($P > 0.05$). w^e to white eye color = 0/5,443 in absence of HD and 12/19,491 (0.06%) in the presence of HD ($P > 0.05$). w^h to white eye color = 0/7,095 in absence of HD and 2/14,172 (0.01%) in presence of HD ($P > 0.05$).
13. T. I. Gerasimova, L. V. Mutyunina, Y. V. Ilyin, G. P. Georgiev, *Mol. Gen. Genet.* **194**, 517 (1984).
14. N. Kleckner, *Annu. Rev. Genet.* **15**, 341 (1981).
15. We are grateful to J. Mason and K. Vessey for their comments on the manuscript and to the technical assistance of J. Boyce and P. Oster. Supported by NSF grant BSR-8540977 to R.C.W., who is the recipient of Research Career Development Award K04-ES-00087.

23 March 1987; accepted 13 July 1987

Corresponding Spatial Gradients of TOP Molecules in the Developing Retina and Optic Tectum

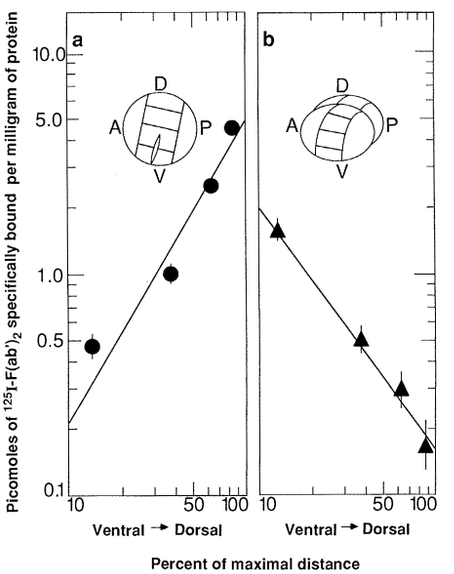
DAVID TRISLER AND FRANK COLLINS

The topographic map of cell position in the avian retina is inverted in its projection to the optic tectum. Dorsal retinal ganglion cell axons project to ventral tectum, and ventral retinal ganglion cells project to dorsal tectum. Topographic gradients of toponymic (TOP) cell surface molecules along the dorsoventral axes of retina and tectum also are inverted. TOP molecules are most abundant in dorsal retina and ventral tectum and least abundant in ventral retina and dorsal tectum during the period of initial retinal-tectal interaction. Thus, TOP molecules may be involved in orienting the retinotectal map.

NEURONAL PROJECTION MAPS ARE formed when one group of neurons connects with a second group so that the spatial order of cells in the first group is represented in the second. In the retinotectal projection, the spatial order of retinal ganglion cells is preserved in the order of their terminals in the optic tectum (*1, 2*). Such projections are thought to require corresponding location-dependent signals. A location-dependent signal could result from a graded distribution of one or more molecules along corresponding axes of each neuronal group. There is a gradient of cell surface molecules, termed TOP for toponymic—a marker of cell position, along the dorsoventral axis of the developing chick retina (*3*). We demonstrate here that the developing chick optic tectum also exhibits a tenfold gradient of TOP antigen along its dorsoventral axis, although inverted with respect to the retinal gradient, as are the cellular projections.

The distribution of TOP molecules in retina and tectum of chick embryos on embryonic day 5 (E5) was determined by radioimmunoassay with a monoclonal antibody to TOP (*3*). Strips of tissue along the dorsoventral axis were removed and cut into pieces, and cells from each region were assayed for TOP. A gradient of TOP was detected along the dorsoventral axis of both retina (Fig. 1a) and optic tectum (Fig. 1b).

Fig. 1. Gradients of TOP molecules in (**a**) retina and (**b**) optic tectum of White Leghorn chicken (*Gallus gallus*) embryos from E5. (a) Strips of retina (5.0 by 2.0 mm), extending from the ventroanterior (0%) to the dorsoposterior (100%) retinal margins, were removed from 48 eyes (24 embryos) and cut into four segments (1.25 by 2.0 mm), as shown (A, anterior; D, dorsal; P, posterior; and V, ventral). The choroid fissure, shown extending from the ventroanterior margin of retina, was used as a landmark for dissection. (b) Strips of optic tectum (3.0 by 2.0 mm), extending from ventral (0%) to dorsal (100%) tectum, were removed from 72 tectal lobes (36 embryos) and cut into four segments (0.75 by 2.0 mm), as illustrated. Radioimmunoassay of the binding of TOP monoclonal antibody was performed at 4°C as described (*3*). Retinal and tectal cells were mechanically dissociated in phosphate-buffered saline (PBS). Cell suspension (100 μl containing 150 μg of cell protein) was added to each well of polyvinyl chloride 96-well V-bottom plates (Dynatech) pretreated with solution B (PBS containing 1 mg of gelatin per milliliter of solution). Plates were centrifuged at 1300*g* for 5 minutes, and the supernatant was decanted. Pellets were washed (resuspended and repelleted) three times in 150 μl of solution B (150 μl each wash) and suspended for 1 hour in 50 μl of solution B containing 1 μl of ascites fluid (6 μg of antibody to TOP or P3X63Ag8 antibody). Cells were washed three times as above, then incubated for 30 minutes in 50 μl of solution B containing 440 n*M* ^{125}I-labeled F(ab′)$_2$ (5×10^4 cpm) fragments of sheep immunoglobulin directed against mouse immunoglobulin and 500 μg of bovine serum albumin. Cells were washed three times as above, wells were separated, and radioactivity was determined. The protein was measured by a modification of the method of Lowry *et al.* (*9*). Specific binding of antibody to TOP was calculated by subtracting the number of picomoles of ^{125}I-labeled F(ab′)$_2$ bound in the presence P3X63Ag8 myeloma antibody from the number of picomoles of ^{125}I-labeled F(ab′)$_2$ bound in the presence of antibody to TOP; 0.2 pmol of ^{125}I-labeled F(ab′)$_2$ bound per milligram of tectal cell protein in the presence of P3X63Ag8 antibody. No topographical differences in P3X63Ag8 antibody binding were detected in retina and tectum. Each point is the mean of nine determinations. Percent maximal circumferential distance from ventral to dorsal retina and tectal lobe is shown on the abscissa. Bars represent the standard error of the mean.



In retina, the highest binding to TOP was present dorsally; in optic tectum, the highest binding was present ventrally. The difference between dorsal and ventral levels of binding to TOP indicates at least a tenfold gradient in both retina and tectum in E5 embryos. Extrapolation of these values to the poles of the gradients suggests that the actual gradient may be considerably larger.

Immunofluorescence of antibody binding to cells of tissue sections taken along the dorsoventral axis of retina and optic tectum of E5 embryos was consistent with the results of radioimmunoassay (Fig. 2). TOP was most abundant in dorsal retina and ventral tectum, intermediate in middle retina and tectum, and least abundant in ventral retina and dorsal tectum. Visual scanning of fluorescently stained transverse sections of whole retina and optic tectum showed that TOP was continuously graded in each. The ring pattern of fluorescence around each cell is consistent with the fact that TOP is a cell surface molecule (*3*). As previously shown in retina (*3*), most or all cells across the thickness of tectum, that is, from the pial surface to the ventricular surface, express similar levels of TOP in any region along the dorsoventral axis (Fig. 2f inset). This

D. Trisler, Laboratory of Biochemical Genetics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892.
F. Collins, Laboratory of Biochemical Genetics, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, and Department of Anatomy, University of Utah School of Medicine, Salt Lake City, UT 84132.

indicates that the dorsoventral gradient is due to differences in the amount of TOP per cell rather than to differences in the number of cells expressing TOP. Thus, TOP can be used to identify cell position along the dorsoventral axis of the developing tectum as well as retina.

TOP was present in retina and optic tectum at all ages tested from E3 to E16 (Fig. 3). The level detected in tectum decreased during this period, whereas that in retina increased. In retina, the TOP gradient is present during the entire embryonic period and in the adult (*3*). In optic tectum, we found five times as much TOP per milligram of protein in the ventral as in the dorsal half of the tectum from E3 to E8, which suggests that the gradient shown at E5 is also present between E3 and E8. Retinal axons arrive at the ventral margin of the tectum on E6 and grow to middle tectum by E8 (*2, 4*). Thus, the TOP gradient is present in tectum during the initial interactions between retinal and tectal cells. The levels of antibody binding to TOP on tectal cells at E10 and later are too low for us to reliably detect dorsal-ventral differences.

The gradient of TOP in the tectum is not a general dorsoventral property of neural tube development because the polarity of the gradient is reversed in retina and tectum, derivatives of neighboring regions of the early neural tube. The tectal gradient of TOP does not require the presence of retinal ganglion cell axons in the tectum; the tectum expresses a gradient of TOP molecules before contact with arriving retinal axons.

A rostroventral to caudodorsal gradient of tectal maturation has been demonstrated (*5, 6*). TOP is most abundant in ventral tectum, the region that matures first. However, TOP does not increase in dorsal tectum as it matures later. The dorsal/ventral ratio of TOP is constant during the early period of tectal maturation. Thus, TOP expression is apparently not correlated directly with maturation.

The gradient of TOP molecules in the optic tectum can be used to identify cell position. Such positional information may be involved in tectal development and in establishing the retinotectal projection. The presence of corresponding TOP gradients in retina and tectum suggests a possible role for the molecule in orienting the dorsoventral axis of the retinal projection onto the tectum. The dorsoventral axis of retina is projected onto the optic tectum with reversed polarity, so that dorsal retina recognizes ventral tectum and ventral retina recognizes dorsal tectum (*1, 2, 7*). The polarities of the TOP gradients also are reversed. Therefore, retinal ganglion cell axons project to regions of tectum that early in development express similar relative levels of TOP antigen. This suggests the possibility of homophilic association between TOP molecules on retinal cell axons (*3, 8*) and tectal cells. Alternatively, the gradient of TOP in the tectum may organize the expression of a second molecule that interacts with retinal TOP. A mechanism of homophilic association between TOP molecules, however, is consistent with the observation that antibody to TOP prolongs the presence of growth cones and inhibits synapse formation in retina where cells with comparable TOP values interact with each other (*8*).

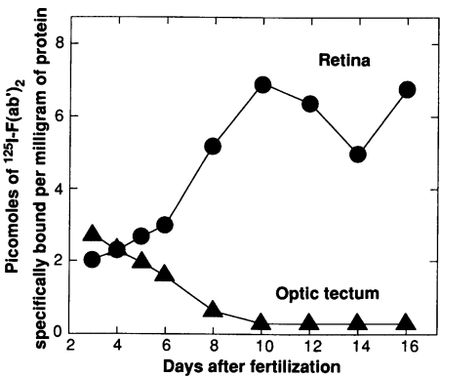


Fig. 3. TOP antigen in chick retina (●) and optic tectum (▲) as a function of developmental age. Assay conditions were as in Fig. 1. The standard error of the mean for all points was less than 6% of the reported value.

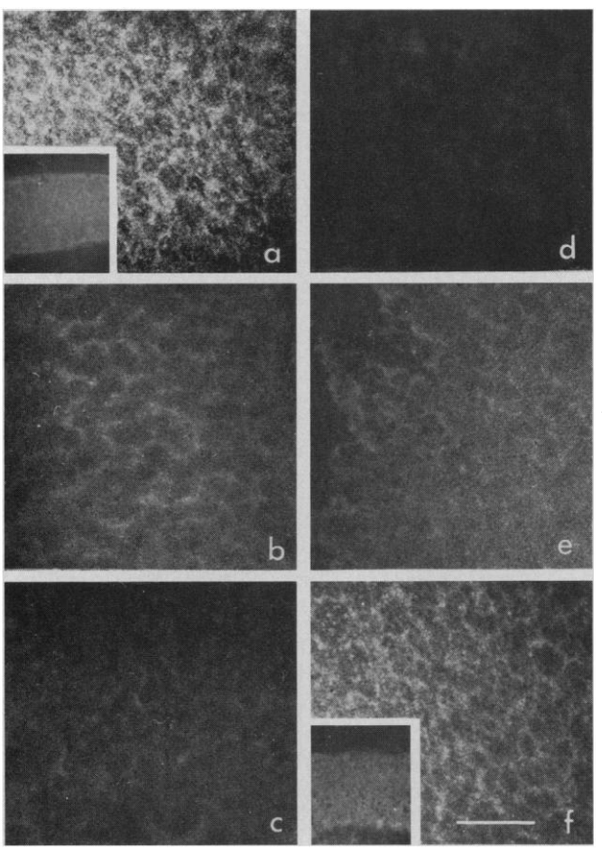


Fig. 2. Cellular distribution of TOP in dorsal, middle, and ventral retina and optic tectum of chick embryos from embryonic day 5. Sections 10 μm thick were cut from different regions of unfixed frozen retina and optic tectum. Retina: (**a**) dorsal; inset shows transverse section from vitreous to pigmented epithelium; (**b**) middle; (**c**) ventral. Tectum: (**d**) dorsal; (**e**) middle; (**f**) ventral; inset shows transverse section from ventricle to pia. Sections were mounted on the wells of dry Teflon-coated microscope slides (Roboz) and dried for 30 minutes at 21°C. Subsequent processing was carried out with 200 μl per well of solutions at 4°C. The sections were washed three times with solution B, then incubated in solution B containing 120 μg of antibody to TOP per milliliter for 2 hours in a humidified chamber. Sections were washed six times in solution B as above over a 20-minute period, then incubated for 1.0 hour in solution B containing 25 μg per milliliter of fluorescein isothiocyanate–conjugated goat immunoglobulin directed against mouse immunoglobulin (Kirkegaard & Perry). Sections were washed six times as above, and cover glasses were mounted in solution B containing 5.5 m*M* *p*-phenylenediamine to retard fluorescence bleaching (*10*). Sections were observed with a Zeiss Universal microscope with epifluorescence illumination, a BP485/2-nm excitation filter, and a LP520-nm barrier filter and were photographed (Kodak ASA 400 Ektachrome color slide film) with a 4.5-minute exposure for all sections. Calibration bar, 15 μm; 110 μm for insets.

REFERENCES AND NOTES

1. R. W. Sperry, *Proc. Natl. Acad. Sci. U.S.A.* **50**, 703 (1963).
2. G. R. DeLong and A. J. Coulombre, *Exp. Neurol.* **13**, 350 (1965).
3. G. D. Trisler, M. D. Schneider, M. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2145 (1981).
4. G. H. Rager, *Adv. Anat. Embryol. Cell Biol.* **63**, 1 (1980).
5. S. Fujita, *J. Comp. Neurol.* **122**, 311 (1964).
6. J. H. LaVail and W. M. Cowan, *Brain Res.* **28**, 421 (1971).
7. A. J. Barbera, R. B. Marchase, S. Roth, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2482 (1973).
8. D. Trisler, J. Bekenstein, M. P. Daniels, *ibid.* **83**, 4194 (1986).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
10. G. D. Johnson and G. M. C. Nogueira-Araujo, *J. Immunol. Methods* **43**, 349 (1981).

11. We thank P. Collins for technical assistance.

13 April 1987; accepted 26 June 1987