and those unlabeled to be on the *cis* side of the Golgi stack (13). The ricin labeled 50% of the total smooth-membraned vesicles of the control cells (Table II). It also labeled the plasma membrane as has been previously reported (13, 38) but other membranes including RER did not label. As a control, the ricin was incubated with 300 mM D-galactose for 10 min before the labeling procedure (13) and this was found to inhibit binding completely (density of gold particles, <2 per μm^2).

The origin of the vesicles which labeled for binding region was determined in double-label experiments in which ricin and binding region labeling were distinguished using gold particles of two sizes (4-nm and 18-nm diameter). In normal chondrocytes (Table III) a high proportion (80%) of labeled vesicles contained both sizes of protein A-gold (Fig. 6), indicating that proteoglycan protein core detected was only in medial/*trans*-Golgi compartments.

IMMUNOLOCALIZATION OF CHONDROITIN SULFATE: Evidence suggests that the synthesis of chondroitin sulfate chains on the protein core occurs in the Golgi complex (12, 29, 41, 45, 71–73) but its precise location within some or all of the Golgi compartments has not previously been determined. A mixture of monoclonal antibodies directed against chondroitinase ABC-digested residues of 6-sulfated, 4-sulfated, and non-sulfated chains was therefore used to localize proteoglycans bearing chondroitin sulfate in the chondrocytes.

In normal cells the immunolabel for chondroitin sulfate was observed only in the Golgi region, and the other cellular compartments including the RER did not contain label above background levels (Table IV). Within the Golgi region, the label was found in 50% of the vesicles and preferentially on one side of the Golgi stack (Fig. 7) (Table II). This distribution of the immunolabel for chondroitin sulfate on proteoglycan was similar to that observed for ricin labeling of the Golgi elements, except that the plasma membrane was not labeled. The origin of the vesicles containing chondroitin sulfate was determined with chondroitin sulfate antibodies and ricin, in double-labeling experiments. In the normal chondrocytes, ricin and chondroitin sulfate labeling showed the same distribution with <5% of the labeled vesicles containing only one size of the protein A-gold (Table V). Chondroitin sulfate was thus shown to be present with the proteoglycan protein core only within the medial/*trans*-Golgi compartments.

Chondrocytes Treated with Monensin

In chondrocytes treated with monensin, the morphology of the Golgi complex changed dramatically (5). After a 4-h incubation with 0.1 μM monensin, the Golgi stacks were often replaced by large vesicles, probably formed by extensive swelling of the Golgi cisternae (Fig. 3*c*) and there was also dilation of some cisternae of the RER. After incubation with 1.0 μM monensin, the dilation of the RER became more pronounced, Golgi stacks were often no longer apparent, and the number of smooth-membraned vesicles increased sixfold (Fig. 3*d*). Other cellular features appeared unaltered except that the mitochondria were more electron dense, as previously reported (64).

IMMUNOLOCALIZATION OF PROTEOGLYCAN PROTEIN CORE: Immunolocalization of binding region showed there to be a dose-dependent increase in the density of labeling

TABLE IV. *Density of Protein A-Gold Labeling of Chondroitin Sulfate over Cellular Compartments (Gold Particles/μm^2 ± SD)*

	Golgi region*	RER	Nucleus	Mitochondria	Cytoplasm
Control	57.7 ± 24.5	2.1 ± 3.0	4.7 ± 3.8	8.6 ± 6.7	2.9 ± 2.4
Monensin (0.1 μM)	76.8 ± 48.1	2.7 ± 4.3	4.1 ± 2.7	6.4 ± 7.5	3.3 ± 1.7
Monensin (1 μM)	51.9 ± 33.6	3.3 ± 3.0	8.5 ± 5.5	5.4 ± 5.7	3.6 ± 2.0

In the untreated and monensin-treated cells, the density of label for chondroitin sulfate in the Golgi region was significantly higher ($P < 0.002$) than in all other compartments, whereas the density of label in the RER was not significantly higher than any other compartment. Comparison of the Golgi region in normal, and in 0.1 μM and 1.0 μM monensin-treated cells showed no significant change in the density of labeling ($P = 0.07$). Comparison of other cellular compartments also showed no significant change ($P > 0.01$) with monensin treatment.

* All smooth-membraned vesicles.

TABLE V. *Double-Label Experiments for Chondroitin Sulphate and Ricin*

	Labeled vesicles (% of total)		
	CS^+/ $ricin^-$	$CS^+/ricin^+$	CS^-/ $ricin^+$
Control	<5	>95	<5
Monensin (0.1 μM)	<5	>95	<5
Monensin (1.0 μM)	<5	>95	<5

CS, chondroitin sulfate.

of protein core within the RER (Table I, Fig. 8). There was also an increase in the volume of the cisternal space of the RER, and thus a marked accumulation of protein core within this compartment. Within the Golgi region there was an increase in the number of vesicles and also an increase in the percentage of vesicles containing immunolabel (Table II, Figs. 9 and 10). The number of gold particles per vesicle also increased by 15% at 0.1 μM monensin and by 40% at 1.0 μM monensin, and this corresponded to a dose-dependent increase in the density of labeling. The proteoglycan protein core was thus shown to accumulate along the intracellular pathway involved in its biosynthesis as previously reported (5).

IMMUNOLOCALIZATION OF MEDIAL AND *TRANS*-GOLGI ELEMENTS: To determine the origin of the vesicles in the Golgi complex in cells treated with monensin, sections were labeled with ricin and anti-ricin antibodies. Although the number of vesicles per cell increased, the proportion that was labeled with ricin did not change significantly: 46% of vesicles was labeled at 0.1 μM and 47% at 1.0 μM (Table II). As a similar proportion of the newly formed vesicles were labeled with ricin, it suggested that these vesicles were derived from both the *cis*- and medial/*trans*-Golgi cisternae.

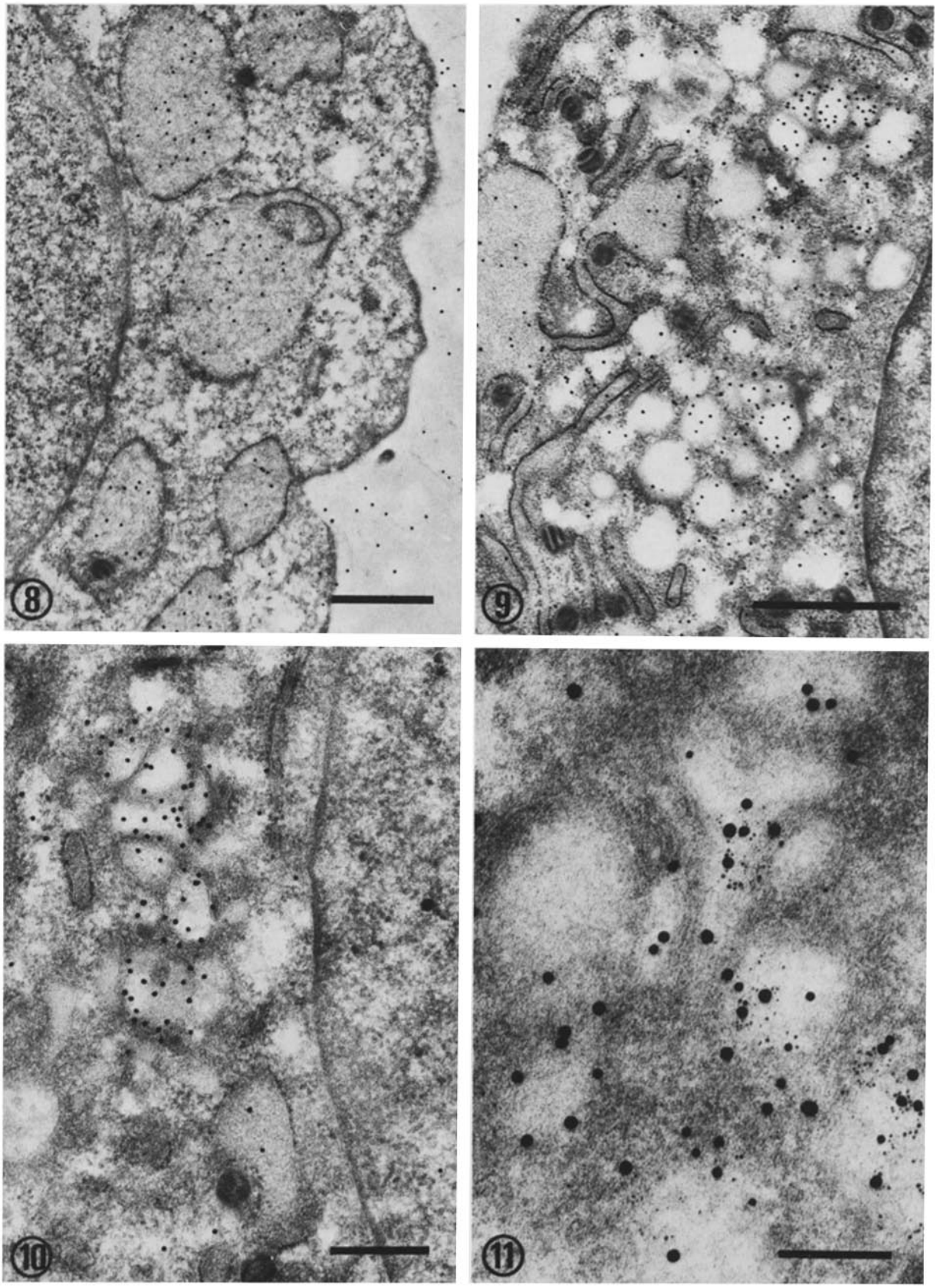
The origin of the vesicles that labeled for binding region in the monensin-treated chondrocytes was determined in double-label experiments for ricin and binding region. With increasing concentrations of monensin, the percentage of labeled vesicles that were double-labeled decreased from 86% in controls to 50% at 0.1 μM monensin and to 46% at 1.0 μM monensin (Table III) (Fig. 11). This corresponded to an increase in the number of vesicles which labeled for binding region alone, which was only 7% in control cells, but increased to 54% at 1.0 μM monensin. Together these results suggested that monensin blocked translocation within or movement out of the medial/*trans*-cisternae of the Golgi stack, producing an accumulation of the proteoglycan protein core in vesicles derived from both the medial/*trans*- and *cis*-Golgi elements in addition to its accumulation within the cisternae of the RER.

IMMUNOLOCALIZATION OF CHONDROITIN SULFATE: In chondrocytes treated with monensin, chondroitin sulfate was localized more abundantly, but the distribution of immunolabel remained the same as that in untreated cells. The immunolabel was only found in Golgi-type vesicles (Table IV) and the proportion of vesicles that contained label was similar to untreated controls being 48% at both 0.1 μM and 1.0 μM monensin (Table II). Furthermore, in double-label experiments the ricin and chondroitin sulfate labeling showed the same distribution as in control cells with >95% of labeled vesicles containing both sizes of gold particles (Table V). These results show that chondroitin sulfate is only detected on proteoglycan protein core within the medial/*trans*-Golgi elements and not within *cis*-Golgi or RER, at earlier sites along the pathway of biosynthesis.

DISCUSSION

Intracellular localization of proteoglycans in both normal cartilage (44) and isolated chondrocytes (5) with antibodies to the binding region showed a limited distribution within some Golgi cisternae and within secretory vesicles. In this study, the binding region was only detected in abundance within the Golgi compartment in normal chondrocytes. The failure to detect protein core extensively within RER of the chondrocytes may be because the concentration of antigen there is too low. The volume of the RER within the cells is much greater than that of the Golgi compartment (approximately ninefold) and so if the normal pathway of secretory protein biosynthesis involves passage from the RER through the Golgi compartment on a route to secretion, then the protein is likely to be much less concentrated within the RER than within the Golgi compartment. A concentration of antigen in RER as low as one-ninth of that in the Golgi compartment might therefore be towards the limit of detection. It is also apparent that proteoglycan accounts for only a small proportion of total protein synthesis in chondrocytes (5) and the labeling density achieved within the ricin-positive Golgi compartments was <20% of that detected in the pericellular matrix of intact cartilage (44).

The detection of proteoglycan binding region within intracellular compartments may also have been hindered by the antigen's inaccessibility to the antibody within the membrane-bound compartments. However, this is unlikely as the localization of chondroitin sulfate within the Golgi vesicles did not give a much higher density of labeling than for binding region, and chondroitin sulfate is unlikely to be inaccessible because of association with membranes as it is very hydrophilic, and there are many copies of the epitope on each proteoglycan that should facilitate its detection. Furthermore, as the anti-binding region antiserum used contained antibodies to both native (43) and unfolded structures (Ratcliffe, A., and T. Hardingham, unpublished results), it is unlikely to have been limited by any delay in the formation of native conformations



FIGURES 8–11 Chondrocytes were treated with various concentrations of monensin, embedded in Lowicryl k4M resin, and the protein core, medial/*trans*-Golgi, and chondroitin sulfate localized with protein A–gold. (Fig. 8) Chondrocyte treated with 10 μM monensin showing labeling (18-nm diameter protein A–gold) for binding region in distended cisternae of the RER. (Fig. 9) Chondrocyte treated with 0.1 μM monensin showing the majority of vesicles labeled for binding region, with some of the immunolabel also in the distended cisternae of the RER (18-nm diameter protein A–gold). (Fig. 10) Chondrocyte treated with 1.0 μM monensin showing vesicles that are heavily labeled for binding region. (Fig. 11) Chondrocyte treated with 0.1 μM monensin and double-labeled for ricin (4-nm diameter protein A–gold) and binding region (18-nm diameter protein A–gold). Dilated Golgi cisternae can be seen, with the vesicles to the right labeled with both ricin and binding region (medial/*trans*-Golgi region) and those to the left labeled for binding region alone (*cis*-Golgi region). Bars, (Figs. 8 and 9) 1 μm; (Fig. 10) 0.5 μm; (Fig. 11) 0.2 μm. × 18,000, 25,700, 38,000, and 108,000, respectively.

in the binding region of the nascent protein core. The low detection of proteoglycan protein core within the RER may thus be an accurate reflection of its low concentration at the site of protein translation.

The carboxylic ionophore monensin interferes with the intracellular translocation of secretory proteins in many cell types without major inhibition of protein synthesis (14, 26, 35, 59, 60, 63–65, 69). In chondrocytes it has been shown to inhibit proteoglycan secretion and ^{35}S incorporation (5, 40) and to lead to an intracellular accumulation of proteoglycan protein core (40) which was localized in many newly formed Golgi vesicles and within the distended cisternae of the RER (5). In the present study, the identity of the vesicles containing protein core and those containing chondroitin sulfate were explored using the lectin from *Ricinus communis* which was shown to distinguish medial/*trans*-Golgi from *cis*-Golgi elements in baby hamster kidney cells (13), and in these normal pig cartilage chondrocytes it showed similar selective labeling. The results obtained by localizing proteoglycan protein core and ricin in double-label experiments showed monensin to block protein core transport into the galactose-rich compartment of the medial/*trans*-Golgi cisternae (Fig. 10). This is the same site of action as reported in baby hamster kidney cells (14) but present results would also suggest some inhibition of translocation from *trans*-Golgi elements into secretory vesicles (42).

The effect of monensin on the detection of chondroitin sulfate was quite different. Although as with binding region it was only detected within ricin-positive smooth membraned vesicles in normal chondrocytes, after monensin treatment it continued to be localized only within the same ricin-positive medial/*trans*-Golgi vesicles. Localization in chondrocytes treated with even low concentrations of monensin (0.1 μM) thus showed a clear distinction between proteoglycan protein core and chondroitin sulfate distribution. The failure to detect chondroitin sulfate outside the medial/*trans*-Golgi compartment, even when protein core was very abundant in *cis*-Golgi and RER compartments, strongly suggests the medial/*trans*-Golgi compartment to be the only site of chondroitin sulfate synthesis on the proteoglycan (see Fig. 12). It also suggests that monensin causes no reversal of normal vectorial transfer, i.e., back from the medial/*trans*-Golgi compartment into *cis*-Golgi and RER compartments. The high coincidence between the dual labeling of chondroitin sulfate and ricin also suggests that synthesis occurs immediately on transfer into medial Golgi elements, before movement into *trans* elements, or throughout the medial/*trans*-Golgi elements, which may function as a single compartment (52, 53). These results extend recent evidence from immunofluorescence studies on chondroitin sulfate synthesis (71) which showed, using the same anti-chondroitin sulfate antibodies as in this study, that chondroitin sulfate was localized in a perinuclear (Golgi) region. This was supported by subcellular fractionation of chondrocytes (11), in which chondroitin sulfate synthesis was localized in smooth membrane (Golgi) fractions although xylosyl transferase activity has been detected more in rough microsomal fractions (25). Vertel and Barkman (71) also demonstrated by immunofluorescence that chondroitin sulfate and keratan sulfate co-localized within Golgi vesicles, suggesting that chondroitin sulfate and keratan sulfate are synthesized in the same compartment. Experiments on the kinetics of biosynthesis of chondroitin sulfate and o-linked oligosaccharides in chondrocytes showed them both to be synthesized as proteoglycan as a late event in intracellular processing (31, 33). The present results on the siting of chondroitin sulfate synthesis within the medial/*trans*-Golgi compartments are thus entirely compatible with other evidence of its location.

Although monensin strongly inhibited (80%) the incorporation of [^{35}S]sulfate into chondroitin sulfate in chondrocytes (5), this was accompanied by increased intracellular localization of chondroitin sulfate in medial/*trans*-Golgi elements. There are two main factors which may account for this. Firstly, the epitopes by which chondroitin sulfate was detected are generated by chondroitinase ABC digestion and occur one per chondroitin sulfate chain irrespective of its initial chain length before digestion. Secondly, in the presence of monensin, the length of chondroitin sulfate chains has been shown to be greatly reduced (37, 60). The presence of chondroitin sulfate within all newly formed ricin-positive vesicles after monensin treatment is therefore compatible with the synthesis of very short chondroitin sulfate chains on the proteoglycan that accumulated in the medial/*trans*-Golgi compartment (see Fig. 12). The main effect of monensin in inhibiting chondroitin sulfate synthesis may not therefore be caused by it restricting the supply of protein core to the site of chondroitin sulfate synthesis, but by direct inhibition of chondroitin sulfate chain elongation. This is supported by results which

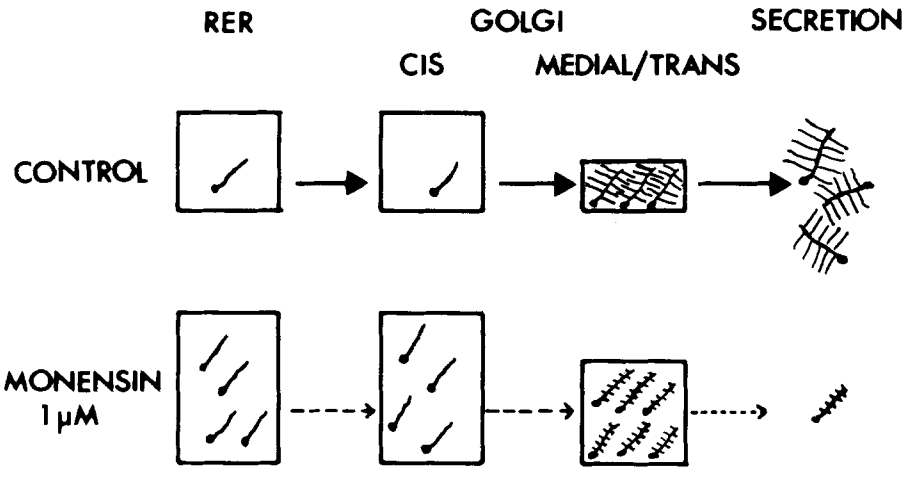


FIGURE 12 Summary of the immunolocalization of proteoglycan protein core and chondroitin sulfate in the RER, *cis*-Golgi (ricin-negative) and medial/*trans*-Golgi (ricin-positive) compartments of control and monensin-treated chondrocytes. In control chondrocytes, low concentration of protein core was detected in the RER, and within the Golgi region the protein core was detected mainly in the medial/*trans*-Golgi compartments, where chondroitin sulfate was also localized. In monensin-treated cells, secretion of proteoglycan was inhibited and protein core accumulated in all Golgi compartments and also in the RER. In contrast, chondroitin sulfate remained restricted to medial/*trans*-Golgi elements.

showed that addition of β-xylosides, which would provide ample substrate for chain elongation (46, 47), failed to give much relief to monensin inhibition of chondroitin sulfate synthesis in chondrocytes (37). However, as chondroitin sulfate synthesis requires the transfer of nucleotide sugar and sulfate intermediates from the cytosol into Golgi compartments (55), one of the effects of monensin may be to inhibit this transfer and thereby deplete the glycosyl transferases and sulphotransferases of these substrates. Proteoglycan protein core may thus continue to arrive in the medial/*trans*-Golgi cisternae, but inhibition of elongation may lead to the premature termination of chondroitin sulfate chains.

In many cell types the medial/*trans*-Golgi cisternae have been shown to be the site of late modifications of N-linked oligosaccharide synthesis involving galactose and sialic acid transfer (7, 51, 52, 57, 66) and also a major site of O-linked oligosaccharide completion (8, 49, 58). As chondroitin sulfate co-distributed with all the galactose-rich cisternae of the medial/*trans*-Golgi cisternae of the chondrocytes in this study, it suggests that chondroitin sulfate synthesis occurs on proteoglycan in a compartment in common with N-linked oligosaccharide processing and O-linked oligosaccharide completion on other secretory glycoproteins. This implies that proteoglycans are not segregated into a separate compartment for glycosaminoglycan synthesis and that the selection of protein core for glycosaminoglycan synthesis may depend primarily upon the specificity of the glycosyl transferases involved in their biosynthesis. This adds to the range of glycosyl transferase activities associated with medial/*trans*-Golgi compartment and suggests that it can function as a site of biosynthesis of all classes of O-linked carbohydrate substituents of secretory glycoproteins.

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REFERENCES

1. Bendayan, M. 1982. Double immunocytochemical labeling applying the Protein A-gold technique. *J. Histochem. Cytochem.* 30:81–85.
2. Bendayan, M., and T. B. Orstavik. 1982. Immunocytochemical localization of kallikrein in the rat exocrine pancreas. *J. Histochem. Cytochem.* 30:58–66.
3. Bendayan, M., and G. C. Shore. 1982. Immunocytochemical localization of mitochondrial proteins in the rat hepatocyte. *J. Histochem. Cytochem.* 30:139–147.
4. Bonnet, F., D. G. Dunham, and T. E. Hardingham. 1985. Structure and interactions of cartilage proteoglycan binding region and link protein *Biochem. J.* 228:77–85.
5. Burditt, L. J., A. Ratcliffe, P. R. Fryer, and T. Hardingham. 1985. The intracellular localization of proteoglycan and its accumulation in chondrocytes treated with monensin. *Biochim. Biophys. Acta.* 844:247–255.
6. Couchman, J. R., B. Caterson, J. E. Christner, and J. R. Baker. 1984. Mapping by monoclonal antibody detection of glycosaminoglycans in connective tissue. *Nature (Lond.).* 307:650–652.
7. Dunphy, W. G., and J. E. Rothman. 1983. Compartmentation of asparagine-linked oligosaccharide processing in the Golgi apparatus. *J. Cell. Biol.* 97:270–275.
8. Elhammer, A., and S. Kornfeld. 1984. Two enzymes involved in the synthesis of O-linked oligosaccharides are localized on membranes of different densities in mouse lymphoma BW5147 cells. *J. Cell. Biol.* 99:327–331.
9. Fellini, S. A., V. C. Hascall, and J. H. Kimura. 1984. Localization of proteoglycan core protein in subcellular fractions isolated from rat chondrosarcoma chondrocytes. *J. Biol. Chem.* 259:4634–4641.
10. Fryer, P. R., C. Wells, and A. Ratcliffe. 1983. Technical difficulties overcome in the use of Lowicryl K4M EM embedding resin. *Histochemistry.* 77:141–143.
11. Geetha-Habib, M., S. C. Campbell, and N. B. Schwartz. 1984. Subcellular localization of the synthesis and glycosylation of chondroitin sulfate proteoglycan core protein. *J. Biol. Chem.* 259:7300–7310.
12. Godman, G. C., and N. Lane. 1964. On the site of sulfation in the chondrocytes. *J. Cell. Biol.* 21:353–366.
13. Griffiths, G., R. Brands, B. Burke, D. Louvard, and G. Warren. 1982. Viral membrane proteins acquire galactose in *trans* Golgi cisternae during intracellular transport. *J. Cell. Biol.* 95:781–792.
14. Griffiths, G., P. Quinn, and G. Warren. 1983. Dissection of the Golgi complex. I. Monensin inhibits the transport of viral membrane proteins from medial to *trans* Golgi cisternae in baby hamster kidney cells infected with Semliki Forest virus. *J. Cell. Biol.* 96:835–850.
15. Hardingham, T. E. 1979. The role of link-protein in the structure of cartilage proteoglycan aggregates. *Biochem. J.* 177:237–247.
16. Hardingham, T. 1981. Proteoglycans: their structure, interactions, and molecular organization in cartilage. *Biochem. Soc. Trans.* 9:489–497.
17. Hardingham, T. 1982. Control of structural diversity in proteoglycans. *Prog. Clin. Biol. Res.* 102A:423–434.
18. Hardingham, T., L. Burditt, and A. Ratcliffe. 1984. Studies on the synthesis, secretion and assembly of proteoglycan aggregates by chondrocytes. *Prog. Clin. Biol. Res.* 151:17–29.
19. Hardingham, T. E., and H. Muir. 1972. The specific interaction of hyaluronic acid with cartilage proteoglycans. *Biochim. Biophys. Acta.* 279:401–405.
20. Hascall, V. C. 1977. Interaction of cartilage proteoglycans with hyaluronate. *J. Supramol. Struct.* 7:101–120.
21. Hascall, V. C., and G. K. Hascall. 1981. Proteoglycans. *In* Cell Biology of Extracellular Matrix. E. D. Hay, editor. Plenum Publishing Corp., New York. 39–63.
22. Hascall, V. C., and S. W. Sajdera. 1970. Physical properties and polydispersity of proteoglycan from bovine nasal cartilage. *J. Biol. Chem.* 245:4920–4930.
23. Heinegard, D. 1977. Polydispersity of cartilage proteoglycans. Structural variations with size and buoyant density of the molecules. *J. Biol. Chem.* 252:1980–1989.
24. Heinegard, D., and M. Paulsson. 1984. Structure and metabolism of proteoglycans. *In* Extracellular Matrix Biochemistry. K. A. Piez and A. H. Reddi, editors. Elsevier, New York, Amsterdam & Oxford. 277–328.
25. Hoffman, H., N. B. Schwartz, L. Roden, and D. Prockop. 1984. Location of xylosyl transferase in the cisternae of the rough endoplasmic reticulum of embryonic cartilage cells. *Connect. Tissue Res.* 12:151–164.
26. Kaariainen, L., K. Hashimoto, J. Saraste, I. Virtanen, and K. Penttinen. 1980. Monensin and FCCP inhibit the intracellular transport of alpha-virus membrane glycoproteins. *J. Cell. Biol.* 87:783–791.
27. Kajiwara, T., and M. L. Tanzer. 1982. Undersulfated proteoglycans are induced by the ionophore monensin: study of possible mechanisms. *Arch. Biochem. Biophys.* 214:51–55.
28. Kruppe, M. 1955. Uber die reaktionsfahigkeit pflanzlicher hamagglutinine mit wasserloslichen blutgruppenmuscoiden und einfacher gebauten kohlenhydratmolekulen. *Z. Physiol. Chem.* 299:277–282.
29. Kimata, K., M. Okayama, S. Susuki, I. Susuki, and M. Hasimo. 1971. Nascent mucopolysaccharides attached to the Golgi membrane of chondrocytes. *Biochim. Biophys. Acta.* 237:606–610.
30. Kimura, J. H., C. B. Caputo, and V. C. Hascall. 1981. The effect of cycloheximide on synthesis of proteoglycans by cultured chondrocytes from Swarm rat chondrosarcoma. *J. Biol. Chem.* 256:4368–4376.
31. Kimura, J. H., T. E. Hardingham, and V. C. Hascall. 1980. Assembly of newly-synthesized proteoglycan and link protein into aggregates in cultures of chondrosarcoma chondrocytes. *J. Biol. Chem.* 255:7134–7143.
32. Kimura, J. H., T. E. Hardingham, V. C. Hascall, and M. Solursh. 1979. Biosynthesis of proteoglycans and their assembly into aggregates in cultures of chondrocytes from the Swarm rat chondrosarcoma. *J. Biol. Chem.* 254:2600–2609.
33. Kimura, J. H., L. S. Lohmander, and V. C. Hascall. 1984. Studies on the biosynthesis of cartilage proteoglycan in a model system of cultured chondrocytes from the Swarm rat chondrosarcoma. *J. Cell. Biochem.* 26:261–278.
34. Kimura, J. H., E. J-M. Thonar, V. C. Hascall, A. Reiner, and A. R. Poole. 1981. Identification of core protein, an intermediate in proteoglycan biosynthesis in cultured chondrocytes from the Swarm rat chondrosarcoma. *J. Biol. Chem.* 256:7890–7897.
35. Ledger, P. W., N. Uchida, and M. L. Tanzer. 1980. Immunocytochemical localization of procollagen and fibronectin in human fibroblasts: effects of the monovalent ionophore, monensin. *J. Cell. Biol.* 87:663–671.
36. Mitchell, D., and T. Hardingham. 1981. The effects of cycloheximide on the biosynthesis and secretion of proteoglycans by chondrocytes in culture. *Biochem. J.* 196:521–529.
37. Mitchell, D., and T. Hardingham. 1982. Monensin inhibits synthesis of proteoglycan, but not of hyaluronate, in chondrocytes. *Biochem. J.* 202:249:254.
38. Nicholson, G. L. 1978. Ultrastructural localization of lectin receptors. *In* Advanced Techniques in Biological Electron Microscopy II. J. K. Koehler, editor. Springer-Verlag. 1–38.
39. Nishimoto, S. K., T. Kajiwara, P. W. Ledger, and M. L. Tanzer. 1982. Effects of the ionophore monensin on type II collagen and proteoglycan synthesis and secretion by cultured chondrocytes. *J. Biol. Chem.* 257:11712–11716.
40. Nishimoto, S. K., T. Kajiwara, and M. L. Tanzer. 1982. Proteoglycan core protein is accumulated in cultured chondrocytes in the presence of monensin. *J. Biol. Chem.* 257:10558–10561.
41. Pacifici, M., R. Soltesz, G. Tahl, D. J. Shanley, D. Boettiger, and H. Holtzer. 1984. Immunological characterization of the major chick cartilage proteoglycan and its intracellular localization in cultured chondroblasts: a comparison with type II proteoglycan. *J. Cell. Biol.* 97:1724–1735.
42. Pesonen, M., and L. Kaariainen. 1982. Incomplete complex oligosaccharides in Semliki Forest virus envelope proteins arrested within the cell in the presence of monensin. *J. Mol. Biol.* 158:213–230.
43. Ratcliffe, A., and T. Hardingham. 1983. Cartilage proteoglycan binding region and link protein. Radioimmunoassays and the detection of masked determinants in aggregates. *Biochem. J.* 213:371–378.
44. Ratcliffe, A., P. R. Fryer, and T. E. Hardingham. 1984. The distribution of aggregating proteoglycans in articular cartilage. Comparison of quantitative immunoelectron microscopy with radioimmunoassay and biochemical analysis. *J. Histochem. Cytochem.* 32:193–201.
45. Revel, J. P., and E. D. Hay. 1964. Light and electron microscopic studies of mucopolysaccharides in developing amphibian and mammalian cartilage. *Anat. Rec.* 148:326–344.

46. Robinson, H. C., M. J. Brett, P. J. Tralaggan, D. A. Lowther, and M. Okayama. 1975. The effect of D-xylose, β-D-xylosides and β-D-galactosides on chondroitin sulfate biosynthesis in embryonic chicken cartilage. *Biochem. J.* 148:25–34.
47. Robinson, J. A., and H. C. Robinson. 1981. Control of chondroitin sulfate biosynthesis. β-D-Xylopyranosides as substrates for UDP-galactose: D-xylose transferase from embryonic chick cartilage. *Biochem. J.* 194:839–846.
48. Roth, J. 1982. The protein A–gold (pAg) technique—a qualitative and quantitative approach for antigen localization on thin sections. *In* Techniques in Immunocytochemistry, Vol. 1. G. Bullock and P. Petrusy, editors. Academic Press, Ltd., London. 108–131.
49. Roth, J. 1984. Cytochemical localization of terminal *N*-acetyl-D-galactosamine residues in cellular compartments of intestinal goblet cells: implications for the topography of O-glycosylation. *J. Cell. Biol.* 98:399–406.
50. Roth, J., M. Bendayan, E. Carlemalm, W. Villiger, and M. Garavito. 1981. Enhancement of structural preservation and immunocytochemical staining in low temperature embedded pancreatic tissue. *J. Histochem. Cytochem.* 29:663–671.
51. Roth, J., and E. G. Berger. 1982. Immunocytochemical localization of galactosyltransferase in HeLa cells: co-distribution with thiamine pyrophosphatase in *trans*-Golgi cisternae. *J. Cell. Biol.* 93:223–229.
52. Rothman, J. E. 1981. The Golgi apparatus: two organelles in tandem. *Science (Wash. DC).* 213:1212–1219.
53. Rothman, J. E., K. L. Miller, and L. J. Urbani. 1984. Intercompartmental transport in the Golgi complex is a dissociative process: facile transport of membrane proteins between two Golgi populations. *J. Cell. Biol.* 99:260–271.
54. Schwartz, N. B. 1982. Regulatory mechanisms in proteoglycan biosynthesis. *In* Glycosaminoglycans and Proteoglycans in Physiological and Pathological Processes of Body Systems. R. S. Varma, R. Varma, and P. Warren, editors. Karger, Basel. 41–54.
55. Schwartz, J. K., J. M. Capasso, and C. B. Hirschberg. 1984. Translocation of adenosine 3′-phosphate 5′-phosphosulphate into rat liver Golgi vesicles. *J. Biol. Chem.* 259:3554–3559.
56. Slot, J., and H. J. Geuze. 1981. Sizing of protein A–colloidal gold in immunoelectron microscopy. *J. Cell. Biol.* 90:533–536.
57. Slot, J., and H. J. Geuze. 1983. Immunoelectron microscopic exploration of the Golgi complex. *J. Histochem. Cytochem.* 31:1049–1056.
58. Strous, G. J., P. V. Kerkhof, R. Willemsen, H. J. Geuze, and E. G. Berger. 1983. Transport and topology of galactosyl transferase in endomembranes of HeLa cells. *J. Cell. Biol.* 97:723–727.
59. Strous, G. J. A. H., and H. F. Lodish. 1980. Intracellular transport of secretory and membrane proteins in Hepatoma cells infected by vesicular stomatitis virus. *Cell.* 22:709–716.
60. Tajiri, K., N. Uchida, and M. L. Tanzer. 1980. Undersulfated proteoglycans are secreted by cultured chondrocytes in the presence of monensin. *J. Biol. Chem.* 255:6036–6039.
61. Tang, L. H., L. Rosenberg, A. Reiner, and A. R. Poole. 1979. Proteoglycans from bovine nasal cartilage. Properties of a soluble form of link protein. *J. Biol. Chem.* 254:10523–10531.
62. Tartakoff, A. M. 1983. Perturbation of vesicular traffic with the carboxylic ionophore monensin. *Cell.* 32:1026–1028.
63. Tartakoff, A. M., and P. Vassalli. 1977. Plasma cell immunoglobulin secretion. Arrest is accompanied by alteration of the Golgi complex. *J. Exp. Med.* 146:1332–1345.
64. Tartakoff, A., and P. Vassalli. 1978. Comparative studies of intracellular transport of secretory proteins. *J. Cell. Biol.* 79:694–707.
65. Tartakoff, A., and P. Vassalli. 1979. Plasma cell immunoglobulin M molecules: their biosynthesis, assembly and intracellular transport. *J. Cell. Biol.* 83:284–299.
66. Tartakoff, A. M., and P. Vassalli. 1983. Lectin-binding sites as markers of Golgi subcompartments: proximal-to-distal maturation of oligosaccharides. *J. Cell. Biol.* 97:1243–1248.
67. Thonar, E. J-M., J. H. Kimura, V. C. Hascall, and A. R. Poole. 1982. Enzyme-linked immunosorbent assay analyses of the hyaluronate-binding region and the link protein of proteoglycan aggregate. *J. Biol. Chem.* 257:14173–14180.
68. Treadwell, B. V., D. P. Mankin, P. K. Ho, and H. J. Mankin. 1980. Cell-free synthesis of cartilage proteoglycans. Partial identification of proteoglycan core and link proteins. *Biochemistry.* 19:2269–2275.
69. Uchida, N., H. Smilowitz, and M. L. Tanzer. 1979. Monovalent ionophores inhibit secretion of procollagen and fibronectin from cultured human fibroblasts. *Proc. Natl. Acad. Sci. USA.* 76:1868–1872.
70. Upholt, W. B., B. M. Vertel, and A. Dorfman. 1979. Translation and characterization of mRNAs in differentiating chicken cartilage. *Proc. Natl. Acad. Sci. USA.* 76:4847–4851.
71. Vertel, B. M., and L. L. Barkman. 1984. Immunofluorescence studies of chondroitin sulfate proteoglycan biosynthesis. The use of monoclonal antibodies. *Collagen Relat. Res.* 4:1–20.
72. Vertel, B. M., and A. Dorfman. 1978. An immunohistochemical study of extracellular matrix formation during chondrogenesis. *Dev. Biol.* 62:1–12.
73. Vertel, B. M., and A. Dorfman. 1979. Simultaneous localization of type II collagen and core protein of chondroitin sulfate proteoglycan in individual chondrocytes. *Proc. Natl. Acad. Sci. USA.* 76:1261–1264.
74. Vertel, B. M., W. B. Upholt, and A. Dorfman. 1984. Cell-free translation of messenger RNA for chondroitin sulfate proteoglycan core protein in rat cartilage. *Biochem. J.* 217:259–263.