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Comparison of Photoreceptor-specific Matrix Domains in the Cat and Monkey Retinas

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Two lectins, wheat germ agglutinin (WGA) and peanut agglutinin (PNA), were used to compare domains within the interphotoreceptor matrices (IPM) of the cat and monkey, two species where the morphological relationship between the retinal pigment epithelium (RPE) and photoreceptors is distinctly different. In the monkey, PNA labeling was heaviest over the cone outer segments and a discrete region of the interphotoreceptor matrix bordering the cone inner and outer segments—a region which has been termed the cone *matrix* sheath. Near the apical border of the RPE, the outer margin of the PNA-labeled matrix is surrounded by a circular array of apical microvilli. In the cat retina, PNA labeling was highest over a region of the IPM lying between the outer margin of the cone sheath processes and surrounding rod matrix. In contrast, intracellular labeling of cone inner and outer segments was sparse. The RPE apical processes forming the cone sheath were not labeled. In the monkey retina, WGA preferentially labeled rod outer segments and the region of the IPM around rod inner and outer segments. The cone matrix sheath was not preferentially labeled using this lectin. Rod inner segments and cone inner and outer segments were labeled moderately. In the cat retina, WGA labeling was dense over both rod outer segments and cone outer segments as well as the cone sheath. Rod and cone inner segments, as well as the IPM around both rods and cones, were moderately labeled. These observations suggest that the specialized processes arising from the apical surface of retinal pigment epithelial cells, together with photoreceptor-specific extracellular matrix domains, contribute to the formation of specific micro-environments around rod and cone photoreceptor cells.

Key words: photoreceptors; extracellular matrix; retinal pigment epithelium; lectin; retina.

1. Introduction

The rod and cone outer segments, comprising the distal portion of the photoreceptor cells, lie in close apposition to a monolayer of epithelial cells, the retinal pigment epithelium (RPE). The apical surface of the RPE has extensive microvillar and sheet-like processes which envelop the distal portion of the outer segments. In the cat retina, the RPE apical processes adjacent to cone photoreceptors form an elaborate array of closely packed lamellae which completely ensheath the outer segments. This concentric array, collectively termed the cone sheath (Steinberg and Wood, 1974; Fisher and Steinberg, 1982), is also present in the retinas of dog (Hebel, 1970) and albino rabbit (Scullica and Tangucci, 1968). The cone outer segments of monkey (Anderson and Fisher, 1979), human (Steinberg, Wood and Hogan, 1977) and numerous other species are also surrounded by elongated microvilli, but they are not organized into the multilayered, lamellar configuration displayed most strikingly in the cat retina.

A heterogeneous matrix, known as the interphotoreceptor matrix (IPM), occupies the extracellular compartment between the RPE and photoreceptors. This intervening matrix has been shown to contain a variety of glycoconjugates, including glycoproteins, glycosaminoglycans and proteoglycans (Adler and Severin, 1981a, b; Adler et al., 1988; Hageman and Johnson, 1990). The IPM is also the site of numerous cellular interactions between the RPE and photoreceptors (Bok, 1985). The extent to which the various IPM components mediate, or actually participate in, these varied interactions has yet to be determined.

Using histochemical and immunocytochemical techniques, it has been shown that some of these matrix constituents are confined to distinct regions of the IPM. Using the lectin peanut agglutinin (PNA) which preferentially binds terminal galactose-*N*-acetyl galactosamine (Gal-GalNAc) disaccharide residues of glycoconjugates (Lotan et al., 1975), Johnson, Hageman and Blanks (1985) observed preferential labeling of the IPM around human, monkey and pig cone inner and outer segments—a region they termed the cone *matrix* sheath. Localization of a matrix domain which appears identical to the cone matrix sheath has also been observed in human retina using the anionic dye cuprolinic blue (Varner et al., 1987). Preferential labeling of the IPM around rod inner and outer segments has been reported by Sameshima, Uehara and Ohba (1987) using wheat germ agglutinin (WGA), a lectin that binds terminal *N*-acetyl glucosamine (GlcNAc) and sialic acid residues (Nagata and Burger, 1974).

The presence of specialized RPE processes surrounding cone outer segments, as well as rod and-cone-specific matrix domains within the IPM, reinforces the concept of the interphotoreceptor space as

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a highly compartmentalized region. In this study, we used the lectins PNA and WGA to examine the IPM in the cat and rhesus monkey retinas. The results confirm that the IPM in both cat and monkey contains at least two photoreceptor cell-type specific matrix domains. However, the morphological relationship between the cones, the cone matrix sheath, and the RPE ensheathing processes is distinctly different in these two species.

2. Materials and Methods

Animals

Cats and Rhesus monkeys used in this experiment were entrained to a 12-hr light:12-hr dark lighting cycle for a minimum of 14 days. Four hours into the light period, animals were brought to a state of deep anesthesia and subsequently killed by i.v. or i.c. injection of sodium pentobarbital (65 mg ml^{-1}; Anthony Products Co.).

Rhesus monkeys were perfused via intracardiac cannulation with 1% paraformaldehyde, 1% glutaraldehyde in 0·10 M sodium phosphate buffer, pH 7·2. Cats were not perfused. Following enucleation, the anterior segments of eyes from both species were removed and the eye-cups immersed in fixative (same as monkey perfusion fixative) at 4°C for 1 hr. Following fixation, eye-cups were divided into quadrants, rinsed in buffer and serially dehydrated in graded methanol solutions. Uranyl acetate was included in the 70% methanol solution to stain the tissue en bloc and improve ultrastructural preservation (Erickson, Anderson and Fisher, 1987). Following dehydration, quadrants were infiltrated overnight at 4°C in a 1:1 mixture of methanol and L.R. White resin (Polysciences). Specimens were then transferred to undiluted L.R. White for several hours and subsequently embedded in L.R. White resin and polymerized at 53°C.

Light-Microscopic Lectin Cytochemistry

Sections 1 μm thick of normal monkey retina were taken with glass knives on a LKB III ultramicrotome and transferred to formvar- (Ladd) coated glass slides. Plexiglass wells were affixed to slides to contain solutions for incubating sections of tissue. Sections were wetted with a Tris-buffered saline (TBS) solution (0·02 M Tris, 0·15 M NaCl, 0·05% NaN_3, 0·1% bovine serum albumen, 0·1 mM Ca^{2+} as $CaCl_2$, 0·01 mM Mn^{2+} as $MnCl_2$, pH 7·3) for 15 min. After wetting, endogenous avidin binding on the sections was blocked by incubating sections in an avidin solution (stock concentration, Vector) for 15 min, followed by a thorough rinse with TBS and incubation in a biotin solution (stock concentration, Vector) for 15 min. After repeated rinsing, sections were incubated in 200 μl of biotinylated peanut agglutinin (200 μg ml^{-1}) in TBS or biotinylated wheat germ agglutinin (200 μg ml^{-1}) in TBS for 15 hr in a humidified chamber at room temperature. Sections on control slides were incubated in either TBS alone or in a lectin solution containing the appropriate inhibitory sugar. D-galactose (300 mM, Sigma) in TBS served as the inhibitor for PNA binding and *N*-acetylglucosamine (500 mM, Sigma) in TBS as the inhibitor for WGA binding. Biotinylated lectin solutions, as well as TBS and control solutions, were centrifuged at 10000 *g* for 5 min prior to use to remove suspended particulates. Following incubation in lectin, sections were thoroughly rinsed with TBS and then soaked in this wash solution for 10 min. This sequence of rinsing and soaking was done twice to remove unbound lectin from the sections. Excess TBS was blotted from the section without allowing the sections to dry. Auro-Probe EM streptavidin G15 (15 nm gold particle, Janssen Life Sciences) was diluted 1:20 in TBS and applied to sections. Sections were incubated in this streptavidin solution at room temperature in a humidified chamber for 2 hr, and rinsed and soaked for 5 min in TBS. This sequence was repeated three times to remove unbound streptavidin gold. The 15-nm gold particles are below the resolution of a light microscope, so to visualize the patterns of lectin binding, colloidal gold particles were silver-enhanced with an IntenSE II silver-enhancing system (Janssen Life Sciences). Briefly, equal quantities of initiator and enhancer solution are mixed and applied to sections. The enhancement process was observed by light microscopy, using a 10 × objective. When the pattern of labeling became apparent on sections, the enhancement process was terminated by rinsing sections with distilled H_2O and the enhancement time recorded. Sections were allowed to air-dry and coverslipped. Selected sections were photographed with Tech Pan black and white negative film (Kodak) on a Zeiss PM III light microscope.

Electron Microscopic Lectin Cytochemistry

Thin sections (approx. 80 nm) of L.R. White embedded cat and monkey retina were taken with a diamond knife and collected on 200-mesh hexagonal nickel grids. Incubation of grids was performed in multi-well porcelain trays in a humidified chamber at room temperature. The blocking and incubation steps as well as the solutions used in these steps were identical to those previously described for light-microscopic localization of lectin label. The resolution afforded by electron microscopy eliminated the need for silver enhancement of the gold label. To facilitate comparisons in labeling intensity, thin sections of cat and monkey retina were processed simultaneously.

At the conclusion of the labeling procedure, grids were rinsed thoroughly in TBS followed by repeated rinsing in distilled H_2O. Sections were then stained with 1% uranyl acetate for 12 min, rinsed in distilled

H_2O, stained in Reynold's lead citrate for 6 min and rinsed in distilled H_2O. Grids were exposed to vapors from a 2% osmium tetroxide solution for 1 hr to enhance contrast, and were carbon coated to improve thermal stability during electron microscopy.

3. Results

Light-Microscopic Lectin Cytochemistry

Light-microscopic examination of PNA labeling in the monkey retina showed that this lectin preferentially labels a discrete region within the interphotoreceptor space associated with cone inner and outer segments [Fig. 1 A]. This PNA-binding matrix surrounding primate cones has been described previously, and has been termed the cone matrix sheath (Johnson et al., 1985). Using a biotinylated lectin–avidin–Au technique followed by silver-enhancement, PNA labeling in the monkey appears as a dense vertical zone at the level of the cone outer segment that terminates at the apical surface of the RPE. This region narrows considerably along the lateral margin of the cone inner segment and ends at the level of the outer limiting lamina. Cone inner segments, rod inner and outer segments, as well as the region of IPM around rods, are only sparsely labeled. The RPE cytoplasm is not labeled.

In contrast to PNA, WGA preferentially labels primate rod outer segments and areas of the IPM surrounding rod inner and outer segments [Fig. 1 B]. A dense and uniform zone of WGA labeling extends from the apical surface of the RPE to the rod outer segment bases. Broad, sparsely labeled vertical zones are interspersed between the heavily labeled rod outer segments and associated matrix. These bands, corresponding to cone outer segments and their surrounding matrix, invariably lie just distal to the cone ellipsoids. Rod inner segments are unlabeled. The WGA-labeled matrix, like the PNA-labeled cone matrix, extends below the outer segment bases and terminates at the outer limiting lamina. The RPE cytoplasm is also labeled, but less densely than the rod outer segments or matrix surrounding rods. Thus, at the light-microscopic level, the WGA labeling pattern in the primate photoreceptor layer can be succinctly described as a reverse image of the PNA pattern.

PNA Labeling: Electron Microscopy

Electron-microscopic localization of PNA labeling at the photoreceptor–RPE interface in monkey and cat retina is shown in Fig. 2 (A), (B). Longitudinal sections of monkey cones show that PNA labeling is most intense over both cone outer segments and an adjacent zone of matrix 0·5–2·0 μm wide on either side of the cone outer segment plasma membrane [Fig. 2 (A)]. The PNA-labeled matrix extends beyond the distal tips of the cone outer segments to the RPE apical surface. The matrix surrounds a group of apical RPE processes that ensheath the distal portion of cone outer segments. These processes are labeled at a much lower level than the matrix which surrounds them.

THe PNA-labeling pattern in the cat is similar to that in the monkey, but there are notable differences [Fig. 2 (B)]. As in the monkey, the matrix surrounding cat cones is preferentially labeled by PNA, but the labeling intensity is much lower despite the fact that

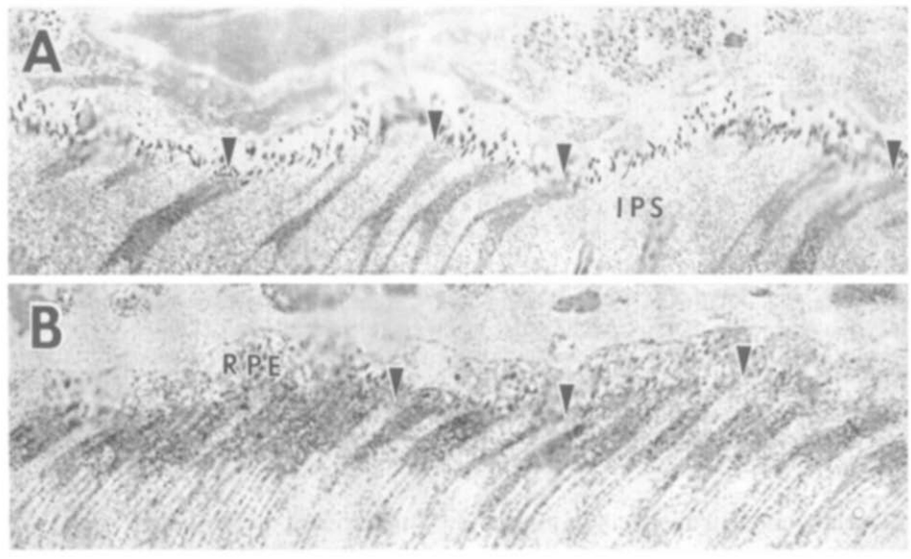

FIG. 1. Light micrographs of silver-enhanced lectin labeling in the monkey interphotoreceptor space (IPS). A, PNA labels a zone associated with cone inner and outer segments (arrowheads). These cone associated matrix domains narrow considerably as they approach the outer limiting membrane. Cone inner segments, rod inner and outer segments, and areas of the interphotoreceptor matrix surrounding rods are lightly labeled by PNA (× 600). B, WGA preferentially labels rod outer segments and areas of the interphotoreceptor matrix around rod inner and outer segments. The RPE is lightly labeled. Rod inner segments and the matrix domains surrounding cones are almost devoid of label, giving these regions a silhouetted appearance (arrowheads) (× 600).

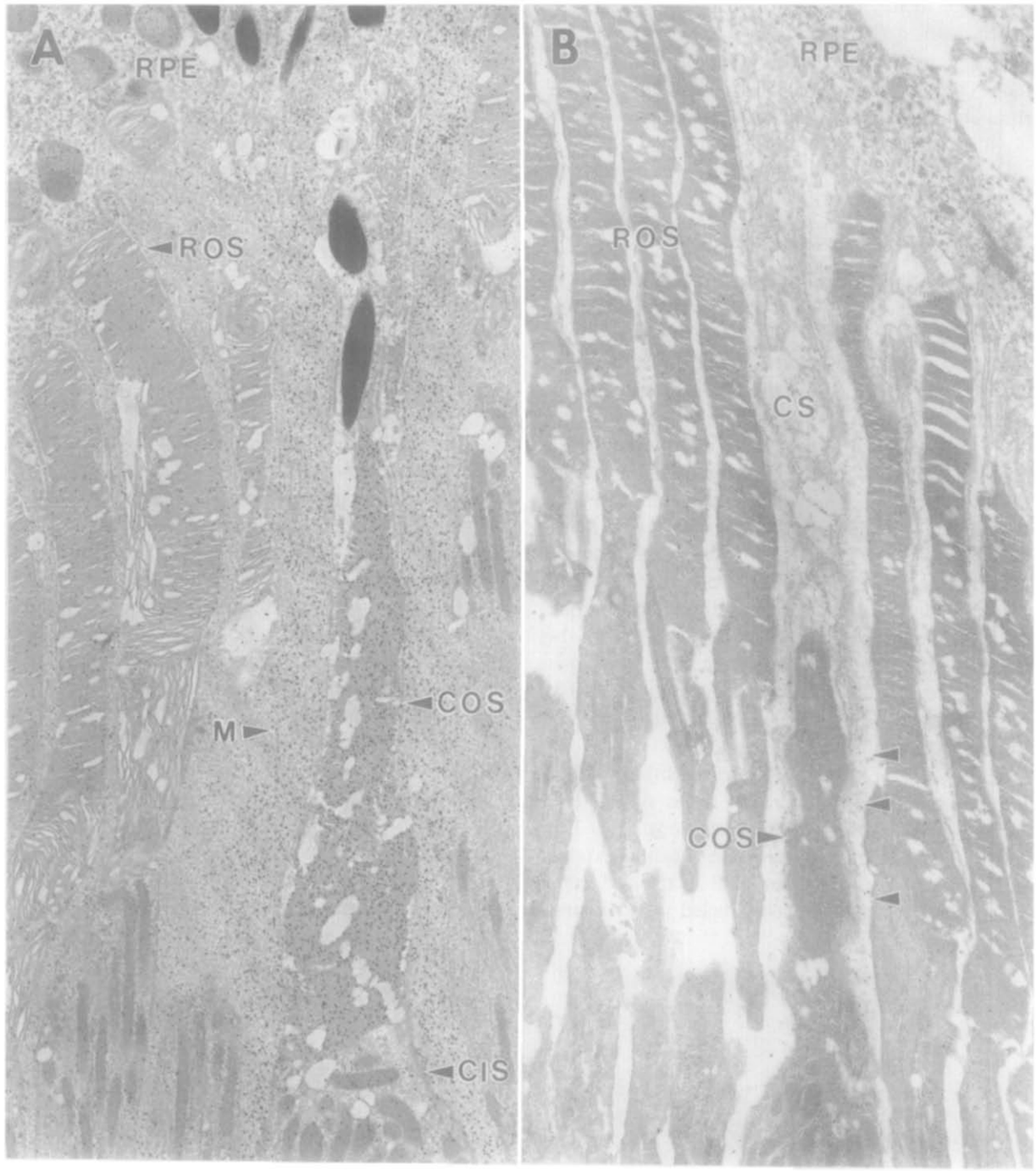

FIG. 2. Electron microscopic localization of PNA-binding glycoconjugates in monkey and cat photoreceptors. A, PNA labeling associated with monkey cones. Au particles (15 nm), denoting PNA labeling, are present over cone outer segments (COS) as well as the matrix (M) that surrounds COSs and cone inner segments (CIS). Adjacent rod outer segments (ROS) and rod inner segments are labeled at a much lower density. Melanin granules are present within the unlabeled RPE apical processes which drape the distal portion of the COS (× 10000). B, PNA labeling associated with cat cones. Cat COSs are ensheathed by a concentric array of apical RPE processes termed the cone sheath (CS). PNA labeling is associated with a region of extracellular matrix termed the cone matrix sheath (arrowheads), interposed between the ensheathing processes and neighboring ROSs and rod associated matrix (× 12000).

identical tissue processing and cytochemical protocols were used. Furthermore, cone outer segments in the cat, unlike those of the monkey, are only sparsely labeled.

In cross-sections through the supracone space, the region between the tip of the cone outer segment and the apical surface of the RPE, PNA labeling in the monkey appears as a circular profile of matrix surrounding rods [Fig. 3(A)]. Vacuolated apical processes occupy the center of this domain while its periphery is surrounded by an array of smaller RPE microvilli, many of which contain melanin granules. The RPE apical processes are very lightly labeled, as are rod outer segments and the matrix surrounding this circular array of processes. In the cat, the supracone space consists of a highly ordered lamellar

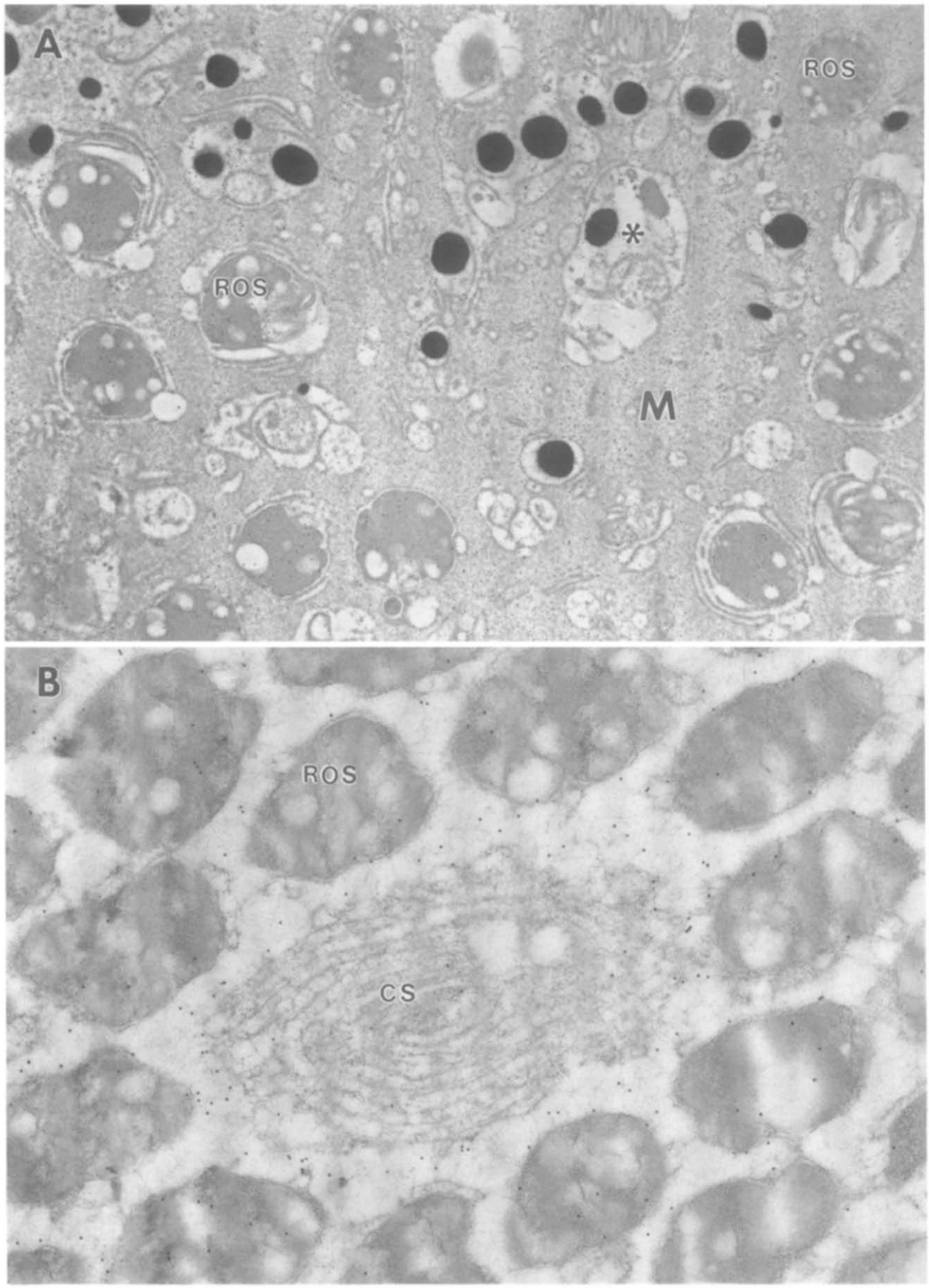

FIG. 3. Cross-sectional views of the interphotoreceptor matrix in the region between the COS tip and the apical RPE surface (the supracone space). A, PNA labeling in the monkey supracone space. In cross-section, a concentration of Au particles defines a circular region of matrix (M) with vacuolated RPE apical processes (*) occupying the center of this domain and smaller melanin-containing processes located at its periphery. These apical RPE processes are only lightly labeled, as are ROSs and rod associated matrix (×13000). B, PNA labeling in the cat supracone space. The supracone space in the cat consists of a concentrically arranged array of unlabeled apical RPE processes (CS) and an adjacent zone of PNA-labeled extracellular matrix. Au particles appear to be associated with filamentous components of this matrix domain that extend from the outermost lamellipodium of the cone sheath to the plasma membrane of neighboring rods (×29000).

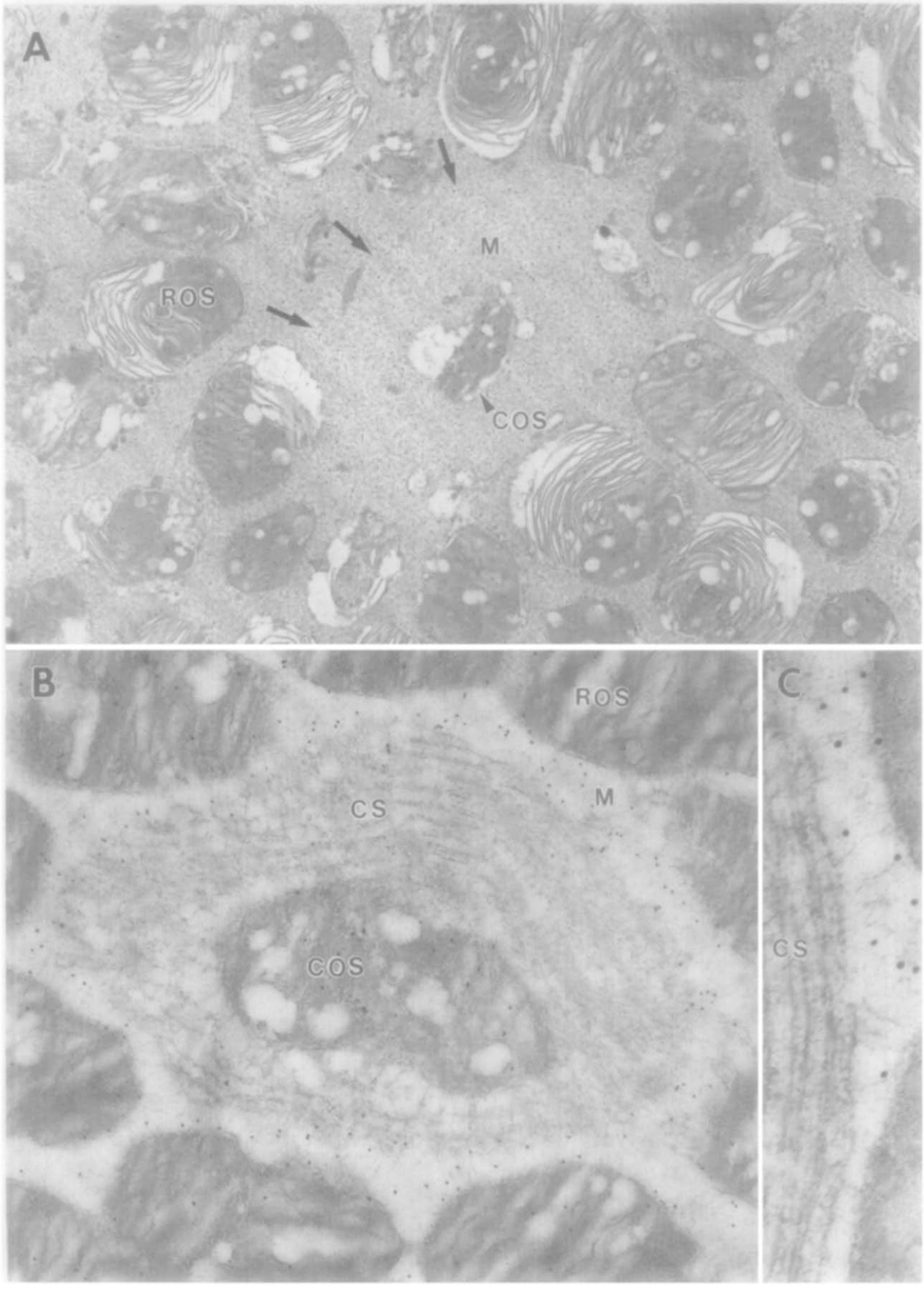

FIG. 4. Cross-sectional profiles of the interphotoreceptor matrix in the region adjacent to the photoreceptor outer segments. A, PNA labeling associated with monkey COSs. COSs are moderately labeled, however the most prominent gold labeling is associated with a zone of matrix (M) that ensheaths the COSs. The PNA binding matrix appears more electron-lucent than the matrix surrounding ROSs. Arrows mark the interface between the cone matrix and the rod associated matrix (× 11 000). B, PNA labeling associated with cat COSs. Au particles are abundant only over a relatively narrow region of matrix (M) between the outer margin of the cone ensheathing processes (CS) and neighboring ROSs (× 29 000). C, High-magnification micrograph of PNA labeling of the cat cone matrix. PNA labeling is associated with filaments in the matrix between the cone ensheathing processes (CS) and the rod outer segments (× 66 000).

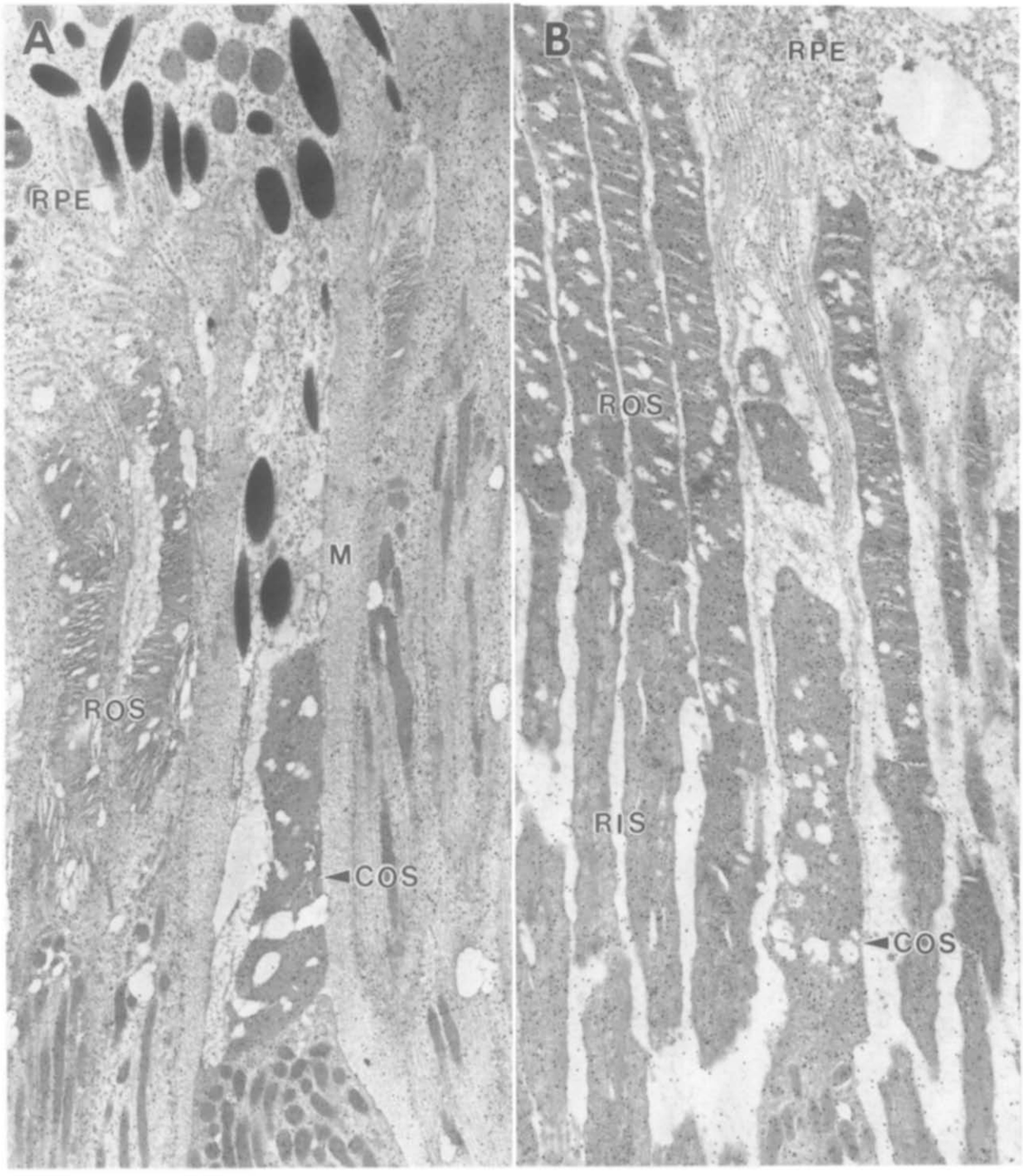

FIG. 5. Longitudinal profiles of WGA labeling associated with monkey and cat photoreceptors. A, Monkey photoreceptors. The IPM surrounding rod photoreceptors is heavily labeled in comparison to the matrix sheath (M) surrounding cones. Rod and cone inner and outer segments are moderately labeled. The RPE cytoplasm and apical processes also show evidence of labeling (×9000). B, Cat photoreceptors. The distribution of WGA labeling is more uniform than in the monkey. Both rods and cones are labeled as are their surrounding matrices. As in the monkey, the RPE apical processes and cytoplasm are labeled. RIS—rod inner segment, COS—cone outer segment, ROS—rod outer segment (×11 500).

array of apical RPE processes and an adjacent region of cone-associated matrix [Fig. 3(B)]. This matrix domain, which is lightly but preferentially labeled by PNA, encircles the array of unlabeled lamellar processes of the cone sheath. The cone associated matrix is surrounded in turn by very sparsely labeled rod outer segments.

Figures 4(A) and (B) illustrate cross-sectional profiles midway through the rod and cone outer segment region in the monkey and cat respectively. At this level, the monkey cone outer segment is surrounded by PNA-labeled matrix that is more electron-lucent than the matrix surrounding rods [Fig. 4(A)]. Unlabeled RPE processes are visible only at the outer margin of this matrix. In contrast, the cat cone outer segment is encircled by a concentric array of RPE

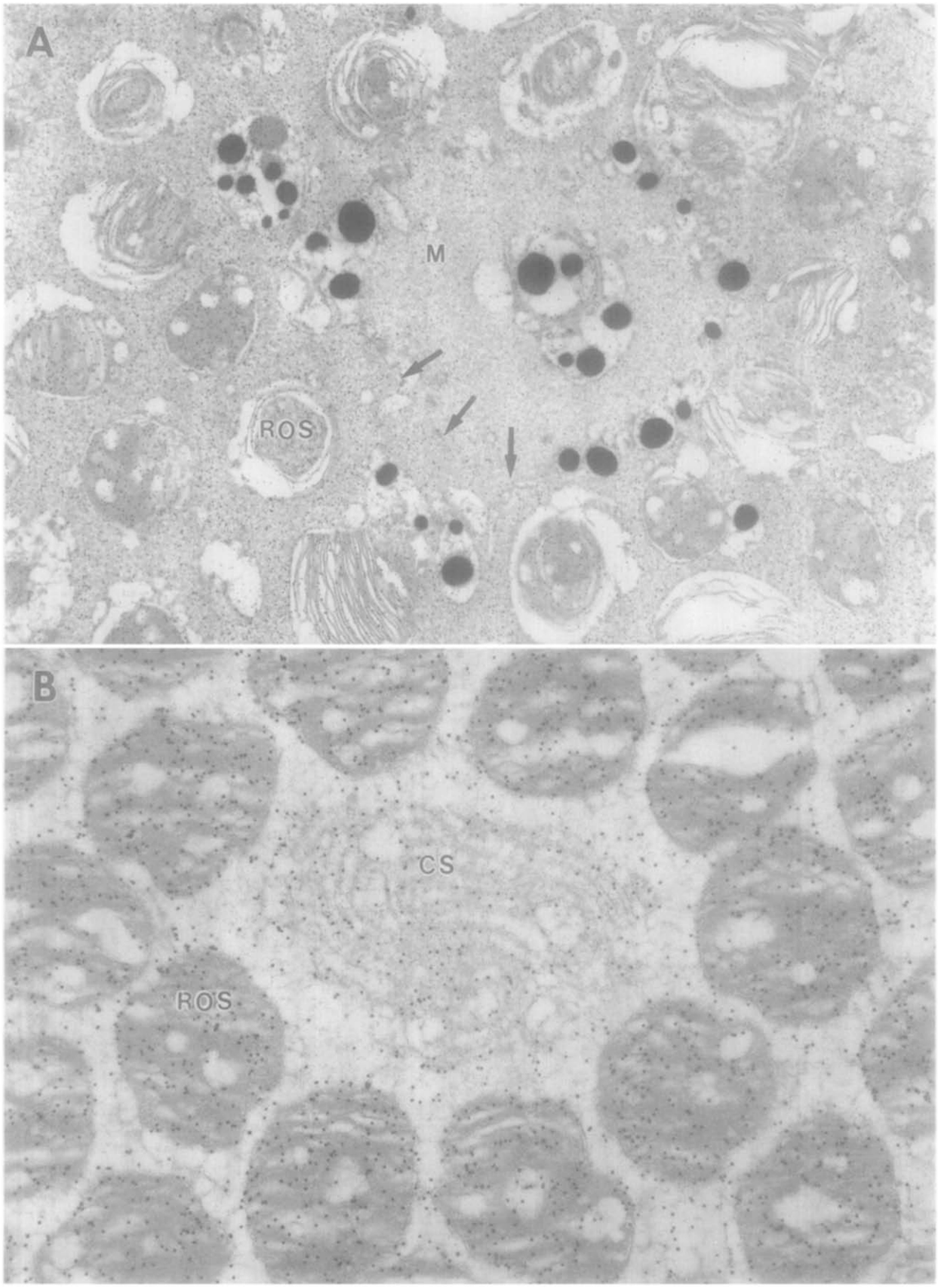

FIG. 6. Cross-sectional profiles of WGA labeling in the supracone space. A, Monkey supracone space. The cone-associated matrix (M) is sparsely labeled in comparison to the matrix surrounding ROSs. The interface (arrows) between the rod and cone-associated matrix domains is as well defined by WGA binding as it is by PNA binding (× 12 000). B, Cat supracone space. All cytoplasmic and extracellular compartments appear to be labeled uniformly. In contrast to the PNA results [see Fig. 3 (B)] the WGA labeling patterns in this region do not indicate the presence of distinct rod and cone matrix domains. CS—cone sheath, ROS—rod outer segment (× 25 000).

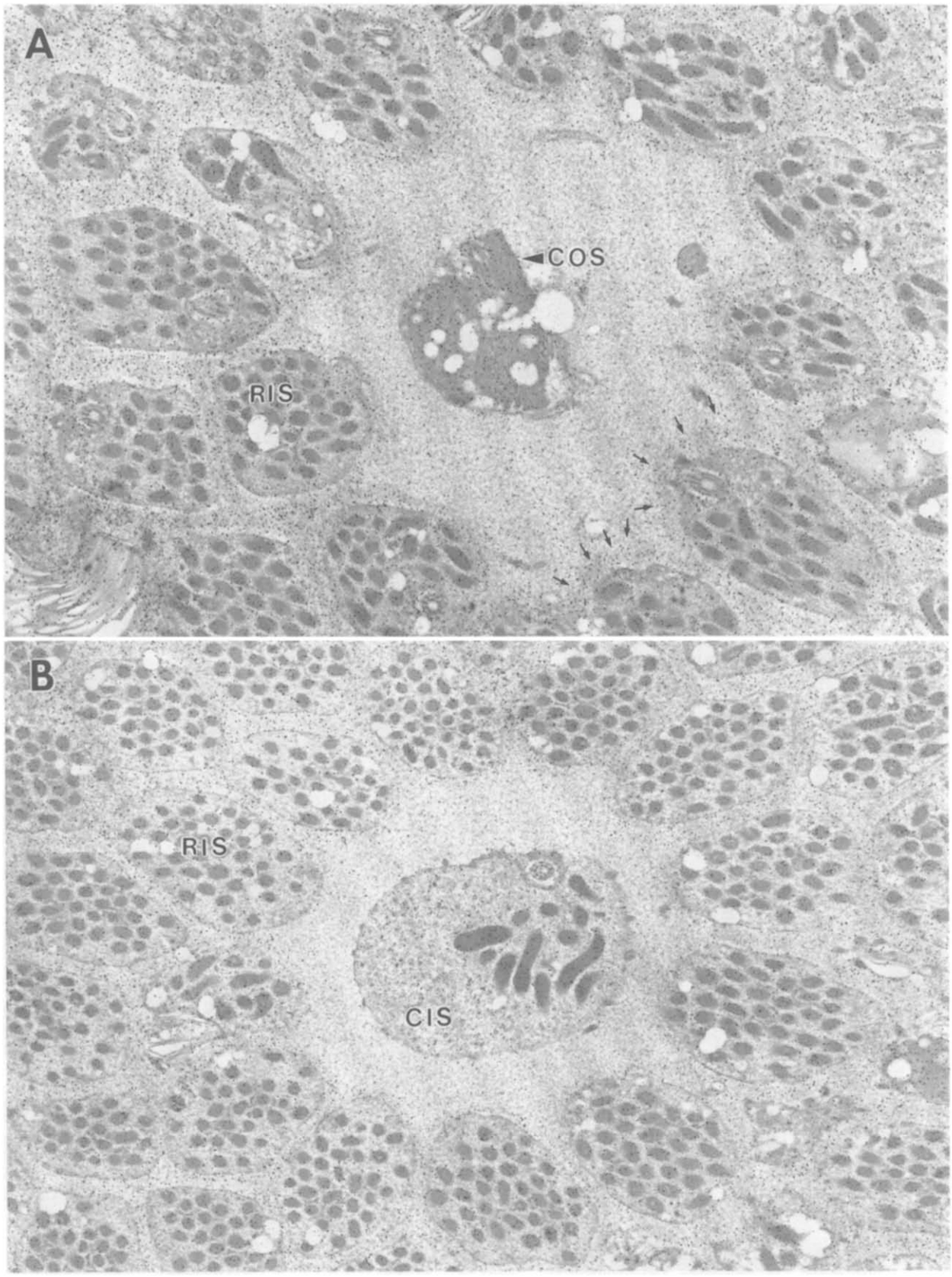

FIG. 7. Cross-sectional profile of WGA labeling in the monkey IPM in the region adjacent to the photoreceptor inner segments. A, WGA labeling of the IPM near the COS base. The matrix near the COS base is labeled but at a low level in comparison to the matrix surrounding RISs. At this level, the outermost border of the cone matrix (arrows) appears scalloped. A narrow band of rod-associated matrix follows the convex profile of the rod inner segments (RIS) (× 11 500). B, WGA labeling at the level of the ellipsoid. Closer to the outer limiting lamina, at a level well below the point at which photoreceptors are contacted by RPE apical processes, the matrix sheath retains its scalloped shape. CIS—cone inner segment, RIS—rod inner segment × 11 000).

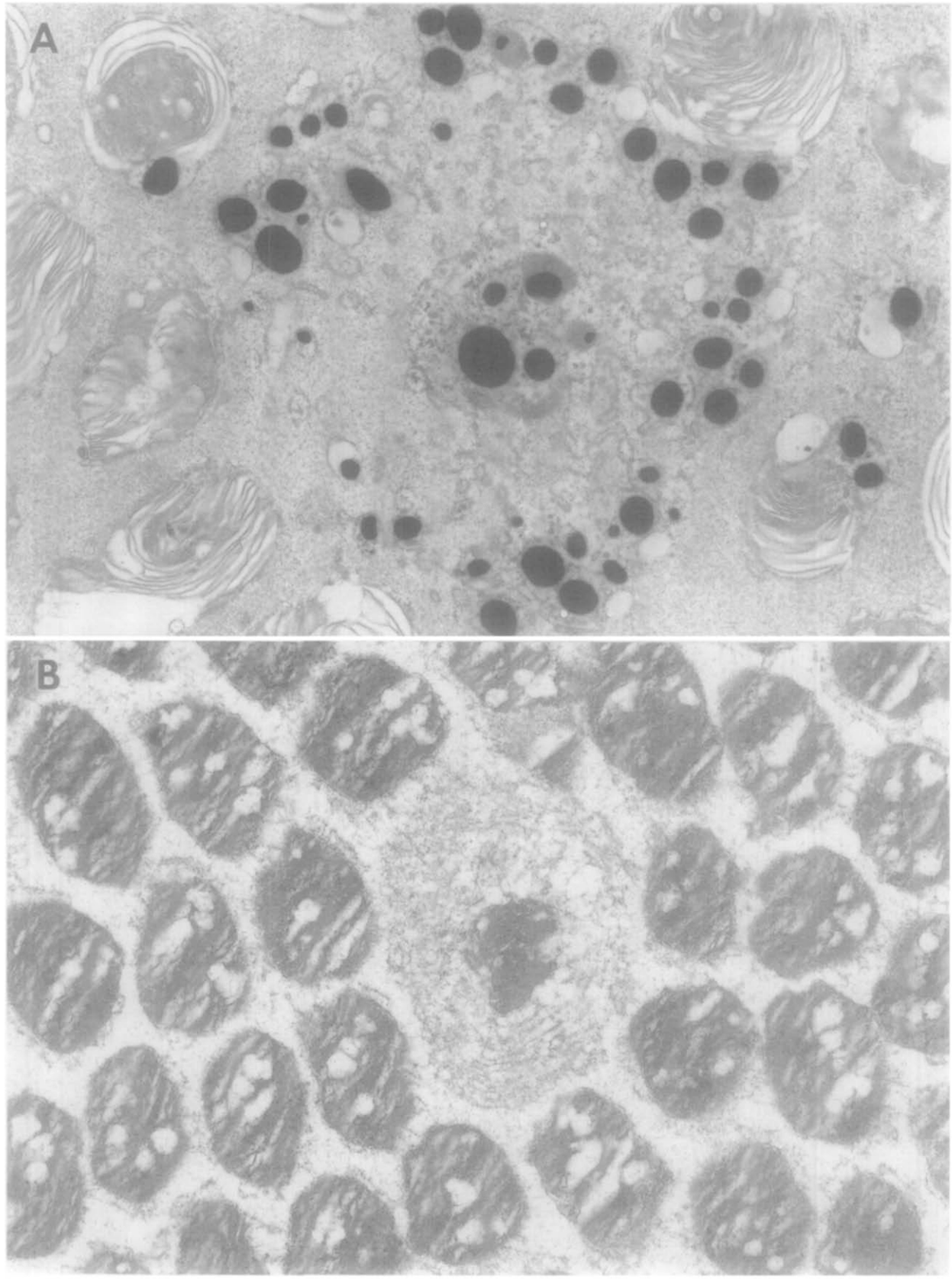

FIG. 8. Negative Controls for PNA and WGA labeling in monkey and cat retina. Cross-sectional views of monkey (A) and cat (B) photoreceptors. When biotinylated lectin was omitted from the incubation solution, avidin–Au labeling was virtually eliminated on sections of either monkey (A) or cat (B) retina. Similarly, when a molar excess of the appropriate inhibitory sugar was included in the primary incubation, Au labeling was reduced to background levels. A, ×13 500; B, ×18 500.

processes also known as the cone sheath (Steinberg and Wood, 1974) [Fig. 4(B)]. The zone of PNA labeled matrix lies between the outer margin of the cone sheath and neighboring rod outer segments. Gold particles are closely associated with filamentous components of the cone-associated matrix. These PNA-labeled filaments radiate across the extracellular space between the outermost lamella of the cone sheath and the rod outer segment plasma membranes [Fig. 4(C)].

WGA Labeling: Electron Microscopy

In the monkey retina, WGA labels a number of cellular and extracellular regions unlabeled, or only lightly labeled, by PNA. Rod outer segments, and the matrix adjacent to them, are heavily labeled by WGA [Figs 5(A), 6(A), 7(A) and (B)]. The RPE cytoplasm and apical RPE processes are also preferentially labeled by this lectin [Fig. 5(A)]. The cytoplasm of rod and cone ellipsoids is lightly labeled [Figs 7(A) and (B). Cone-associated matrix is labeled but at a distinctly lower density than its rod counterpart [Fig. 5(A)]. In general, WGA labeling is less intense over cone outer segments than rod outer segments (data not shown). Near the apical surface of the RPE, a circular array of microvilli is positioned at the interface between the rod matrix and the cone-associated matrix [Fig. 6(A)]. The interface between these two zones of matrix appears scalloped in cross-sections through the photoreceptor inner segments [Fig. 7(A), (B)].

In the cat, rod and cone inner and outer segments are moderately labeled with WGA [Fig. 5(B)]. The interphotoreceptor matrix also appears lightly, but uniformly, labeled, with no indication of any specialized domains [Figs 5(B) and 6(B)]. WGA labeling is present over filamentous structures in both rod- and cone-associated matrix [Fig. 6(B)]. The RPE processes forming the cone sheath, like those that interdigitate with rod outer segments, are also labeled.

When control sections from each species were incubated in the absence of lectin, or in the presence of biotinylated lectin plus an appropriate inhibitory sugar, labeling was eliminated [Fig. 8(A), (B)].

4. Discussion

The apical surface of the RPE elaborates numerous microvilli which interdigitate with the outer segments of rod and cone photoreceptors. In most mammalian species, the apical processes contacting cones are morphologically distinct from those contacting rods (Anderson, Fisher and Steinberg, 1978). In the cat and rabbit retinas particularly, this distinction is reinforced by the presence of a concentric array of microvilli and lamellipodia, termed the *cone sheath* (Steinberg and Wood, 1974), that envelops the cone outer segment for virtually its entire length. The *cone sheath* is known to participate in the shedding and phagocytosis of disc packets from the cone outer segment tips (Fisher and Steinberg, 1982: Fisher, Pfeffer and Anderson, 1983) and it may have additional, but still unconfirmed, functions as well. For example, the multilamellar ensheathment of the cone outer segment may define a cone-specific path for metabolic exchange with the RPE. Ensheathment could also serve to influence the distribution of current along, or reduce the spread of current from, the cone outer segment. The sheath could have optical properties that influence the absorption of light quanta by cone photopigment molecules, or it could contribute to retinal adhesion by maintaining close apposition between the photoreceptor outer segments and the RPE (Steinberg and Wood, 1974).

Cone photoreceptors in many species are known to be ensheathed by another structure, a domain of extracellular matrix called the *cone matrix sheath*. The matrix sheath was identified definitively on the basis of its PNA binding characteristics in pig, monkey and human cones (Johnson, Hageman and Blanks, 1985, 1986). More recently, it has been shown to be a discrete and heterogeneous extracellular structure composed of a number of PNA binding glycoproteins (Hageman and Johnson, 1986; Johnson and Hageman, 1987) and chondroitin-6-sulfate-containing proteoglycan (Hageman and Johnson, 1987, 1990). Like the ensheathing processes, the functions of the matrix sheath are largely unknown, although many of the functions proposed for the processes (see above) have also been advanced as potential functions for the matrix sheath (Hageman and Johnson, 1990).

In this study, we find that the lamellar processes which form the elaborate cone sheath in the cat are, in turn, enveloped by a matrix sheath (as defined by its PNA labeling pattern). However, the cone matrix sheath of the cat is different from other mammalian species in several respects. Firstly, it is not immediately adjacent to the cone outer segment, but instead is interposed between the outermost lamellipodia of the cone sheath and the surrounding rods and rod associated matrix. There is no evidence of PNA labeling immediately adjacent to the cone outer segment plasma membrane, between individual ensheathing processes, or over the ensheathing processes themselves. This suggests that insoluble PNA binding glycoconjugates within the matrix sheath are probably excluded from the extracellular space between individual processes. That is not the case for all IPM constituents however, since interphotoreceptor retinoid binding protein (IRBP), a soluble IPM protein, has been localized to the space between the processes which comprise the cat cone sheath (Anderson and Erickson, unpubl. res.).

Secondly, the level of PNA labeling in the matrix sheath of the cat appears relatively weak in comparison to that in the monkey. This may represent an actual species difference in the molecular properties of the sheath because similar observations have been

made in rabbit cones where the fluorescence associated with PNA labeling also appears relatively weak in comparison to that in pig, monkey, and human cones (Johnson et al., 1986). Taken together, these results imply that there may be functional similarities between the ensheathing processes and the matrix sheath such that species with well-developed matrix sheaths may not require an elaborate array of ensheathing processes (and vice versa).

The identification of WGA and PNA binding matrix domains in two species where the relationship between the cone outer segment and the RPE ensheathing processes is distinctly different reinforces the concept of photoreceptor-specific heterogeneity within the IPM as a general phenomenon (Hageman and Johnson, 1990). Furthermore, it is also clear that, despite interspecies differences in organization, the ensheathing processes themselves contribute to the structural definition of these photoreceptor-specific matrix domains.

Preferential WGA labeling of primate rod outer segments and the IPM around rod inner and outer segments has been reported by Sameshima et al. (1987). They reported that areas of the primate IPM corresponding to cone matrix sheaths appeared moderately electron-dense, while the IPM around rods appeared electron-lucent. In contrast, our observations in monkey cones show just the opposite appearance. Such differences illustrate the fact that significant modifications in the appearance of the IPM may be dependent on the methods and reagents used in tissue preservation. Variations in fixatives (Blanks et al., 1988; Hageman and Johnson, 1990), buffers (Hageman and Anderson, unpubl. res.), or treatment with various enzymes including trypsin (Hageman and Johnson, 1986), chondroitinase (Hageman and Johnson, 1987), and neuraminidase (Uehara et al., 1985) have all been shown to influence the appearance or labeling patterns in the IPM. To reduce such variability, the retinal samples from cats and monkeys used in this study were fixed and processed similarly. Consequently, the variations which we observe in the ultrastructural appearance of the IPM are likely to reflect inherent differences between these two species.

Rod and cone photoreceptors differ significantly in both anatomical organization and physiological function, and yet they lie in extremely close proximity to each other. Segregation of the matrix domains which surround each cell type, as well the RPE processes which contact them, define two photoreceptor-specific microenvironments within the IPM. It is virtually certain that these two domains mediate rod- and cone-specific interactions with RPE, the scope of which we are just beginning to appreciate.

Acknowledgments

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