

## A topographic gradient of molecules in retina can be used to identify neuron position

(cell membrane/antigen/embryo/synapses/hybrid cells)

G. DAVID TRISLER, MICHAEL D. SCHNEIDER, AND MARSHALL NIRENBERG

Laboratory of Biochemical Genetics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205

Contributed by Marshall Warren Nirenberg, December 24, 1980

**ABSTRACT** A monoclonal antibody was obtained that binds to cell membrane molecules distributed in a topographic gradient in avian retina. Thirty-five-fold more antigen was detected in dorsoposterior retina than in ventroanterior retina. Most of the antigen was associated with the synaptic layers of the retina. Less antigen was detected in cerebrum, thalamus, cerebellum, and optic tectum, but little or none was found in non-neural tissues tested. The antigen was found on most or all cell types in retina, and the concentration of antigen found is a function of the square of the circumferential distance from the ventroanterior pole of the gradient toward the dorsoposterior pole. Thus, the antigen can be used as a marker of cell position along the ventroanterior-dorsoposterior axis of the retina.

Topographic relationships between retina ganglion neurons are conserved, forming point-to-point representations of the retina, when ganglion neurons synapse in tectum or certain other regions of brain. Thus, the retina is a favorable model system for studying the formation of synaptic circuits. However, the molecular basis for spatial order has not been defined. Sperry (1) hypothesized that two orthogonal gradients of molecules on retina ganglion neurons and corresponding gradients of complementary molecules in the optic tectum might determine the specificity of synaptic connections between retina and tectum neurons. Other mechanisms proposed include adhesive interactions between migrating neurites, myelination of bundles of axons, and formation of extracellular channels by glia to guide axons in appropriate directions (2).

Antibodies provide a means of identifying surface molecules and reactions required for neural function (3-7). Rabbit antiserum to clonal retina hybrid cells was used to detect an antigen with a restricted domain in retina (8).

Our objective was to detect cell surface molecules with topographic specificity in the retina, as candidates for neuronal recognition molecules. Monoclonal antibodies were obtained by fusing P3X63 Ag8 mouse myeloma cells (9) with spleen cells from mice immunized with small portions of dorsoposterior or ventral chicken embryo retina. A hybridoma antibody was obtained that binds to cell membrane molecules that are distributed in a dorsoposterior → ventroanterior gradient in retina.

### RESULTS

The rationale for the experiments is shown in Fig. 1. Spleen cells from mice immunized with small portions of dorsoposterior or ventral neural retina from the left eyes of 14-day chicken embryos were fused with P3X63 Ag8 mouse myeloma cells, and the binding of hybridoma antibody to cultured cells from the sector of the retina used for immunization was compared with

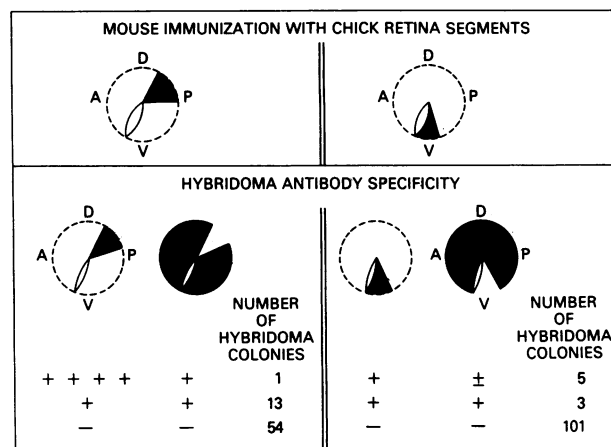


FIG. 1. (Upper) Hybridoma cell lines were derived from mice immunized with cells mechanically dissociated from dorsoposterior (Left) or ventral (Right) retina from the left eyes of 14-day White Leghorn chicken embryos (*Gallus gallus*) (sections labeled 4 and 1, respectively, in Fig. 2). Symbols, A, D, P, and V correspond to anterior, dorsal, posterior, and ventral, respectively. The choroid fissure, through which axons exit or enter the retina, shown extending from the ventroanterior margin of the retina, was used as a landmark for dissection. Female BALB/c mice were injected intraperitoneally at 0, 7, and 14 days with  $8 \times 10^6$  retina cells in 0.4 ml of Dulbecco's phosphate-buffered saline and intravenously with  $2 \times 10^6$  cells in 0.1 ml of the saline. On day 17,  $10^8$  dissociated spleen cells from each mouse were fused (10) with  $2 \times 10^7$  P3X63 Ag8 mouse myeloma cells. After fusion, the cells were suspended with  $5 \times 10^6$  spleen cells from a mouse that had not been immunized in 50 ml of medium A. [Medium A is selective medium D20SHAT of Berzofsky *et al.* (11) except that it contains 10% fetal bovine serum, 1  $\mu$ M aminopterin, 2 milliunits of insulin per ml, and each nonessential amino acid at 0.1 mM.] Cells were added to 96-well plates (Falcon) (186,000 cells per 74  $\mu$ l of medium A per well), incubated at 37°C in a humidified atmosphere of 10% CO<sub>2</sub>/90% air, and fed additional medium A (0.1 ml per well) on the 5th and 10th days of incubation. (Lower) To identify hybridomas synthesizing antibodies to regional antigens in retina, cells from the portion of 12-day chicken embryo retina (left eye) used for mouse immunization, or the rest of the retina, were dissociated with trypsin and collagenase, and transfer plates (12) were inoculated with  $1.2 \times 10^6$  of the cells per well. Cells were cultured for 2 days in 90% Eagle's minimal essential medium/10% fetal bovine serum; the medium was replaced with 50  $\mu$ l of medium conditioned by hybridoma cells, and plates were incubated for 30 min at 37°C. Each well was washed three times with 150  $\mu$ l of solution B (1 mg of pigskin gelatin per ml of phosphate-buffered saline) at 4°C. Each cell monolayer was incubated for 30 min at 37°C with 50  $\mu$ l of solution B containing 1.6 nM rabbit <sup>125</sup>I-labeled F(ab')<sub>2</sub> antibody fragment [<sup>125</sup>I-F(ab')<sub>2</sub>] directed against mouse IgG heavy and light chains (9799 cpm) and 500  $\mu$ g of bovine serum albumin. Cells were washed as above, and bound radioactivity was determined. F(ab')<sub>2</sub> (Cappel) was purified on a mouse IgG-Sepharose 4B column and iodinated with mono-<sup>125</sup>I Bolton-Hunter reagent (Amersham) (13).

binding to cells from the remainder of the retina. Cultured retina cells were used to increase the probability of detecting antibodies directed against cell surface molecules. Sixty-eight of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

672 wells inoculated contained hybridoma colonies derived from a mouse immunized with dorsoposterior retina cells. One cell line (14H3) synthesized antibody that bound more to dorsoposterior retina cells than to cells from the rest of the retina, whereas 13 hybridoma antibodies bound equally to cells from both portions of retina. Antibodies to retina were not detected with 54 additional cell lines. Hybridoma colonies derived from a mouse immunized with ventral retina cells were detected in 109 of the 672 wells plated. Five antibodies of the 87 tested bound somewhat more to cells from ventral retina than to cells from the rest of the retina, and 3 antibodies bound equally. Thus, 14% of the 155 hybridoma cell lines examined synthesize antibodies to retina; however, only one antigen was detected, termed TOP for toponymic (i.e., a marker of position), with an asymmetric distribution, more abundant in dorsoposterior retina than in the remainder of the retina.

**Distribution of TOP Molecules in Retina.** Retinas from right or left eyes of chicken embryos were cut into eight sections as shown in Fig. 2, and cells from each section were assayed for [ $^{125}\text{I}$ -F(ab') $_2$ ·anti-TOP antibody·TOP antigen] complexes. Bilaterally symmetrical gradients of TOP were found in retina from right and left eyes. The highest concentrations of antigen detected were in dorsoposterior or dorsal retina and the lowest, in ventroanterior or ventral retina. Little  $^{125}\text{I}$ -F(ab') $_2$  bound to

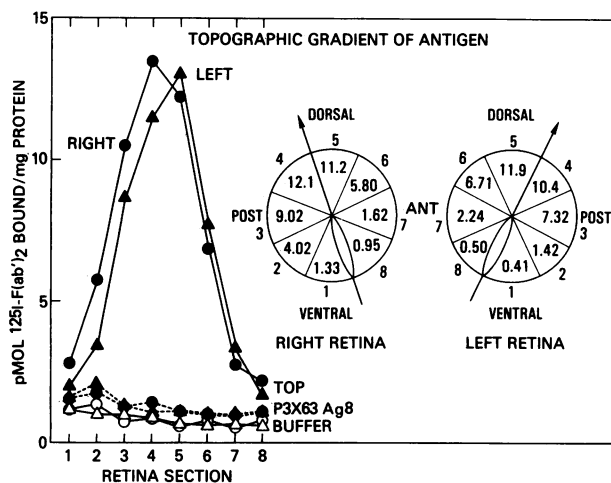


FIG. 2. Gradients of TOP molecules in retina from right (●) and left (▲) eyes of 14-day chicken embryos. ●—● and ▲—▲, anti-TOP antibody; ●—●—● and ▲—▲—▲, P3X63 Ag8 antibody; ○—○ and △—△, buffer B without antibody to TOP. Values shown within appropriate sections of retina are pmol of  $^{125}\text{I}$ -F(ab') $_2$  specifically bound per mg protein (i.e., pmol of  $^{125}\text{I}$ -F(ab') $_2$  bound in the presence of anti-TOP antibody minus pmol of  $^{125}\text{I}$ -F(ab') $_2$  bound in the presence of P3X63 Ag8 antibody). The assay conditions for TOP antigen used in this and subsequent experiments, except where stated, were as follows. Each retina was cut into eight sections as shown. Retina cells were mechanically dissociated in phosphate-buffered saline at 4°C by trituration (10 times) with a 200  $\mu\text{l}$  micropipette tip (Medical Laboratory Automation), and 100  $\mu\text{l}$  of a cell suspension (usually 160  $\mu\text{g}$  of protein; range, 50–250  $\mu\text{g}$ ) was added to each well of polyvinyl chloride 96-well V-bottom plates (Dynatech) treated with solution B. Cells were centrifuged at  $1300 \times g$  for 5 min at 4°C and supernatant solutions were decanted. Each pellet was washed three times in solution B at 4°C (150  $\mu\text{l}$  each wash), suspended in 50  $\mu\text{l}$  of an antibody solution containing 1.0  $\mu\text{l}$  of ascites fluid in solution B (except where specified), and incubated for 30 min at 4°C. Retina cells were washed three times as above, suspended in 50  $\mu\text{l}$  of solution B containing 440 nM rabbit  $^{125}\text{I}$ -F(ab') $_2$  ( $5\text{--}10 \times 10^4$  cpm) and 500  $\mu\text{g}$  of bovine serum albumin, and incubated at 4°C for 30 min, unless otherwise specified. Pellets were washed three times as described above, wells were separated, and radioactivity was determined. Each value shown is the mean of two or three determinations. Protein was determined by a modification of the method of Lowry *et al.* (14).

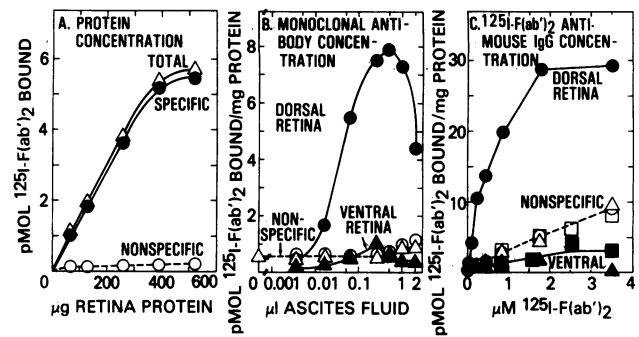


FIG. 3. (A) Effect of retina protein concentration on  $^{125}\text{I}$ -F(ab') $_2$  binding in the presence of anti-TOP antibody ( $\Delta$ ) or P3X63 Ag8 antibody ( $\circ$ ). ●,  $^{125}\text{I}$ -F(ab') $_2$  bound specifically. (B) Effect on hybridoma antibody (ascites fluid) concentration on  $^{125}\text{I}$ -F(ab') $_2$  binding. Solid symbols, specific binding of  $^{125}\text{I}$ -F(ab') $_2$  due to anti-TOP antibody; open symbols, nonspecific binding with P3X63 Ag8 antibody. ●,  $\circ$ , Dorsal retina, sections 4 and 5; ▲,  $\Delta$ , ventral retina, sections 1 and 8. (C) Reaction mixtures contained 220 nM  $^{125}\text{I}$ -F(ab') $_2$  ( $3.68 \times 10^{-3}$   $\mu\text{Ci}/\text{pmol}$ ). (C) Effect of concentration of rabbit  $^{125}\text{I}$ -F(ab') $_2$  anti-mouse IgG. Solid symbols, specific  $^{125}\text{I}$ -F(ab') $_2$  binding; open symbols, nonspecific binding to cells from dorsal retina (●,  $\circ$ ) or ventral retina (▲,  $\Delta$ ).

retina cells when antibody to TOP was omitted or was replaced with P3X63 Ag8 antibody. Other hybridoma antibodies including A2B5 (6) bound equally to cells from different regions of retina (not shown).

**Assay Conditions.** The effects of varying retina protein, hybridoma antibody, or  $^{125}\text{I}$ -F(ab') $_2$  anti-mouse IgG are shown in Fig. 3. Specific binding of  $^{125}\text{I}$ -F(ab') $_2$  was proportional to retina protein in the range 65–300  $\mu\text{g}$  of protein (Fig. 3A). Half-maximal and maximal specific binding of  $^{125}\text{I}$ -F(ab') $_2$  to dorsoposterior retina cells were obtained with 1:1350 and 1:100 dilutions of ascites antibody to TOP, respectively; higher concentrations of antibody to TOP reduced specific  $^{125}\text{I}$ -F(ab') $_2$  binding (Fig. 3B). Specific  $^{125}\text{I}$ -F(ab') $_2$  binding to ventral retina was low; however, the concentrations of anti-TOP antibody required for half-maximal and maximal specific binding did not differ greatly from those found with dorsal retina. Thus, no obvious difference was detected in the affinity of the antibody for antigen in dorsal and ventral retina. Nonspecific binding of  $^{125}\text{I}$ -F(ab') $_2$  to retina cells was low at all concentrations of P3X63 Ag8 antibody tested. The antibody to TOP was identified as an IgG1 with  $\kappa$  light chains (not shown). Half-maximal and maximal specific binding of  $^{125}\text{I}$ -F(ab') $_2$  were obtained with approximately 0.5 and 2  $\mu\text{M}$   $^{125}\text{I}$ -F(ab') $_2$ , respectively, both to dorsal and ventral retina cells (Fig. 3C). Nonspecific  $^{125}\text{I}$ -F(ab') $_2$  binding in the presence of P3X63 Ag8 ascites antibody was proportional to  $^{125}\text{I}$ -F(ab') $_2$  concentration and was not saturating at 3.52  $\mu\text{M}$ , the highest  $^{125}\text{I}$ -F(ab') $_2$  concentration tested.

**Geometry of the Gradient.** The retina grows by accretion of concentric rings of neurons at the periphery; thus, central retina is the oldest portion of the retina and peripheral retina is the youngest. To determine whether the antigen gradient is a polar gradient that rotates around the center of the retina with uniform antigen concentration along any arc from center to periphery or is a circumferential gradient extending from dorsoposterior to ventroanterior retina, left retinas of 14-day chicken embryos were cut into eight central and eight peripheral sections (Fig. 4A) which were assayed for TOP. A 35-fold gradient of antigen was found extending from dorsoposterior to ventroanterior margins of the retina aligned parallel to the long axis of the choroid fissure.

As shown in Fig. 4B, strips of retina extending from the dorsoposterior to ventroanterior margins, or perpendicular to this axis from anterior to posterior margins, were removed and each

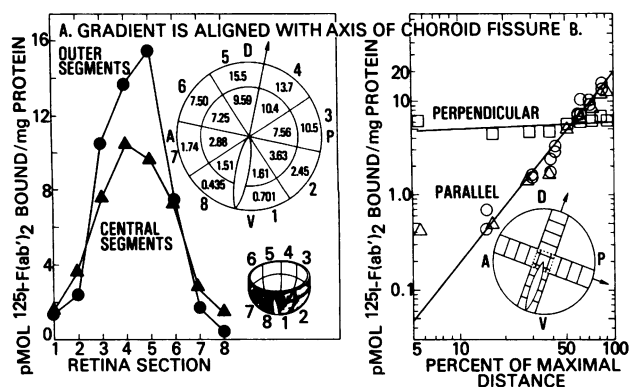


FIG. 4. Geometry of the TOP gradient in 14-day chicken embryo retina. Specifically bound  $^{125}\text{I-F(ab')}_2$  (pmol/mg of protein) is shown on the ordinate in A and B and within the appropriate segment of retina tested in A. (A) Each retina (left eye) was cut into eight  $45^\circ$  sections (7.25 mm in length from the center to periphery of retina) and each was divided into central (4.9 mm) and outer (2.35 mm) segments. (B) Demonstration that TOP concentration detected depends on the square of distance from the ventroanterior margin of the retina. Percentage of maximal circumferential distance is shown on the abscissa; 100% corresponds to 14.5 mm.  $\Delta$ , Strips of retina from ventroanterior (0%) to dorsoanterior (100%) retina margins,  $14.5 \times 2.5$  mm, were removed from eight retinas (left eyes), and each was cut into nine segments ( $1.6 \times 2.5$  mm) as shown; each segment was assayed for TOP antigen.  $\square$ , Strips of retina from dorsoanterior (0%) to posterior (100%) margins of the retina perpendicular to the choroid fissure were prepared and assayed as above.  $\circ$ , Data from A. The length of the arc from the ventral pole of the gradient to the center of each segment was calculated by assuming the retina to be a hemisphere and using equations for spherical triangles.

was cut into nine pieces which were assayed for antigen. The concentration of TOP molecules detected varied continuously and logarithmically with the logarithm of distance along the circumference of the retina from the ventroanterior pole of the gradient to the dorsoanterior pole, with a slope of 2. In contrast, little or no change was detected in retina cells along a perpendicular axis from anterior to posterior margins of the retina. The data are described somewhat better by a power function than by a logarithmic function, but a logarithmic function has not been ruled out.

The concentration of [ $^{125}\text{I-F(ab')}_2$ :anti-TOP antibody:TOP antigen] complex detected is described by the relationship:

$$[\text{TOP complex}] = \text{distance}^2.$$

Thus, cell position along a ventroanterior–dorsoanterior axis of retina can be identified by the concentration of TOP detected:

$$D_x = D_{\text{max}} (F_x/F_{\text{max}})^{0.5}$$

in which [TOP complex] is the fraction of maximal pmol of  $^{125}\text{I-F(ab')}_2$  specifically bound per mg of protein,  $F_x/F_{\text{max}}$ , and distance is the fraction of maximal circumferential distance from the ventroanterior to the dorsoanterior poles of the gradient,  $D_x/D_{\text{max}}$ . Under the conditions used,  $F_{\text{max}}$  is 20 pmol of  $^{125}\text{I-F(ab')}_2$  bound specifically per mg of protein and  $D_{\text{max}}$  is 14.5 mm. Thus, the calculated mean position in retina of cells that bind 5 pmol  $^{125}\text{I-F(ab')}_2$  specifically per mg protein is 7.25 mm from the ventroanterior pole of the gradient, which agrees well with the experimental values.

**Autoradiography and Immunofluorescence.** Autoradiography revealed most antigen in dorsoanterior chicken embryo retina in the inner and outer synaptic layers of the retina (Fig. 5 A and B). The antigen was detected in lesser amounts on the soma of most, or all, cell types in dorsoanterior retina. Little antigen was detected in ventroanterior retina (Fig. 5 C and D). Similar results were obtained by immunofluorescence (not shown). All cells mechanically dissociated from 8-day chicken embryo dorsoanterior retina exhibited punctate ring fluorescence. All cells from middle retina also were fluorescent, but less intensely than dorsoanterior retina cells. At each location, no obvious heterogeneity in cell population was seen. No fluorescent cells were detected in ventroanterior retina, although low levels of TOP were detected in ventroanterior retina by  $^{125}\text{I-F(ab')}_2$  binding.

**Expression of Antigen During Development.** The concentration of TOP antigen detected was higher in dorsoanterior retina than in ventroanterior retina at every age tested from the 4-day embryo through the adult (Fig. 6A *Inset*), and the axis and polarity of the gradient did not change during development (Fig. 6A). The concentration of antigen detected in the dorsal half of the retina increased 3-fold between the 4th and 12th days of embryonic development and then decreased slightly the adult. Antigen concentration detected in ventral retina did not vary with development. The amount of protein per retina and

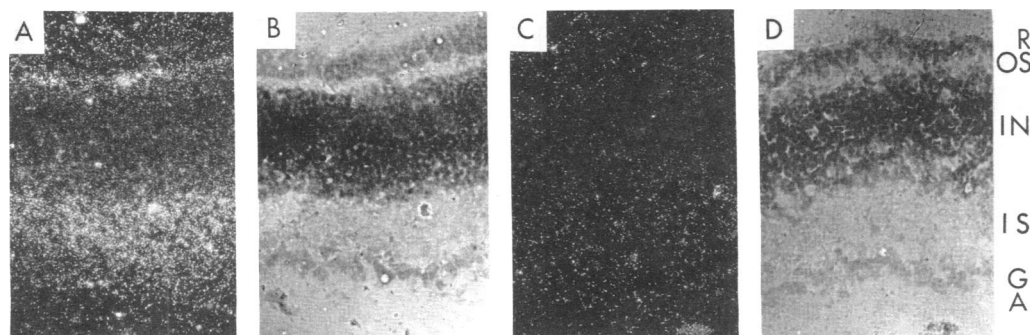


FIG. 5. Autoradiographs of 14-day chicken embryo retina. (A and B) Dark-field (A) and phase-contrast (B) views of dorsal retina. In A, some silver grains over cell soma in the inner nuclear layer appear dim due to staining of cells by toluidine blue. (C and D) Dark-field (C) and phase-contrast (D) views of ventral retina. R, photoreceptor layer; OS, outer synaptic layer; IN, inner nuclear layer; IS, inner synaptic layer; G, ganglion cell layer; A, ganglion cell axon layer. ( $\times 630$ .) Outer halves of 14-day chicken embryo dorsal or ventral retina were immersed in liquid Freon and then in liquid nitrogen and were cut in sections  $16 \mu\text{m}$  thick. Each section was incubated with antibody to TOP or P3X63 Ag8 antibody (ascites fluid diluted 1:50 with solution B) for 45 min at  $4^\circ\text{C}$ , washed six times (15 min) with solution B ( $50 \mu\text{l}$  per wash), incubated with  $50 \mu\text{l}$  of solution B containing  $20 \mu\text{g}$  of fluorescein conjugate of rabbit IgG anti-mouse IgG (Cappel) for 45 min at  $4^\circ\text{C}$ , and washed as above. For autoradiography, each section was incubated with  $50 \mu\text{l}$  of solution B containing  $440 \text{mM } ^{125}\text{I-F(ab')}_2$  ( $1 \times 10^5$  cpm) and  $500 \mu\text{g}$  of bovine serum albumin for 30 min at  $4^\circ\text{C}$ , washed as above, and coated with NTB-2 nuclear track emulsion (Kodak). Slides were exposed in the dark (22 days) at  $4^\circ\text{C}$  in the presence of a dessicant and stained with 0.02% toluidine blue.

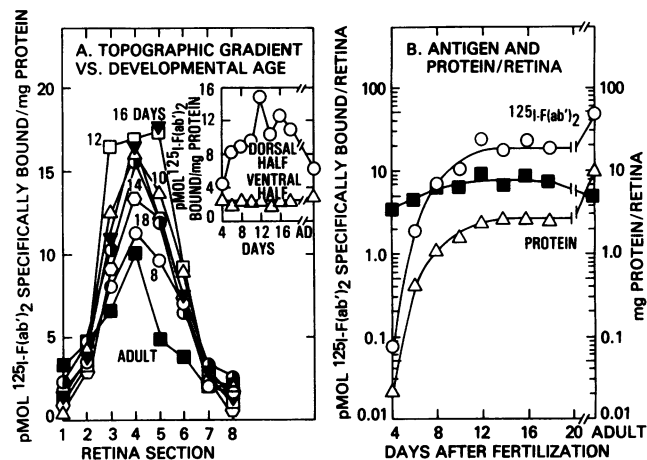


FIG. 6. TOP antigen in chick retina as a function of developmental age. (A) Each retina (left eye) was cut into eight sections as shown in Fig. 2. Symbols and days *in ovo* are:  $\circ$ , 8;  $\Delta$ , 10;  $\square$ , 12;  $\square$ , 14;  $\nabla$ , 16;  $\bullet$ , 18; and  $\blacksquare$ , adult. (Inset)  $^{125}\text{I-F(ab')}_2$  bound specifically is shown on the ordinate; days *in ovo* and adult (AD) are shown on the abscissa.  $\circ$ , dorsal half of retina (sections 3–6);  $\Delta$ , ventral half of retina (sections 1, 2, 7, and 8). Data for retina from 4- and 6-day embryos are shown only in Inset. (B)  $\circ$ ,  $^{125}\text{I-F(ab')}_2$  bound specifically per retina;  $\Delta$ , protein per retina;  $\blacksquare$ , ordinate represents pmol  $^{125}\text{I-F(ab')}_2$  bound specifically per mg of protein.

TOP antigen detected per retina increased 470- and 620-fold, respectively, between the 4-day embryo and the adult; the amount of antigen detected per mg of protein remained relatively constant (Fig. 6B). These results show that a gradient of TOP molecules is formed early in retina development, during the period of active neuroblast proliferation and neuron genesis, and that the gradient is maintained after neuron genesis ceases.

**Tissue Specificity.** Highest concentrations of TOP antigen detected were in regions of the nervous system derived from prosencephalon (forebrain): retina > cerebrum > thalamus (Table 1). Low levels of antigen were found in dorsal and ventral retina pigment epithelium, optic nerve, optic tectum, and cerebellum; little or no antigen was detected in heart, liver, kidney, or cells from blood.

**Chicken Embryo with an Ectopic Eye.** During the course of these studies, a chicken embryo with three eyes was found (Fig. 7). The third eye was situated in the middle of the forehead, facing in a dorsoanterior direction. Retinas from right, middle, and left eyes contained gradients of TOP molecules with normal polarity and alignment with the choroid fissure.

Table 1. Distribution of TOP antigen in chicken tissues

Tissue	$^{125}\text{I-F(ab')}_2$ specifically bound, pmol/mg protein	
	14-day embryo	Adult
Dorsal neural retina	12.0	7.70
Ventral neural retina	1.08	2.70
Cerebrum	3.30	3.12
Thalamus	2.13	—
Optic nerve	—	0.58
Optic tectum	0.15	—
Cerebellum	0.23	0.46
Dorsal retina pigment epithelium	0.26	—
Ventral retina pigment epithelium	0.25	—
Heart, liver, kidney, or blood cells	0.002–0.055	

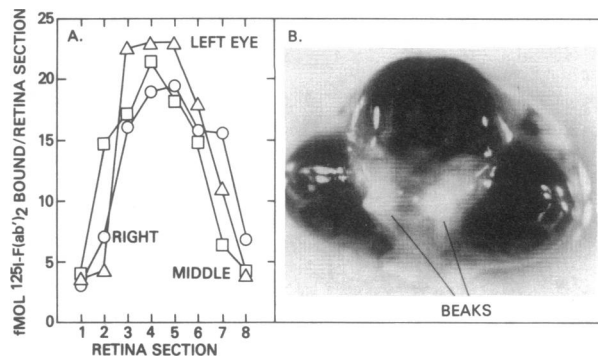


FIG. 7. (A) TOP antigen gradients in retinas from the right ( $\circ$ ), middle ( $\square$ ), and left ( $\Delta$ ) eyes of a 14-day chicken embryo with three eyes. Total  $^{125}\text{I-F(ab')}_2$  bound per retina section is shown. Reaction mixtures contained 2.38 nM  $^{125}\text{I-F(ab')}_2$ . (B) Frontal view of head of embryo. The third eye is situated on the forehead facing in a dorsoanterior direction. The embryo had two pairs of beaks, two brains in one head, and one body.

Thus, a gradient of TOP antigen was generated with normal orientation in the supernumerary eye despite the abnormal orientation of the eye in the embryo.

**Species Specificity.** Gradients of TOP molecules with similar orientation and symmetry were detected in turkey, quail, and duck embryo retina, 17, 15, and 16 days after fertilization, respectively (Fig. 8).  $^{125}\text{I-F(ab')}_2$  concentrations in reaction mixtures were low (0.074–0.26 nM); thus, bound  $^{125}\text{I-F(ab')}_2$  also was low. With 440 nM  $^{125}\text{I-F(ab')}_2$ , 7.5 pmol of  $^{125}\text{I-F(ab')}_2$  bound specifically per mg of dorsoposterior quail retina protein, comparable to chicken retina. The antigen was not detected in retina of goldfish, *Xenopus laevis*, *Rana pipiens*, or Fisher rats (not shown).

**Properties of TOP Antigen.** A dorsal→ventral gradient of GM<sub>2</sub> ganglioside molecules (II<sup>3</sup>NeuAc-G<sub>6</sub>Ose<sub>3</sub>Cer) in chicken embryo retina has been postulated but not detected (15). Bovine brain gangliosides (10–10,000  $\mu\text{M}$ ) did not inhibit the binding of anti-TOP antibody to retina (not shown). Rabbit antisera to mono-, di-, and trisialogangliosides (gift of C. Alving) bound to retina; however, a ganglioside gradient was not detected.

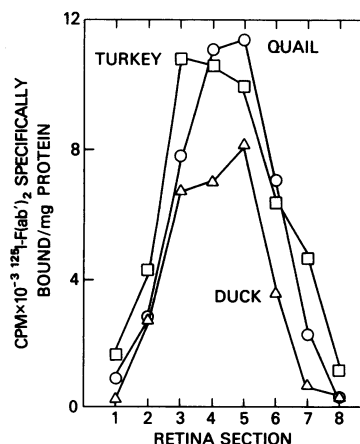


FIG. 8. TOP gradients in retina from Japanese quail ( $\circ$ ), *Coturnix coturnix japonica* (15-day embryo); White Pekin duck ( $\Delta$ ), *Anas platyrhynchos* (16-day embryo); and turkey ( $\square$ ), *Meleagris gallopavo* (17-day embryo). The eggs hatch 17, 28, and 28 days after fertilization, respectively.  $^{125}\text{I-F(ab')}_2$  concentrations and  $\mu\text{Ci}/\text{pmol}$  were as follows: quail, 0.26 nM, 0.90  $\mu\text{Ci}/\text{pmol}$ ; duck, 0.074 nM, 2.92  $\mu\text{Ci}/\text{pmol}$ ; and turkey, 0.078 nM, 2.38  $\mu\text{Ci}/\text{pmol}$ .

Table 2. Effect of trypsin or heat on TOP antigenicity

Exp.	Treatment of retina cells		<sup>125</sup> I-F(ab') <sub>2</sub> bound specifically to retina cells	
	0–30 min	30–40 min	cpm	%
1	Control	+ Trypsin inhibitor	1700	100
	Trypsin	+ Trypsin inhibitor	93	6
	Trypsin + trypsin inhibitor	—	1803	106
2	4°C, 30 min		1607	100
	100°C, 30 min		132	8

Inactivation of TOP retina molecules in 14-day chicken embryo dorsal retina by trypsin or heat. In Exp. 1, retina cells were incubated for 30 min at 37°C, in phosphate-buffered saline alone or with 11 μM trypsin (crystallized three times, Worthington) or with 11 μM trypsin inactivated with 12 μM soybean trypsin inhibitor (Worthington), and then for 10 min in soybean trypsin inhibitor. TOP ascites fluid was diluted 1:1000; 1.86 nM <sup>125</sup>I-F(ab')<sub>2</sub> (90.5 nCi/pmol) was used. In Exp. 2, TOP and P3X63 Ag8 antibodies were diluted 1:100; 1.82 nM <sup>125</sup>I-F(ab')<sub>2</sub> (96.9 nCi/pmol) was used.

TOP antigenicity was upon incubation at 100°C or by incubation with trypsin (Table 2). All TOP antigenicity in retina cell homogenates was recovered from the 100,000 × g particulate fraction; soluble antigen was not detected (not shown).

## DISCUSSION

Fusion of spleen cells from a mouse immunized with dorso-posterior chicken embryo retina with P3X63 Ag8 mouse myeloma cells yielded a line of hybridoma cells that synthesizes antibody to molecules that are distributed in a topographic gradient in the retina. At least 35-fold more antigen was detected in dorso-posterior than in ventroanterior retina. The antigen was found by autoradiography in highest concentration in 14-day chicken embryo retina on neurites in the inner and outer synaptic layers. The antigen was found by immunofluorescence on most, or all, cells from dorso-posterior and middle portions of 8- and 14-day chicken embryo retina. These results suggest that antigen molecules are distributed in the retina on the basis of cell position, rather than cell type.

Neurons in dorsal and ventral retina differ in several ways. For example, *Xenopus* retina is composed of at least three clonal domains (16). One cell on each side of the 16-cell embryo gives rise to dorsal retina, another cell gives rise to middle retina, and a third cell gives rise to cells that migrate across the midline of the embryo and form ventral retina on the opposite side. In addition, dorsal retina ganglion neurons synapse in ventral tectum, whereas ventral retina ganglion neurons synapse in dorsal tectum (1, 17, 18), establishing a continuous, point-to-point retino-tectal map.

Neurons are generated in chicken embryo retina between the 2nd and 12th days after fertilization; central retina is the oldest portion of the retina, and peripheral retina is the youngest. The concentration of TOP molecules detected in dorso-posterior retina increased as the diameter of the retina increased, and it varied with cell position at every stage tested, from the 4-day embryo through the adult, suggesting that the gradient of TOP is established as neurons and glia (and possibly their precursors) are generated in retina. Proliferation of clonal populations of cells with different genotypes in mosaic mice results in radial patterns of cells in neural retina and retina pigment epithelium (19); whether the gradient of TOP molecules is due to clonal inheritance remains to be determined.

Dorsal and ventral retina cells also differ in adhesive specificity—i. e., cells from dorsal retina adhere preferentially to cells from ventral retina or tectum and vice versa (20–22). However, the properties of TOP molecules differ from those reported for preferential adhesion of dorsal retina cells and for retina adhesion factors such as cognin (4), CAM (5), and ligand and agglutinin (23).

The dorso-posterior portion of chicken or pigeon retina contains a fovea, 3- to 5-fold more amacrine synapses than ventral retina (24), and a relatively high concentration of red droplets in photoreceptor cells and functions as a binocular visual field for pecking (25). Thus, dorso-posterior retina differs from other portions of retina in embryologic development, migration of cells and axons across the midline of the embryo, synapse specificity, adhesive specificity, and function.

The results suggest that a gradient of TOP molecules is formed by a gradient of cells which have different numbers of antigenic TOP molecules depending on cell position in the retina. No evidence was found for topographically distributed differences in antigen affinity for antibody or in the proportion of cells expressing antigen. Variation in antigen accessibility has not been excluded. The mechanism of generating and maintaining the gradient of TOP, highly ordered with respect to the axis of the retina, remains to be determined.

The function of TOP molecules has not been determined. However, since TOP antigen concentration detected is continuously graded and distributed on the basis of cell position not cell type, our working hypothesis is that TOP molecules play a role in the coding of positional information in the retina.

- Sperry, R. W. (1963) *Proc. Natl. Acad. Sci. USA* **50**, 703–710.
- Silver, J. & Sidman, R. L. (1980) *J. Comp. Neurol.* **189**, 101–111.
- Goldschneider, I. & Moscona, A. A. (1972) *J. Cell Biol.* **53**, 435–449.
- Hausman, R. E. & Moscona, A. A. (1979) *Exp. Cell Res.* **119**, 191–204.
- Thiery, J.-P., Brackenbury, R., Rutishauser, U. & Edelman, G. M. (1977) *J. Biol. Chem.* **252**, 6841–6845.
- Eisenbarth, G. S., Walsh, F. S. & Nirenberg, M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4913–4917.
- Barnstable, C. J. (1980) *Nature (London)* **286**, 231–235.
- Trisler, G. D., Donlon, M. A., Shain, W. G. & Coon, H. G. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 2368–2373.
- Köhler, G. & Milstein, C. (1975) *Nature (London)* **256**, 495–497.
- Galfre, G., Howe, S. C., Milstein, C., Butcher, G. W. & Howard, J. C. (1977) *Nature (London)* **266**, 550–552.
- Berzofsky, J. A., Hicks, G., Fedorko, J. & Minna, J. (1980) *J. Biol. Chem.* **255**, 11188–11191.
- Schneider, M. D., & Eisenbarth, G. S. (1979) *J. Immunol. Methods* **29**, 331–342.
- Bolton, A. E. & Hunter, W. M. (1973) *Biochem. J.* **133**, 529–539.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
- Marchase, R. B. (1977) *J. Cell Biol.* **75**, 237–257.
- Hirose, G. & Jacobson, M. (1979) *Dev. Biol.* **71**, 191–202.
- Hamdi, F. A. & Whitteridge, D. (1954) *Quant. J. Exp. Physiol.* **39**, 111–119.
- McGill, J. I., Powell, T. P. S. & Cowan, W. M. (1966) *J. Anat.* **100**, 5–33.
- Sanyal, S. & Zeilmaker, G. M. (1977) *Nature (London)* **265**, 731–733.
- Barbera, A. J., Marchase, R. B. & Roth, S. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2482–2486.
- Gottlieb, D. I., Rock, K. & Glaser, L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 410–414.
- Cafferata, R., Panosian, J. & Bordley, G. (1979) *Dev. Biol.* **69**, 108–117.
- Rutz, R. & Lilien, J. (1979) *J. Cell Sci.* **36**, 323–342.
- Yazulla, S. (1974) *J. Comp. Neurol.* **153**, 309–324.
- Galifret, Y. (1968) *Zeitschrift für Zellforschung* **86**, 535–545.