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Retinal photoreceptor neurons and pinealocytes accumulate mRNA for interphotoreceptor retinoid-binding protein (IRBP)

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We have utilized cDNA probes and in situ hybridization techniques to define the subcellular localization of interphotoreceptor retinoid-binding protein (IRBP) mRNA in bovine and monkey retinas. Results suggest that the mRNA is mainly localized in rod photoreceptor neurons within the outer nuclear layer of the retina. IRBP mRNA is also abundant in cells of the pineal gland, strengthening the analogy between rod photoreceptor cells and pinealocytes.

Interphotoreceptor retinoid-binding protein *cDNA* *Hybridization* *Rod photoreceptor cell* *Pinealocyte*

1. INTRODUCTION

Interphotoreceptor retinoid-binding protein (IRBP), also known as 7S receptor or interstitial retinol-binding protein [1–4], is a novel, extracellular retinoid-binding protein which appears to be present in uniquely high concentrations in photosensitive tissues, i.e. neural retina and pineal organ [5]. In the retina, IRBP is now known to be a large (~140 kDa) lipoglycoprotein, primarily localized in the extracellular matrix between the photoreceptors and retinal pigment epithelium [5,6], where it may act as an intercellular retinoid-transport vehicle [7].

The specific retinal cell that contains the active gene(s) for IRBP which encode and produce a mature IRBP mRNA has been the subject of much conjecture [8–10]. Using an authentic cDNA probe for IRBP, we have previously shown the presence of IRBP mRNA in bovine retina by RNA blot analysis [11]. In the present study, we utilize labeled cDNA probes and in situ hybridization techniques to precisely define the cellular localization of IRBP mRNA in retina and also demonstrate the presence of specific mRNA species in cells of the pineal gland.

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2. METHODS

2.1. *Tissue preparation*

Bovine retinas and pineal glands were collected from animals immediately following killing. Monkey eyes were obtained immediately following exsanguination of Rhesus monkeys under deep anesthesia. For in situ hybridization, the tissue was fixed for 1 h at room temperature in Carnoy's fixative (70:30:10, ethanol/chloroform/acetic acid). The fixed tissue was rinsed in absolute alcohol and used for preparation of cryostat sections.

2.2. *In situ hybridization*

The IRBP cDNA clones utilized for these studies were prepared as follows: a 1.5 kb *Eco*RI fragment of a bovine IRBP cDNA clone (λIRBP3) [11] was

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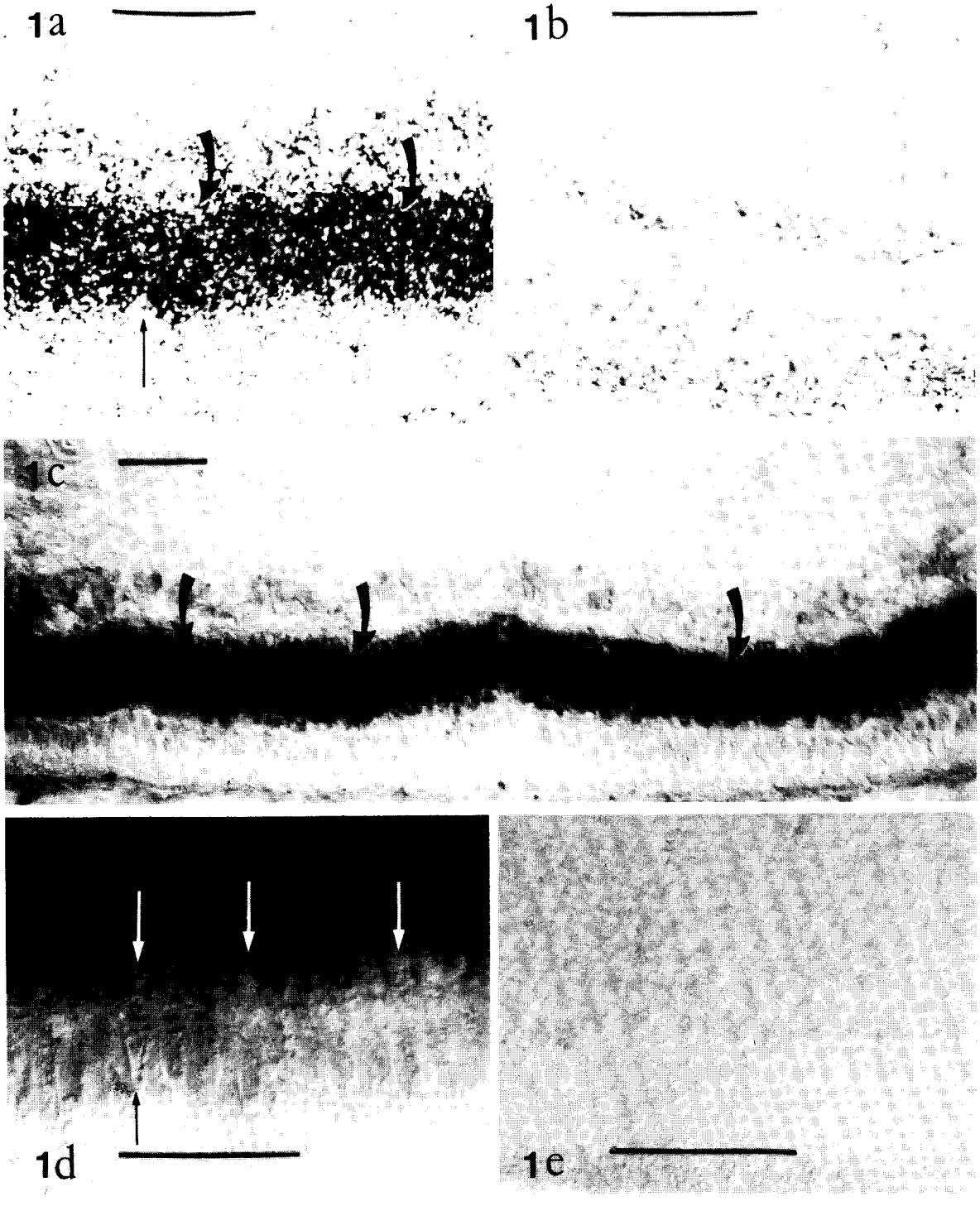
1a
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subcloned into pGEM2. The resulting clone was named rIRBP3-1.5. A radioactive probe designated (−) which is complementary to IRBP mRNA was prepared using T7 RNA polymerase and rIRBP3-1.5 that had been linearized with *Sph*I which cuts this plasmid at a single site. A control probe, the same sense (+) as the IRBP mRNA, was prepared using SP6 RNA polymerase and the rIRBP3-1.5 plasmid linearized with *Bam*HI (which cuts it only once). Labeling with [^{35}S]ATP and UTP was carried out using a Promega Biotech kit according to the manufacturer's instructions. A number of smaller DNA fragments derived from the 1.5 kb *Eco*RI fragment were blunt-end ligated into the *Sma*I site of M13mp8. These clones were characterized by the dideoxy sequencing method [12]. Two of the clones containing opposite orientations (+ and − strands) of the insert were used for these studies. The + strand served as the negative control for the in situ experiments. Both orientations of the single-stranded cDNA probe were labeled with HRP (horseradish peroxidase) using the Digene labeling system (Digene, College Park, MD). Prehybridizations, hybridizations, and washings were carried out by standard procedures [13–15].

3. RESULTS

The retina is a highly stratified tissue with well-defined nuclear and plexiform layers. Also, within the photoreceptor (outer) nuclear layer, rods and cones are easily differentiated due to the distinctive size and shape of the cone cells. To define the cells that harbor the active genes for IRBP, therefore, we utilized Nomarski micrographic techniques and in situ hybridizations to specifically identify the cells synthesizing and containing IRBP mRNA in bovine retina. Using an ^{35}S-labeled RNA probe, intense labeling was seen in the outer nuclear layer, indicating the presence of substantial amounts of IRBP mRNA in photoreceptor perikarya (fig.1a). No hybridization was detected in other cells of the neural retina or pigment epithelium. When a radiolabeled probe of the same sense as the IRBP mRNA was used, no specific hybridization was detected (fig.1B).

In situ hybridization with a second, HRP-labeled probe was also performed (fig.1c–e). Hybridization to bovine retina exhibited a positive reaction with the IRBP-M13 (− strand) probe which is complementary to endogenous mRNA contained within the cells (fig.1c). A higher magnification of the same section highlights the absence of hybridization to cones, or, at least to a major population of cones (fig.1d). For use as a control, sections were also hybridized with an IRBP-M13 (+ strand) probe which is of the same sense as IRBP mRNA. This showed no hybridization with bovine retina sections (fig.1e).

We also examined monkey retinas and bovine pineal organs for specific hybridization with bovine IRBP sequences (fig.2). Using HRP-labeled probes, hybridization was seen only in the photoreceptor nuclear layer of the monkey retina (fig.2a). This signal was less intense, when compared to bovine retina, probably due to cross-species specificity. Importantly though, the less intense reaction allowed for an even better view of the relative absence of reaction product coincident with cone cell perikarya as revealed by Normarski microscopy. No reaction product was seen when an HRP-labeled M13 vector lacking the insert was used as a probe (fig.2b), again demonstrating the specificity of the IRBP-encoding insert. HRP-reactivity was apparent in the bovine pineal gland (fig.2c) indicating the accumulation of mRNA for IRBP in a population of pineal cells. No hybridization was observed when a probe of the same sense as IRBP mRNA was used (fig.2d).

Fig.1. Nomarski micrographs of in situ hybridization of 12 μm cryostat tissue sections of bovine retina. (a) Positive hybridization of a bovine ^{35}S-labeled cRNA probe to outer nuclear (photoreceptor) layer of bovine retina (thick arrows). Cone perikaryon shows no specific hybridization signal (thin arrow). (b) Negative reaction in retina using cRNA strand that is the same sense as IRBP mRNA. (c) Positive reaction with HRP-labeled probe (thick arrows). (d) Higher magnification of same section; arrows show cone perikarya (white) or outer segments (black) that lack reaction product. (e) Negative reaction in control section hybridized with probe of same sense as IRBP mRNA. Retinal sections are oriented with vitreous to the top. Bars = 50 μm.

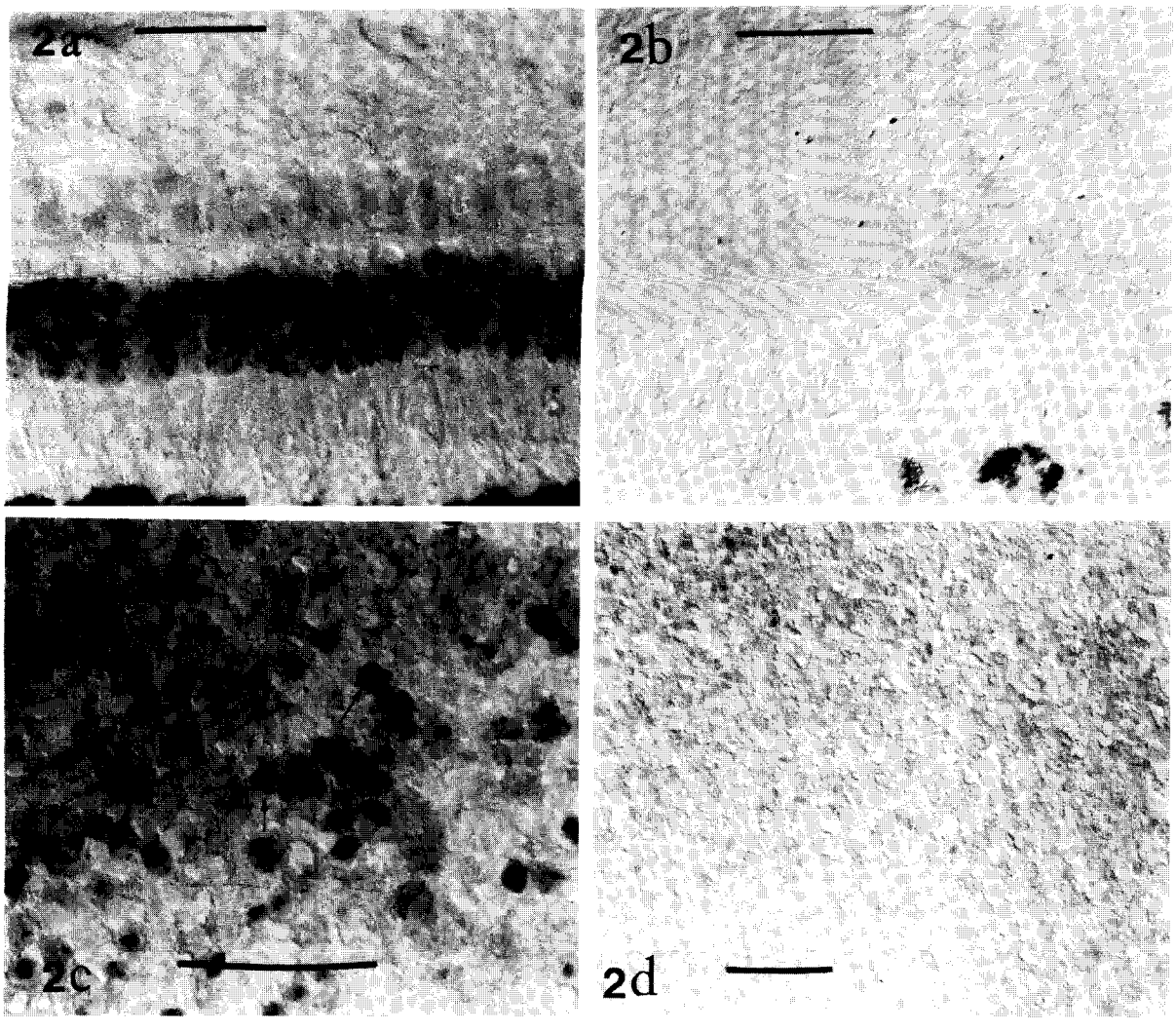

Fig.2. Hybridization with HRP-labeled probe to tissue sections. (a) Positive reaction restricted to outer nuclear layer of monkey retina. Reaction product is absent most often above cone cells. (b) Negative reaction with HRP-labeled M13 vector containing no insert. (c) Hybridization in bovine pineal organ with HRP-labeled probe. Arrows indicate intensely stained cells. (d) Lack of hybridization with single-stranded probe of the same sense as the IRBP mRNA. Bars = 50 μm.

4. DISCUSSION

Four cell types border the retinal interphotoreceptor matrix space: rod and cone photoreceptor neurons, Muller glial cells and pigment epithelial cells. Previous studies [8–10], focusing on the synthesis and secretion of the IRBP protein moiety, have been unable to conclusively pinpoint the cell of synthesis, although it was determined that IRBP was of neural retinal rather than of pigment epithelial origin. From our present study, it is clear that IRBP mRNA accumulates in substantial amounts in rod photoreceptors of both bovine and monkey retinas. It appears that IRBP mRNA accumulates to a much lesser extent if at all in glia, conventional neurons or in cone perikarya in these species. Cone photoreceptor neurons could yet be involved significantly in IRBP synthesis (at least in some

species) since IRBP has been reported in the cone-dominant ground squirrel retina [16].

Of interest also is our demonstration of IRBP synthetic capabilities in pinealocytes. This is consistent with the idea that mammalian pinealocytes share several important characteristics with photoreceptors as well as raising intriguing questions as to the possible function of IRBP as a retinoid transport molecule in the pineal organ. Moreover, the substantial labeling pattern of mRNA in rod photoreceptor cells and pinealocytes and only sparse labeling in cone cells brings up interesting evolutionary questions as to the possible biochemical divergence of rods and pinealocytes on one hand and cones on the other.

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