

CELL RECOGNITION AND PATTERN FORMATION IN THE DEVELOPING NERVOUS SYSTEM

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Summary

The topographic map of cell position in the avian retina is conserved and inverted when retinal ganglion neurons synapse with neurons in the optic tectum. Developmental mechanisms based on molecular gradients that specify positional information and pattern formation have been postulated in the establishment of these topographic maps of cells in retina and optic tectum. Two cell surface proteins in retina, TOP_{DV} and TOP_{AP}, are distributed in dorsoventral and anteroposterior topographic gradients, respectively. Corresponding gradients of TOP molecules present in the tectum are inverted with respect to the retinal gradients. These orthogonal gradients of TOP_{DV} and TOP_{AP} molecules provide a possible Cartesian coordinate system for designation of cell position at all points in the retinotectal map.

Introduction

Development and function of the nervous system involves the formation of a complex network of cellular organization. Highly stereotyped patterns of synaptic connections are formed between diverse classes of neurons in various and distant locations in the nervous system. In the retinotectal system of lower vertebrates, for example, the topographic order of retinal cells is conserved when retinal ganglion cells project to the optic tectum and other regions of the brain. Attardi and Sperry (1963), using the goldfish, demonstrated that after optic nerve transection regenerating axons from dorsal retina project to ventral tectum and those from ventral retina project to dorsal tectum (Fig. 1A). Posterior retina grows to anterior tectum and anterior retina to posterior tectum (Fig. 1B). Similar topography is present in the avian visual system (DeLong and Coulombre, 1965). Resolution of the topographic map in greater detail, determined by measuring electrophysiological responses of tectal cells to stimulation of discrete retinal fields, revealed a continuous point-to-point correspondence between cells of the retina and cells of the tectum (Fig. 1C; for a review, see Fraser and Hunt, 1980). The intricate specificity of synapses in the continuum map develops in a stereotyped pattern. However, the map is flexible to a degree. The retinal map will compress or expand

Key words: retinotectal development, growth cones, synapses, cell membrane molecule, topographic gradient, cell position.

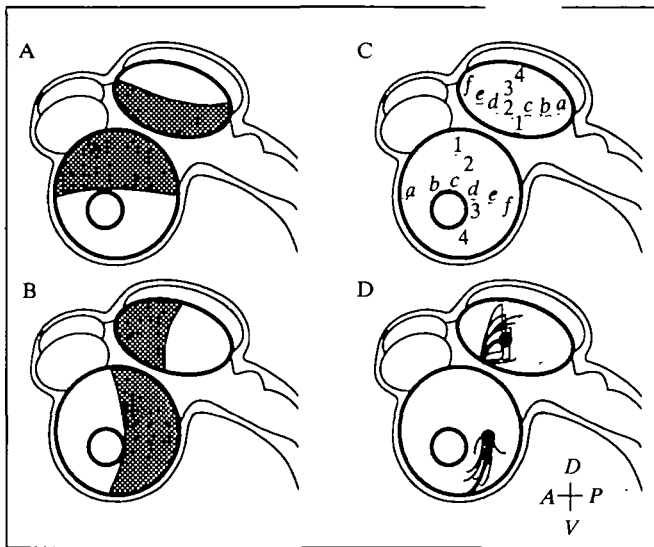


Fig. 1. Topographic projection of retinal ganglion cell axons to the optic tectum in lower vertebrates. (A) Dorsal retinal neurons innervate ventral tectum and ventral retina innervates dorsal tectum. (B) Anterior retina projects to posterior tectum and posterior retina projects to anterior tectum. (A, B after Attardi and Sperry, 1963; DeLong and Coulombre, 1965). (C) Continuous point-to-point topographic map of retinal projection onto tectum demonstrated by electrophysiological data (after Gaze *et al.* 1963; Gaze and Sharma, 1970; Yoon, 1971; Chung and Cooke, 1975; Schmidt *et al.* 1978). (D) Ganglion cell axons misrouted after disruption in retina and optic nerve can reorient on the tectum and project to the correct target site (after Thanos *et al.* 1984). *D*, dorsal; *V*, ventral; *A*, anterior (nasal); *P*, posterior (temporal/caudal).

to fill the available synaptic field of tectum after ablation of part of the retina or tectum, yet maintain the topographic order of the map (Gaze *et al.* 1963; Gaze and Sharma, 1970; Yoon, 1971; Chung and Cooke, 1975; Schmidt *et al.* 1978). The orderly retinotectal projection apparently relies, in part, on the ability of retinal axons to read tectal cues. In the developing chick retina, misrouted ganglion cell axons, disrupted in the retina and optic nerve, can reorient on the tectal surface and project to the correct target site (Fig. 1D; Thanos *et al.* 1984). Some misrouted growth cones migrate to the appropriate anterior–posterior tectal longitude, make a 90° turn and project to the correct position along the dorsoventral axis of the tectum.

Developmental mechanisms involved in axonal guidance that result in synaptic specificity are not fully understood. Molecular gradients have been proposed as a mechanism for encoding cell positional information and establishing pattern in the embryo (Boveri, 1901; Dalcq and Pasteels, 1937; Child, 1941; Sperry, 1963; Grierer and Meinhardt, 1972; Fraser and Hunt, 1980; Whitelaw and Cowan, 1982; Trisler, 1982). Topographically distributed molecules have been reported in retina

and optic tectum (Trisler *et al.* 1981; Constantine-Paton *et al.* 1986; Rabacchi and Dräger, 1987; Trisler and Collins, 1987; Müller *et al.* 1990).

Topographically graded molecules in retina and optic tectum

Topographically graded molecules in retina were detected with monoclonal antibodies generated by the fusion of spleen cells from mice immunized with a small portion of dorsal or posterior retina to P3X63Ag8 mouse myeloma cells (Fig. 2). One antibody to a cell surface molecule bound more abundantly to cells from dorsal retina than to cells from the remainder of the retina. A second antibody bound preferentially to cells of posterior retina. These molecules, termed TOP for toponymic (i.e. a marker of position), are present throughout the retina but are distributed in topographic gradients (Fig. 3). TOP_{DV} is graded dorsoventrally and TOP_{AP} is graded anteroposteriorly. Bilaterally symmetrical gradients of TOP molecules are present in the retinas of both right and left eyes.

A 35-fold gradient of TOP_{DV} was found extending from the dorsal to the ventral margins of the retina aligned parallel to the long axis of the choroid fissure (Fig. 4), and a 16-fold gradient of TOP_{AP} is present from the anterior retinal margin to the posterior margin perpendicular to TOP_{DV}. The concentration of TOP molecules detected varied continuously and logarithmically with the logarithm of distance along the circumference of retina.

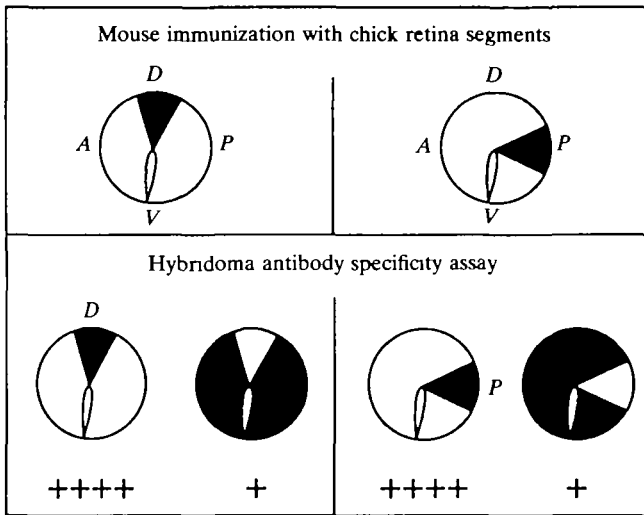


Fig. 2. Strategy for detecting topographically distributed retinal antigens. Mice were immunized with dorsal or posterior retina segments from E14 chicken (*Gallus gallus*) embryos. Hybridomas were produced with spleen cells from the hyperimmune mice and monoclonal antibodies were screened for preferential binding to dorsal or posterior retinal cells over cells from the remainder of retina. D, V, A and P correspond to dorsal, ventral, anterior and posterior, respectively. The choroid fissure shown extending from the ventral margin to central retina was used as a landmark for dissection (after Trisler *et al.* 1981).

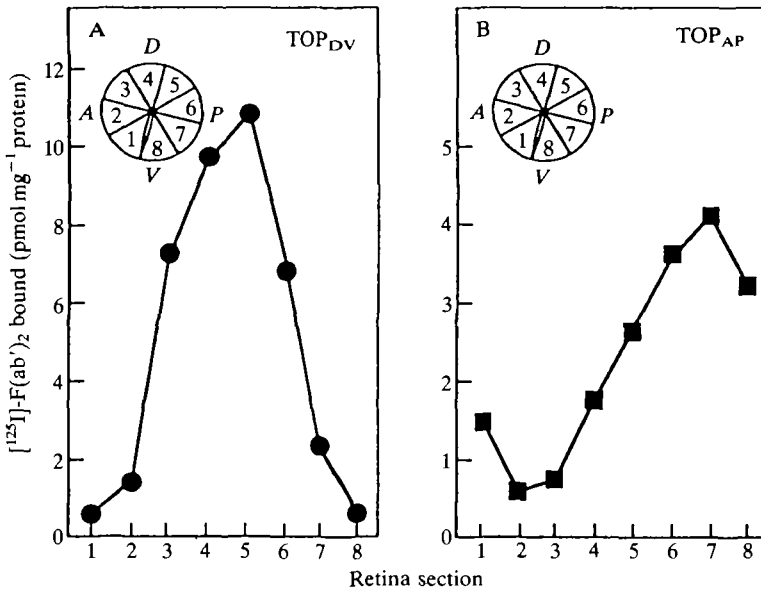


Fig. 3. Topographic gradients of (A) TOP_{DV} (●) and (B) TOP_{AP} (■) molecules in E14 chick retina. Molecular gradients detected with mouse monoclonal anti-TOP_{DV} and anti-TOP_{AP} antibodies. Values shown are picomoles of $[^{125}\text{I}]\text{-F(ab')}_2$ fragment of rabbit IgG directed against mouse IgG specifically bound to retinal cells exposed to anti-TOP antibody per milligram of retina protein (A after Trisler *et al.* 1981).

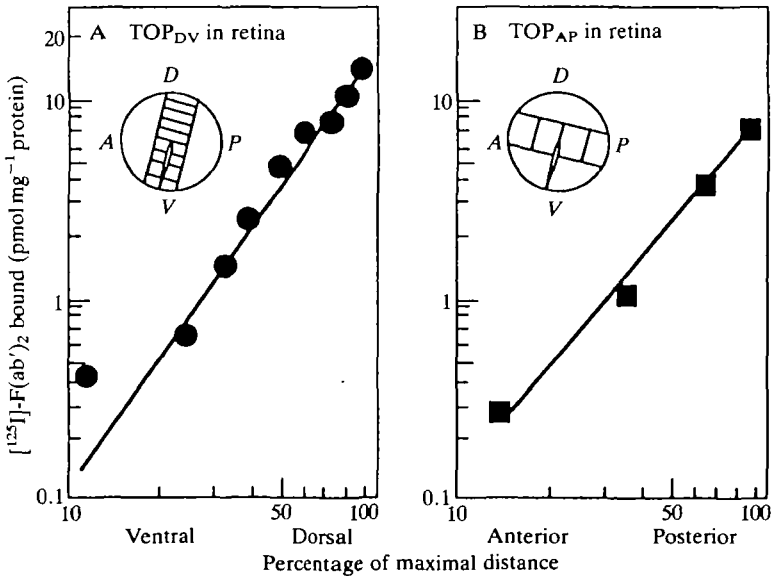


Fig. 4. Geometry of (A) TOP_{DV} and (B) TOP_{AP} gradients in E14 retina. TOP concentrations were determined from strips of retina cut along the axes of the respective gradients.

Indirect immunofluorescence of anti- TOP_{DV} antibody binding to cells in tissue sections taken along the dorsoventral axis of retina of E5 embryos revealed TOP_{DV} was most abundant in dorsal retina, intermediate in middle, and least abundant in ventral retina (Fig. 5). No obvious heterogeneity was observed in the cell population from each location. Most or all cells across the thickness of retina from the vitreal surface to the pigmented epithelium stained. Visual scanning of fluorescently stained transverse sections of whole retina showed that TOP_{DV} was continuously graded. This indicates that the dorsoventral gradient is due to differences in the amount of TOP_{DV} per cell rather than to differences in the number of cells expressing TOP_{DV} . The ring fluorescence pattern around each cell is consistent with the fact that TOP_{DV} is a cell surface molecule. In older embryos and in adult retina, TOP_{DV} is most abundant in the synaptic layers and in the ganglion cell axon layer (Fig. 5G).

Similarly, TOP_{AP} is distributed in a graded pattern along the anteroposterior axis (Fig. 5). The ring fluorescence of cells from anterior retina was weakest, that of middle cells was intermediate and that of cells from posterior retina exhibited the greatest binding of anti- TOP_{AP} in E5 embryos. Autoradiography of anti- TOP_{AP} binding along the anterior–posterior equatorial meridian of the entire E8 retina revealed the TOP_{AP} gradient. Since TOP_{DV} and TOP_{AP} molecules are graded on the basis of number of molecules per cell, TOP_{DV} can be used to identify cell position along the dorsoventral axis, while TOP_{AP} identified cell position along the anteroposterior axis of retina.

Gradients of TOP molecules were present in retina at all developmental ages tested from E4 to E18 and in hatchling chicks (Fig. 6). The orientation of the gradients remained constant throughout development; however, the magnitude of the TOP_{DV} gradient increased 10 times from a threefold gradient at E4 to a 35-fold gradient at E12. The TOP_{AP} gradient increased fivefold from E5 to E18. Thus, the TOP gradients are present early during blast cell proliferation and persist throughout development when both neurogenesis and gliogenesis occur and tissue organization is established.

Both TOP_{DV} and TOP_{AP} are cell surface proteins. TOP_{DV} has an apparent relative molecular mass of 47×10^3 (Moskal *et al.* 1986) and TOP_{AP} of 40×10^3 . Topographic differences in TOP_{AP} distribution were found in immunoblots of retina cell proteins resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Approximately 16-fold more TOP_{AP} was detected in cells from the posterior margin of retina than from the anterior margin (Fig. 7). Analogously, more TOP_{AP} was present in the posterior half-retina than in the anterior half-retina. These results suggest that the graded nature of TOP_{AP} demonstrated by radioimmuno-binding assay and by indirect immunofluorescence reflects the distribution of the antigen itself and not that of a masking molecule or of antigen accessibility. However, a gradient of post-translational modification of evenly distributed TOP_{AP} molecules has not been ruled out.

TOP_{DV} and TOP_{AP} molecules were also detected in the optic nerve (Fig. 8). Their distribution matched the organization of ganglion cell axons in the nerve.

Axons from peripheral dorsal retina cells of E12 embryos were shown by fluorescent dye-tracing to be present in dorsal nerve. Indirect immunofluorescence revealed a greater abundance of TOP_{DV} in dorsal nerve than in ventral

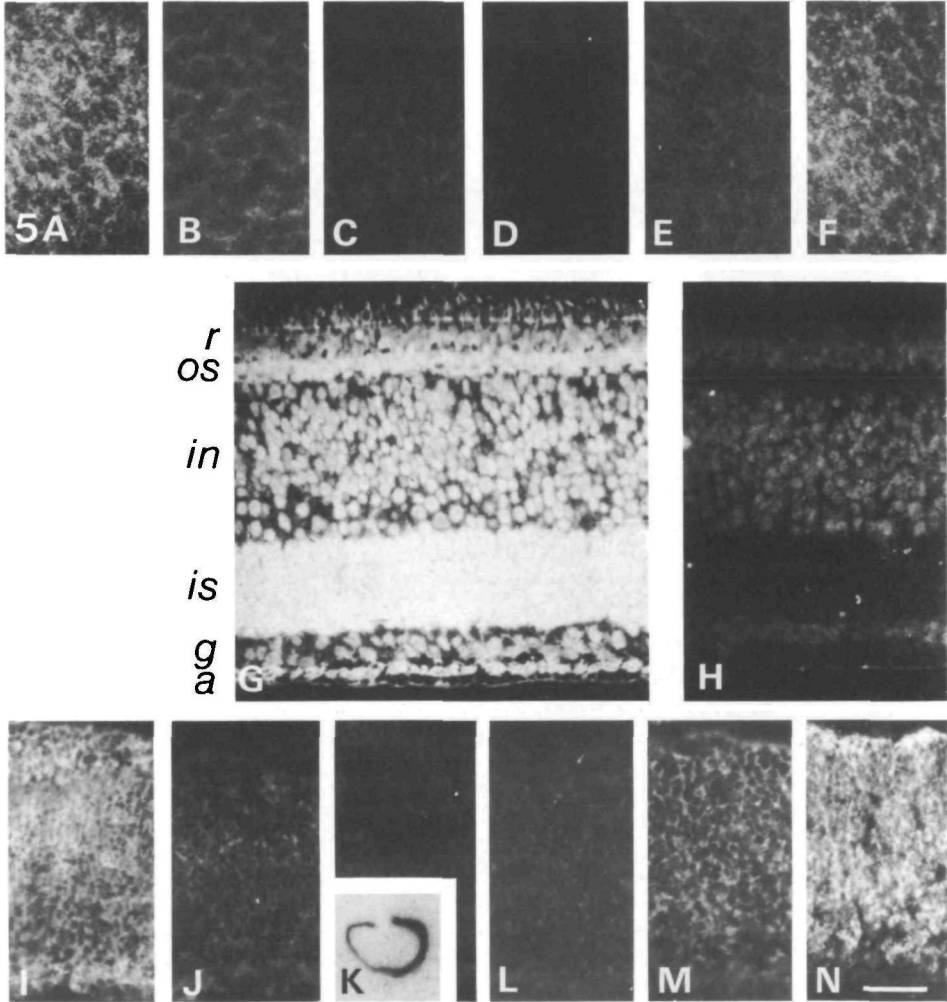


Fig. 5. Cellular distribution of TOP molecules. Indirect immunofluorescence of anti-TOP_{DV} and anti-TOP_{AP} binding to cells in 10 μm thick sections of retina and optic tectum. (A,B,C) Anti-TOP_{DV} binding to dorsal, middle and ventral E5 retina, respectively; (D,E,F) anti-TOP_{DV} binding to dorsal, middle and ventral E5 optic tectum, respectively; (G) anti-TOP_{DV} in E18 dorsal 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); (H) P3X63Ag8 myeloma antibody binding to E18 retina; (I,J,K) anti-TOP_{AP} binding to posterior, middle and anterior E5 retina, respectively (inset in K is an autoradiogram of anti-TOP_{AP} binding to cells in a section along the equatorial meridian of E8 retina); (L,M,N) anti-TOP_{AP} binding to posterior, middle and anterior tectum, respectively. Scale bar, 12 μm A-F; 25 μm , G and H; 35 μm , I-M; 6.5 mm, inset).

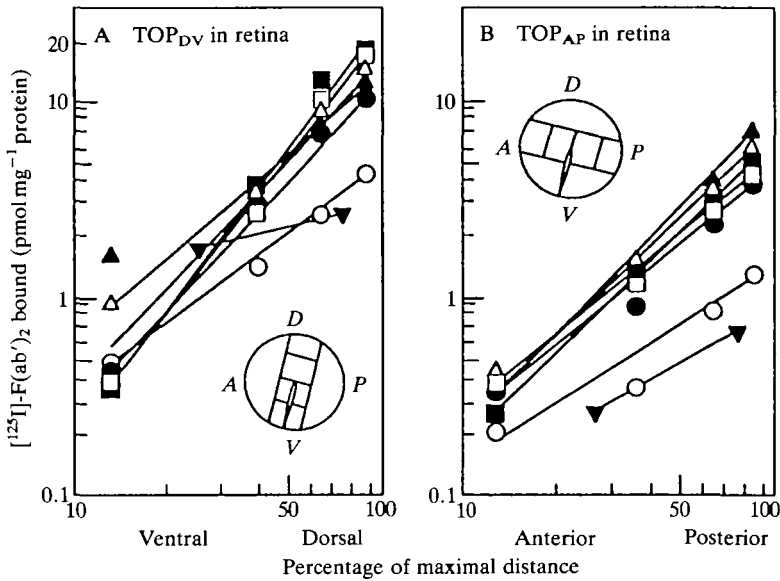


Fig. 6. TOP antigen gradients in chick retina as a function of developmental age. (A) TOP_{DV} ; (B) TOP_{AP} in (\blacktriangledown), E4; (\circ), E5; (\bullet), E8; (\square), E10; (\blacksquare), E12; (\triangle), E14; (\blacktriangle), E18 retinas.

nerve. Ganglion cell axons from posterior retina were in anterior nerve, as were higher levels of TOP_{AP} . Although the distribution of TOP molecules reiterates the organization of ganglion cell axons in the nerve, the question of whether TOP molecules in the nerve are associated with axons, with glia or with both is not resolved. In E12 embryos, at the age when retinal axons have covered the tectal surface, TOP_{AP} was most abundant on the anterior medial portion of tectum (Fig. 8) where posterior retinal axons project (DeLong and Coulombre, 1965).

TOP molecules are present on optic tectum cells as well as in retina (Trisler and Collins, 1987). They are distributed in gradients in E5 embryos 1 day before ganglion cell axons arrive at the tectum (Fig. 9). TOP_{DV} and TOP_{AP} gradients in tectum are inverted with respect to the retinal gradients. TOP_{DV} is 10-fold higher in ventral than in dorsal tectum and TOP_{AP} is eightfold higher in anterior than in posterior tectum. The quantities of TOP_{DV} and TOP_{AP} detected per cell by indirect immunofluorescence varied continuously along the axes of the tectal gradients (Fig. 5). Little or no heterogeneity of TOP_{DV} or TOP_{AP} expression was found among cells from ventricular to pial surfaces at a given position along the respective gradients. Thus, as in retina, TOP molecules can be used to mark cell position in tectum.

The orthogonal monotonic gradients of TOP_{DV} and TOP_{AP} topographic molecules constitute a possible Cartesian coordinate system that can be used to designate cell position at all points in the plane of the retina and optic tectum (Fig. 10). The presence of corresponding TOP gradients in retina and tectum

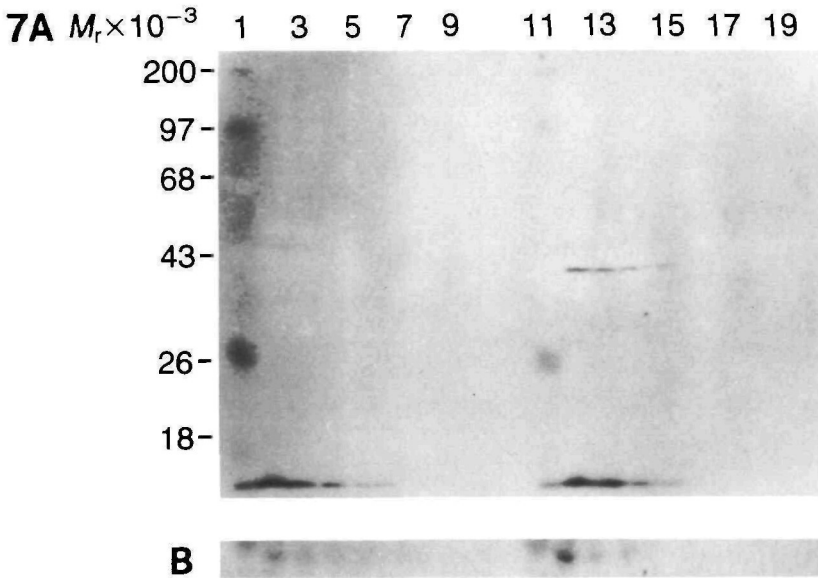


Fig. 7. Demonstration of TOP_{AP} topographic distribution in retina and determination of TOP_{AP} relative molecular masses by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis. (A) Lanes 1 and 11, relative molecular mass standards; lanes 2-10, serial twofold dilutions of 25 μ g of E8 protein from the anterior retinal margin; lanes 12-20, serial twofold dilutions of 25 μ g of E8 protein from the posterior retinal margin; (B) detail of autoradiogram of anti-TOP_{AP} binding to serial dilutions of 25 μ g of retina protein from anterior half-retina (lanes 2-10) and posterior half-retina (lanes 12-20).

suggests a possible role for the molecules in orienting the dorsoventral and anteroposterior axes of the retinal projection onto the tectum.

Cell lineage and regulation of TOP expression

The retina grows by accretion of rings of cells in the proliferative zone at the peripheral margin (Coulombre, 1955; Kahn, 1974). Expression of the TOP gradient during retinal growth was examined by comparison of the amount of TOP_{DV} in the proliferative zone at the poles of the gradient in E4-E10 retinas with the amount of TOP_{DV} at the corresponding distances along the gradient axis in E12 retina (Fig. 11). The cells in the proliferative zone at the dorsal margin of retina expressed progressively more TOP_{DV} with retinal growth, while those in the proliferative zone at the ventral margin expressed progressively less TOP_{DV}, thereby increasing the magnitude of the gradient (Trisler, 1987). There was close agreement in the amount of TOP_{DV} detected on cells at a given distance along the gradient axis from the fundus, the oldest portion of the retina, throughout the developmental period tested. Thus, each position along the axis has a constant TOP_{DV} value through these developmental ages.

The level of TOP_{DV} expressed in the progeny of cells dividing at various angles

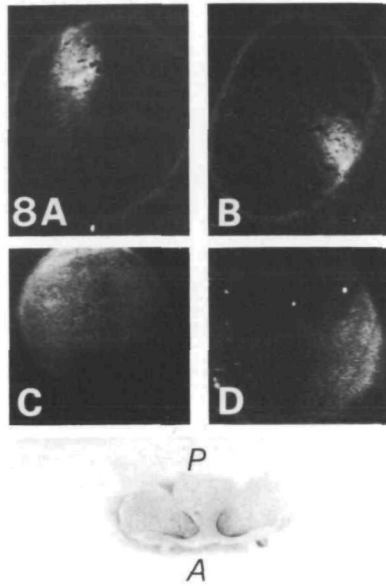


Fig. 8. TOP molecules in optic nerve and optic tectum. (A,B) Anterogradely transported rhodamine isothiocyanate from dorsal and posterior retina in E12 optic nerve from within 1 mm of the nerve head, respectively; (C,D) image-enhanced video micrographs of TOP_{DV} and TOP_{AP} binding in E12 optic nerve, respectively; (E) dorsal view of a whole mount of E12 optic tectal lobes stained with anti-TOP_{AP} antibody and horseradish-peroxidase-labeled rabbit antibody to mouse IgG.

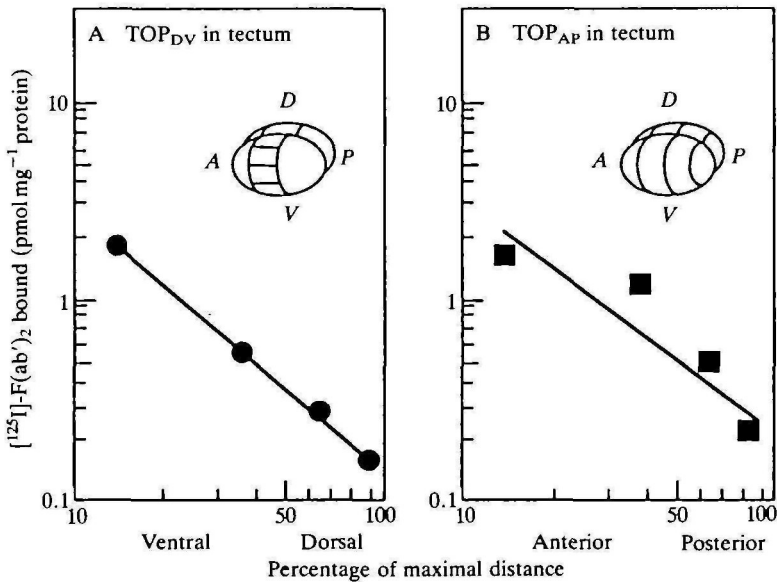


Fig. 9. Topographic gradients of (A) TOP_{DV} and (B) TOP_{AP} in E5 optic tectum.

to the axis of the antigen gradient was determined by comparing TOP_{DV} expression in cells of the proliferative zone of E4 and E12 retinas at different positions around the peripheral margin (Fig. 12). Both the magnitude and the sign of change in TOP_{DV} expression during development varied depending on the position of the parental cells. Progeny cells in dorsal retina expressed more TOP_{DV} than parental cells while those in ventral retina expressed less. The greatest magnitude of change in expression was along the axis of the gradient (0°). Little or no change in TOP_{DV} expression occurred in cells with retinal growth along the perpendicular axis (90°). Intermediate rates of change in TOP_{DV} expression were found during cell division at 45° to the gradient axis.

Ablation of parental cells in the proliferative zone at the dorsal and ventral poles of the gradient in embryos 60h after fertilization altered the level of TOP_{DV} expression in dorsal and ventral retina later in development (Fig. 13). Progeny cells that replaced the ablated dorsal region expressed 60% less TOP_{DV} than normal in E16 retina. After ventral ablation, cells in ventral retina expressed 300% more TOP_{DV}. These values are similar to that expected for the progeny of parental cells from regions neighboring the ablated portion. This suggests that by 60h of development the level of TOP_{DV} expressed by the retina cells and their progeny is already determined.

Function of TOP_{DV} in retinal development

The role of molecular markers of cell position in the development of the nervous system was examined using a monoclonal antibody to TOP_{DV} (Trisler, 1983; Trisler *et al.* 1986). Antibodies provide a means of blocking molecular function (Levi-Montalcini and Booker, 1960; Crawford *et al.* 1982; Schwartz and Spirma, 1982; Warner *et al.* 1984). Several groups have demonstrated effects of antibodies against nervous system molecules on growth cone behavior, neurite outgrowth and tissue organization *in vivo* and *in vitro*. Antibodies against chicken cognin and N-CAM inhibit cell-cell adhesion (Hausman and Moscona, 1979; Thiery *et al.* 1977, respectively) and anti-N-CAM disrupts axonal fasciculation (Thanos *et al.* 1984; Fraser *et al.* 1984). Antibody T61/3/12 blocks neurite outgrowth of chick retina cells *in vitro* (Henke-Fahle and Bonhoeffer, 1983), whereas antibody to Thy-1 stimulates neurite outgrowth of rat retinal ganglion cells *in vitro* (Leifer *et al.* 1984). Antibody L1 inhibits granular cell migration in rat cerebellar explants (Lindner *et al.* 1983). Our objectives were to determine the accessibility of TOP_{DV} to antibody in the *in vivo* retina, to determine the persistence of antibody in retina after injection into the embryo, and to identify changes in the development of retina continuously exposed to anti-TOP_{DV} antibody.

Antibody to TOP_{DV} injected into the amniotic cavity of *in ovo* chick embryos 2–4 days after fertilization was detected on retina cells 1 day after injection (Fig. 14). The concentration of [Anti-TOP_{DV}·TOP_{DV}] complexes (Ab·TOP_{DV}) detected was higher in dorsal retina than in ventral retina. Anti-TOP_{DV} antibody was injected intraocularly into the vitreal space of E7–E19 eyes. A dorsal to

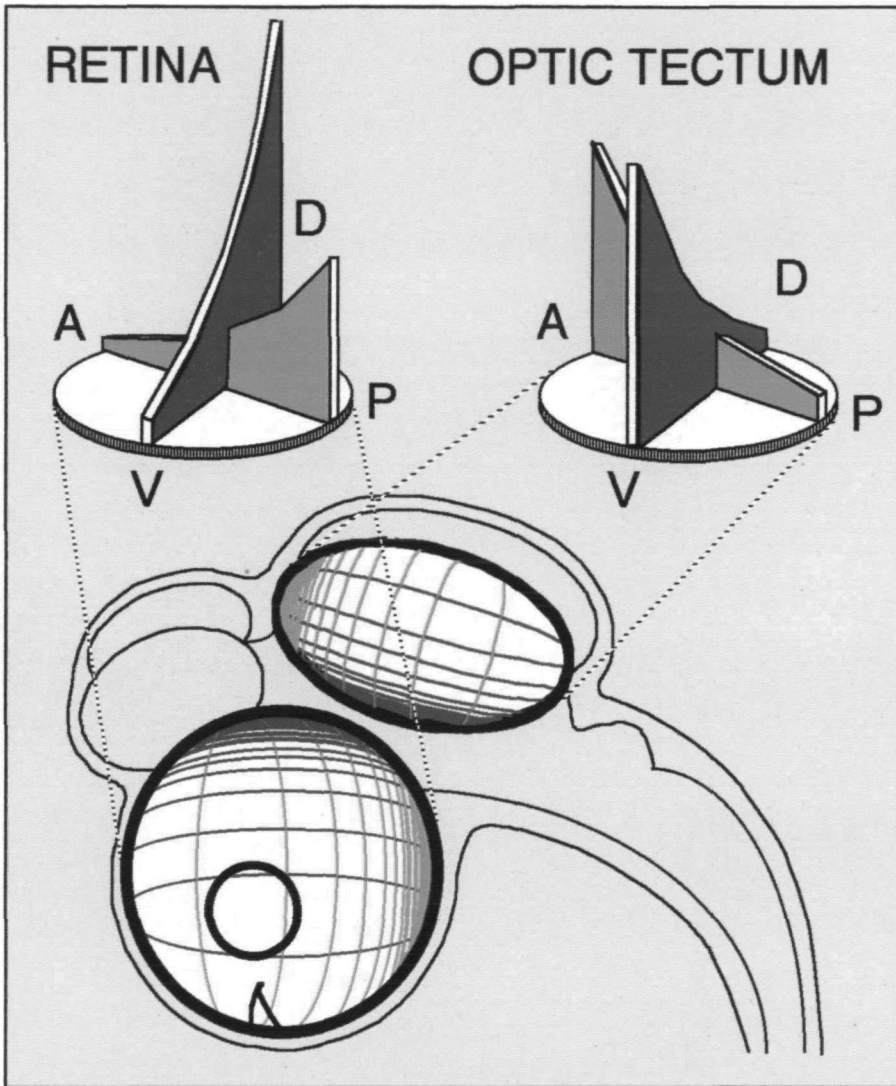


Fig. 10. Schematic diagram of the corresponding orthogonal gradients of TOP_{DV} and TOP_{AP} in retina and optic tectum. D, dorsal; V, ventral; A, anterior (nasal); P, posterior (temporal/caudal).

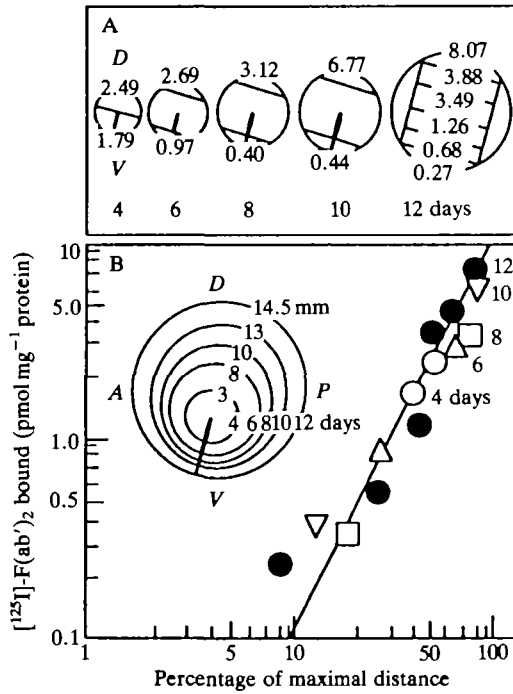


Fig. 11. Increase in TOP_{DV} gradient with retinal growth. (A) TOP_{DV} detected at the poles of the gradient of E4–E10 retinas and along the axis of the gradient of E12 retina. (B) TOP_{DV} values at these positions for each age as a function of distance along the axis of the gradient in E12 retina. Circumferential distances from dorsal to ventral poles for each age are shown: ○, E4; △, E6; □, E8; ▽, E10; ●, E12 (after Trisler, 1987).

ventral gradient of Ab·TOP_{DV} complexes of the same magnitude and orientation detected by *in vitro* binding studies was present 24 h after intraocular injection of antibody.

A gradient of Ab·TOP_{DV} complexes in retina persisted for 4 days after intraocular injection of mouse ascites fluid containing anti-TOP_{DV} antibody (Fig. 15). The Ab·TOP_{DV} gradient was maintained for 10 days when hybridoma cells, that synthesize anti-TOP_{DV} antibody, were injected. The hybridoma cells provide a continuous source of antibody for long-term maintenance of Ab·TOP_{DV} complexes in the retina. The decrease in Ab·TOP_{DV} complexes in retina after E18 represents a loss in accessibility of TOP_{DV} to intraocular antibody, perhaps due to developmental changes in permeability of the inner limiting membrane of the retina.

Two aspects of the process of synapse formation – the disappearance of growth cones and the appearance of synapses – were measured in retina from embryos exposed to anti-TOP_{DV} antibody. Antibody was injected into eyes of E11 embryos at the time neuron process layers are forming and 2 days before the first

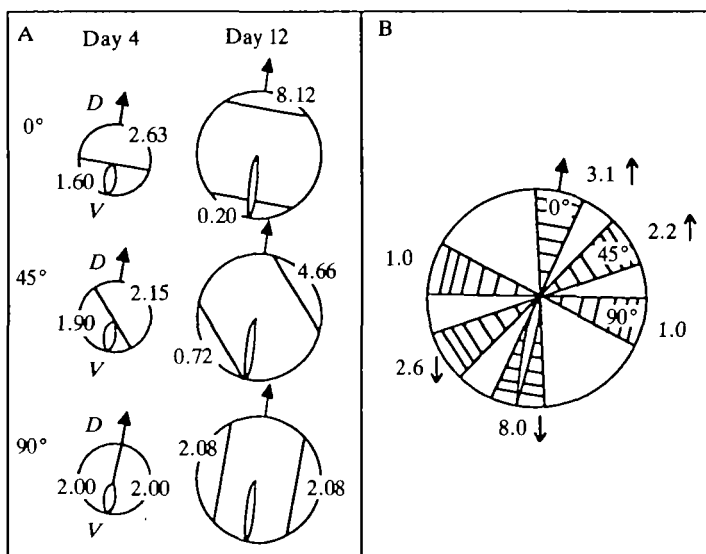


Fig. 12. Comparison of TOP_{DV} expression in parental cells (E4) and progeny cells (E12) during embryonic development as a function of the angle of growth from the axis of the TOP_{DV} gradient in retina. (A) TOP_{DV} detected at the peripheral margins of E4 and E12 retina along the axis (0°) and at 45° and 90° to the axis. (B) Change of TOP_{DV} expression at each angle from E4 to E12 retinal growth (after Trisler, 1987).

structurally identifiable synapses appear (Sheffield and Fishman, 1970; Hughes and LaVelle, 1974; Daniels and Vogel, 1980). For the electron microscopic analysis of injected retinas, growth cones were defined as bodies containing large, irregular membrane cisternae or vesicles (Del Cerro and Snider, 1968; Kawana *et al.* 1971). These bodies were larger in section than most neurites and were sometimes seen in continuity with neurites and filopodia. Anti-TOP_{DV} antibody from the injected hybridoma source reached maximal binding in the retina 4 days after injection (E15 embryos). At this time, retinal development appeared normal. However, with continued exposure to anti-TOP_{DV} antibody retinal development was altered. The normal progressive loss of growth cones during development was delayed in retinas containing Ab·TOP_{DV} complexes and synapse formation was inhibited (Fig. 16). The inhibition of synapse formation was not restricted to any particular cell type. Both conventional synapses and bipolar cell ribbon synapses were present in reduced numbers from 5 to 7 days after injection of anti-TOP antibody. Synapse formation appeared to be interrupted across the entire inner synaptic layer in both dorsal and ventral retina. Our working hypothesis is that TOP_{DV} molecules mark cell position in the retina and that, in the presence of Ab·TOP_{DV} complexes, growing neurites fail to detect the TOP_{DV} gradient. This could result in the persistence of growth cones and the delay in synaptogenesis. Synapses eventually form after exposure to anti-TOP antibody. It is not known whether these synapses are positionally correct.

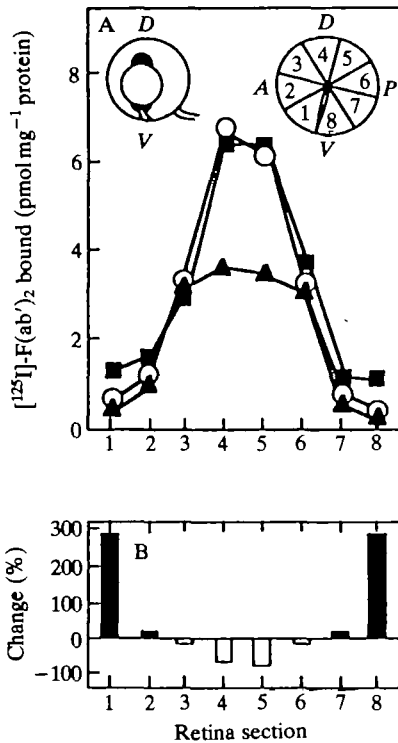


Fig. 13. TOP_{DV} gradients in E16 retinas after ablation of parental cells at the dorsal or ventral poles of the gradient in the cell proliferative marginal zone 60 h after fertilization. (A) TOP_{DV} in ○, normal retina; ▲, retina after dorsal pole ablation; ■, retina after ventral pole ablation. (B) Percentage change of TOP_{DV} expression for each region of retina after ablation.

Conclusions

The topographic map of cell position in retina is conserved and inverted when retinal axons project to and synapse with neurons of the optic tectum. Developmental mechanisms based on molecular gradients that specify positional information and pattern formation have been postulated in the establishment of the topographic map of cells in avian retina and optic tectum. Orthogonal gradients of toponymic (TOP) cell membrane molecules are present in the retina and optic tectum. TOP_{DV} molecules are distributed dorsoventrally and TOP_{AP} molecules are graded anteroposteriorly. The polarities of the gradients are inverted in tectum with respect to retina. TOP_{DV} is most abundant in dorsal retina and ventral tectum and TOP_{AP} is most abundant in posterior retina and anterior tectum. The quantities of TOP_{DV} and TOP_{AP} detected per cell vary continuously along the axis of the respective gradient. Thus, TOP molecules can be used to identify cell position along the dorsoventral and anteroposterior axes of the developing retina and optic tectum.

The orthogonal monotonic gradients of TOP_{DV} and TOP_{AP} molecules consti-

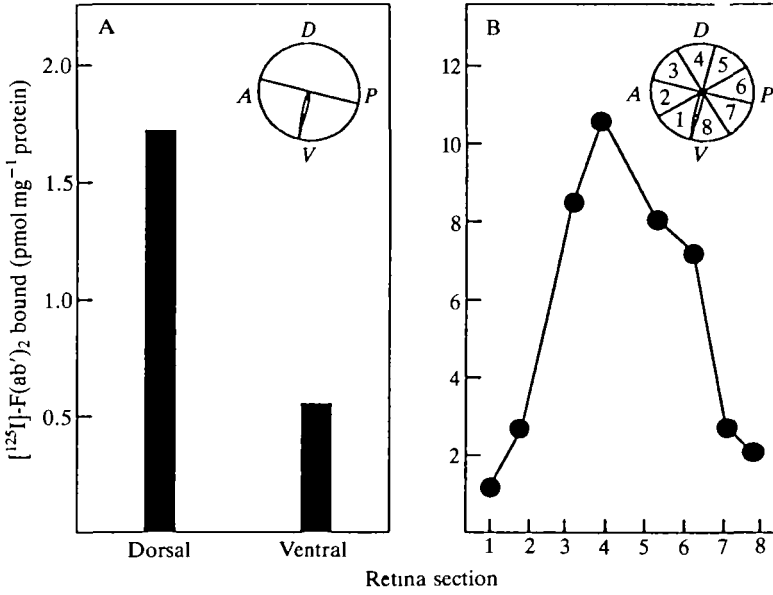


Fig. 14. Anti-TOP_{DV} distribution in retina after *in ovo* injection. (A) Anti-TOP_{DV} in E3 retina 1 day after injection of antibody into the amniotic cavity. (B) Anti-TOP_{DV} in E12 retina 1 day after intraocular injection of antibody (after Trisler *et al.* 1986).

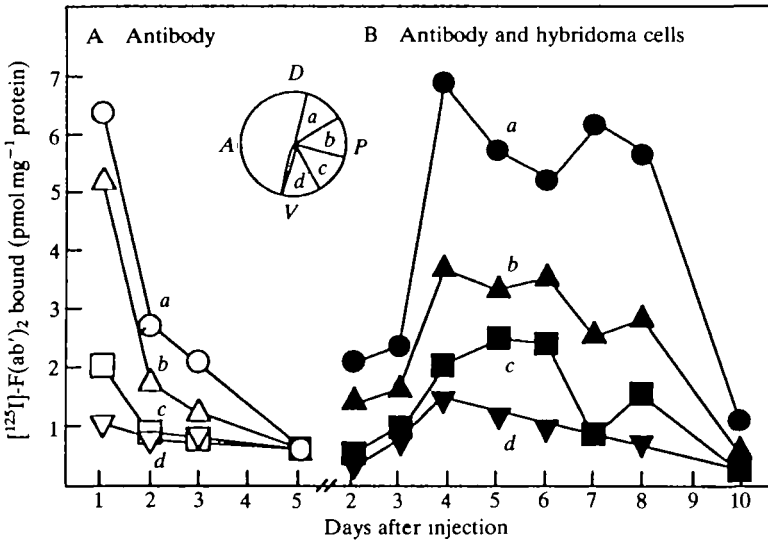


Fig. 15. Duration of antibody to TOP_{DV} gradient in retina after intraocular injection of (A) mouse ascites fluid containing anti-TOP_{DV} antibody and (B) hybridoma cells producing anti-TOP_{DV} antibody. ○ and ● section a of retina; △ and ▲, section b; □ and ■, section c; ▽ and ▼, section d (after Trisler *et al.* 1986).

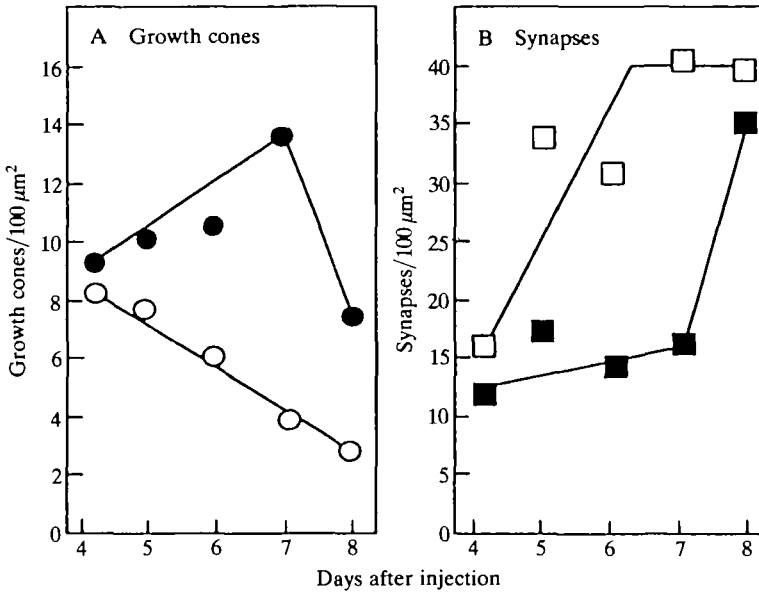


Fig. 16. Time course of synapse formation in the inner synaptic layer of retina after intraocular injection of hybridoma cells producing anti-TOP_{DV} antibody. (A) Growth cone and (B) synapse density as a function of developmental age after exposure to anti-TOP_{DV} (● and ■, respectively) and exposure to no antibody and to control antibodies P3X63Ag8 and 57D8 (○ and □, respectively) (after Trisler *et al.* 1986).

tute a possible Cartesian coordinate system that can be used to identify cell position in the topographic map of retina and optic tectum. Synapse formation in retina was inhibited in the presence of anti-TOP_{DV} antibody. This suggests that TOP_{DV} may be involved in the recognition of cell position that is required for normal synapse formation. The inverted configuration of the gradients in retina and tectum corresponds to the inverted topographic map of cell position in the retinotectal projection. Thus, TOP molecules may be involved in orienting the retinotectal map.

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