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Neuronal Locus Specificity: Trans-Repolarization of *Xenopus* Embryonic Retina After the Time of Axial Specification

Abstract. *Signaling within an embryonic* Xenopus *eye comprised of two fused eye fragments can reprogram, in turn, the anteroposterior and dorsoventral axes of one of the fragments. The responding fragment, subsequently isolated and allowed to round up and innervate the brain, shows corresponding inversions in its retinotectal map. This is the first evidence for trans-repolarization of presumptive retina and provides an assay system for analysis of positional signaling within the retinal field.*

Differentiating retinal ganglion cells undergo position-dependent diversification, acquiring properties (*locus specificities*) that enable each cell's axon to reach its appropriate locus in the retinotectal map (*1, 2*). Such differentiations presumably continue to occur throughout tadpole life in *Xenopus*, as new ganglion cells are added to the ciliary margin of the growing retina (*3*). Yet, even before the first optic axons appear, a developmental program is finalized in the stage 28 to 31 optic cup which *specifies* the permanent anteroposterior (AP) and dorsoventral (DV) reference axes for positional information (*4*) in the retinal field, and establishes the spatial blueprint for patterning of locus specificities across the entire future ganglion cell population (*5*). Axial specification occurs in two steps (AP first) over 5 hours, and is triggered under retinal control (*6*). Before specification, a rotated eye can interact with the axial cues of the embryo, rapidly replace its labile retinal axes with a new pair of (properly aligned) axes (*7*), and assemble a normally oriented retinotectal map from the rotated position (*7, 8*). After specification, the stage 31 eye is unaffected by the embryo's axial cues and, even when grafted in rotated orientation into a pre-stage 28 host, retains its specified axes and assembles a correspondingly rotated retinotectal map (*5, 8*).

This developmental program is exceedingly stable and was expressed with fidelity when stage 31/32 eyes were submitted to a variety of serial transplantation procedures, prolonged tissue culture, chronic deprivation of tectal connections, or temporary suppression of retinal growth (*5, 9*). Likewise, individual stage 31/32 nasal, temporal, or ventral eye fragments, which round up and form morphologically "whole" eyes, are insensitive to the extraocular microenvironment, and retain (or, occasionally, reduplicate) their specified axes (*10*). The first hint of modifiability came from surgically constructed eyes, formed by fusing specific stage 31/32 eye fragments together (*11, 12*); but without knowing which regions of the adult retina arose from which fragments of the recombinant eye, inferences about frank modification of retinal axes remained speculative (*2*).

Here we show that when allowed to fuse and interact with a right-nasal fragment, a left-temporal fragment (subsequently isolated, allowed to round up, and assayed after it has mapped into the tectum alone) undergoes a stepwise reprogramming of first its AP and then its DV axis. This is the first clear evidence for trans-repolarization of retinal tissue and for axis reversal after the time of specification, and provides an assay system for analysis of positional signaling within the retinal field. Published accounts exist for all methods used, including those for staging, surgical management, and rearing of *X. laevis* clawed frog embryos (*5*); preparation of eye fragments and recombinant eyes (*10, 11*); testing of visually guided strike responses of the frogs during metamorphosis [to confirm the existence of functional synapses between the experimental eye and the brain (*2, 5*)]; and electrophysiologic analysis of the visual field projection from the experimental right eye to the left optic tectum, 5 to 18 weeks after eye surgery, in the juvenile frog (*2*).

Four control series were prepared, concurrent with the experimental series and using siblings of the experimental embryos. Normally oriented retinotectal maps (Fig. 1a) developed in all 11 frogs whose right eye was removed and replaced intact in normal orientation at stage 27 ± 1, 31/32, 38/39, or 43/44. Thus, simple surgical intervention at these stages did not produce map inversions. Normally oriented maps developed in seven frogs after grafting a stage 27 ± 1 right eye, in 180°-rotated orientation, into the completely vacated right orbit of a stage 27 ± 1 host; but the map was inverted in both axes in all eight frogs, after grafting a stage 31/32 right eye in 180°-rotated orientation into the vacated right orbit of a stage 27 ± 1 host. These controls confirm that the stage 31/32 eyes used in our experiments (since their axes were not modified by interaction with host embryos of proven competence) had in fact undergone axial specification prior to stage 31/32. Finally, the retinotectal map was normally oriented in the AP axis but inverted in the DV axis in all but 11 frogs after grafting a stage 31/32 left eye (in AP-normal, DV-inverted orientation) into the vacated right orbit of a stage 31/32 host, with no further surgery or with subsequent (after 15 to 30 minutes or after 13 to 14 hours) extirpation of its nasal region (see Fig. 1b). Thus, the retinal axes of a specified left eye are stable in left-temporal fragments, isolated in the right orbit, and allowed to map into the left tectum. Reversal of these axes in the experimental series (in which the left-temporal fragments were similarly isolated after contact with a right-nasal fragment) must have resulted from interaction with the right-nasal fragment.

In the four experimental series, a (donor) stage 31/32 left-temporal eye fragment was apposed to a (host) stage 31/32 right-nasal fragment, by grafting the donor fragment in place of the extirpated temporal region to the host right eyes. The right-nasal (host) fragment was either (i) completely removed after 15 to 30 minutes (sham fusion); (ii) left undisturbed as part of a permanent recombinant eye; (iii) completely removed after 13 to 16 hours (host stage 39 ± 1; 22°C) when the two fragments were composite halves of a "dumbbell-shaped" eye; or (iv) completely removed after 30 to 32 hours (host stage 43/44), when the two fragments were no longer visibly discrete but remained easily separable by cutting along the fusion scar. Nasal extirpations were confirmed histologically (*10*).

The first two experimental series, which define the boundary conditions for the time of fragment interaction (sham fusion and indefinite fusion), gave consistent results: all seven frogs in the sham-fusion series developed AP-normal, DV-inverted maps (Fig. 1b), identical to those seen in the fourth control group; with only minor vari-

ations, all 12 frogs with undisturbed recombinant eyes developed mirror-symmetrical, redundant maps (Fig. 1c) in which the axes in the temporal region of the adult retina were AP-inverted DV-normal. The third and fourth series gave mixed results, but a clear progression emerged. In the third series (13 to 16 hours fusion), the predominant retinotectal map (eight frogs) was inverted in both axes (Fig. 1d), which reflects a reprogramming of the AP axis (from normal in the original left-temporal graft to inverted in the final retina) but no change in the DV axis from its original (inverted) state. In the fourth series (30 to 32 hours fusion), the predominant retinotectal map (nine frogs) was AP-inverted, DV-normal (Fig. 1e), which reflects a reprogramming of both the AP axis (from normal in the original left-temporal fragment to inverted in the final retina) and the DV axis (from inverted in the original left-temporal fragment to normal in the final retina). Significantly, both of these predominant patterns show exaggerated curving of the reprogrammed axes, as is seen in the temporal region of the recombinant retina. Minority results in the third series included: three AP-normal, DV-inverted maps, identical to those in the first (sham-fusion) series; two AP-inverted, DV-normal maps, identical to the majority result in the fourth series (30 to 32 hours fusion); and one AP-inverted, DV-disorganized map. Minority results in the fourth series included four completely inverted maps; one AP-normal, DV-inverted map; and two AP-inverted, DV-disorganized maps.

The DV pattern of nonneural eye tissues (fissure position, choroidal pigmentation, thickness of iris, and so forth) developed normally in accord with the anatomical polarity of the eyes or eye fragments, and held constant to electrophysiologic recording. Thus, this pattern of regional differentiation was right-side up in eyes of the first control group, a "patchwork" composite in the permanent recombinant eyes (*11*), and upside down in all other eyes.

Although these results leave many questions unanswered (Is the AP-inverted, DV-disorganized pattern also a transitory intermediate? Why does the rounded-up fragment map across the whole tectum?), they also have several clear implications. First, they show that the retinal axes can be reprogrammed after the time of axial specification, indicating that specification renders the retinal cells refractory to the axial cues of the extraocular microenvironment but not to axial signaling per se. Second, the altered patterning of specificities in the temporal region of the recombinant retina is not a function of the recombinant state; rather it is sequel to a rapid reprogramming of one fragment by the other, generating a new and stable program in the left-temporal fragment which is executed to completion, whether or not the fragments remain together. Third, the fact that signals from one retinal region can reorganize position-dependent differentiation of all the ganglion cells arising in another region argues that cell interactions play a normal role in integrating new ganglion cells into the pattern program of the growing retina. Finally, some fragments

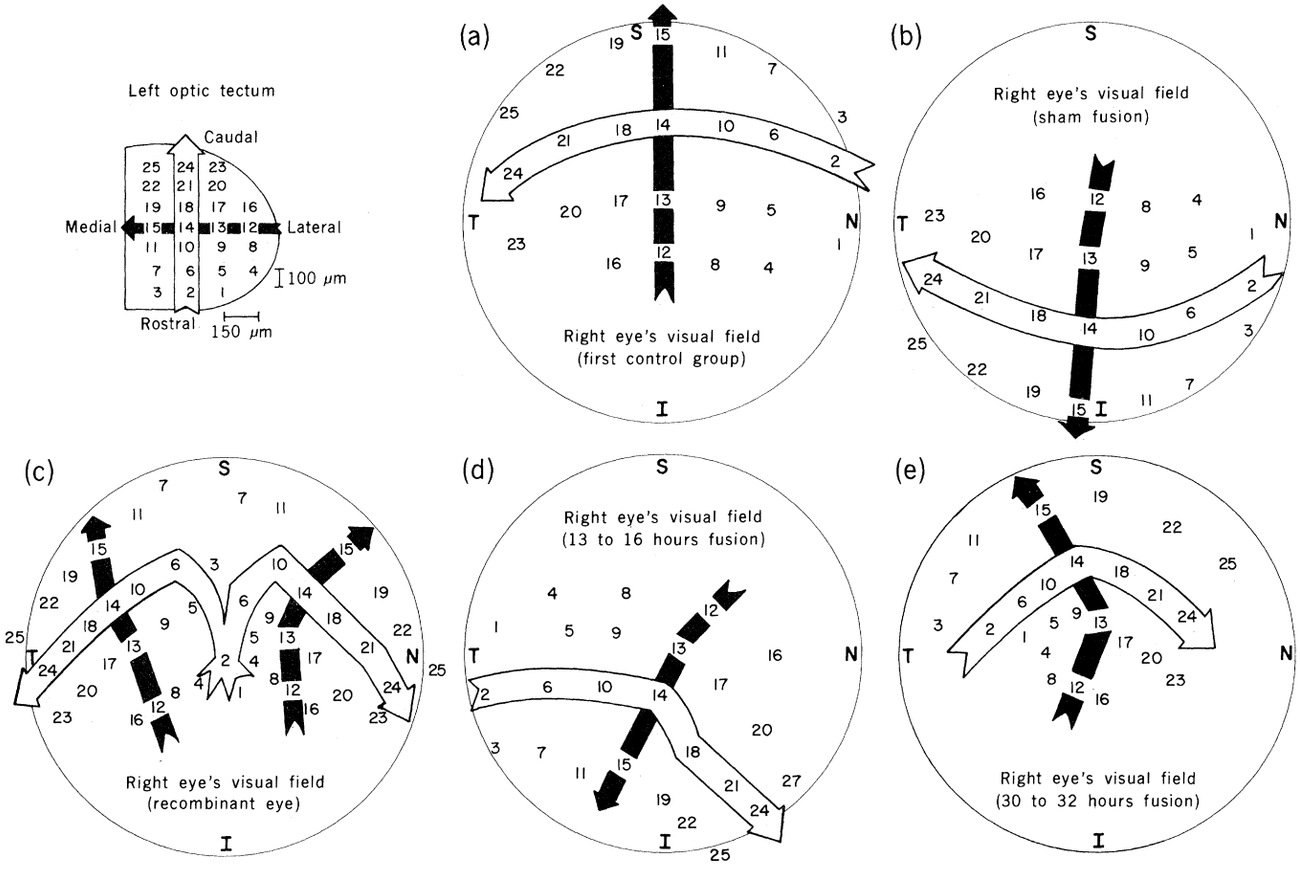

Fig. 1. Projection of the right eye's visual field to the left optic tectum in five adult *Xenopus* (a–e). Each number in the visual field shows the position of the stimulus that optimally evoked potentials recorded by a microelectrode at the position shown by the same number on the tectum. The distance between tectal electrode positions is shown by the bars in the drawing at top left; the visual field extends 100° from center to periphery in all directions: *S*, superior; *I*, inferior; *N*, nasal; *T*, temporal. A constant set of electrode positions was probed in all five frogs, and only one tectum is shown (top left). (a) Visual field projection from a typical frog in the first control series. (b) Visual field projection from a frog in the first experimental series. (c) Visual field projection from a frog in the second experimental series. (d) Visual field projection from a frog in the third experimental series. (e) Visual field projection from a frog in the fourth experimental series.

were not yet reprogrammed in one or both retinal axes by stage 43/44 (separation after 30 to 32 hours fusion); yet reprogramming in both axes was observed in the temporal retina of all recombinant eyes. This suggests that reprogramming can occur at least as late as stage 44, well into larval life.

The discovery of a stable intermediate in reprogramming (both axes inverted) is particularly telling, in that it virtually excludes wholesale "derotation" of the retinal field (*12, 13*) as the mechanism of axis reversal, and shows that the retina never reverts to a completely blank intermediate state. The curvature of only one axis, when only one axis has been reprogrammed (Fig. 1d), also shows that the two reference axes for position-dependent differentiation need not be strictly orthogonal. That the AP axis is reprogrammed first (even though it is the DV axis which is misaligned) provides strong support for the hypothesis (*12*) that the transmission or processing (or both) of AP signals is more rapid than that of the DV signal. Yet, DV misalignment may help to trigger the AP reprogramming, for recombinant eyes comprised of a right-temporal fragment and a right-nasal fragment nearly always integrate to form a single normal pattern (*12, 14*).

Finally, this work introduces what we hope will be a powerful assay system for analysis of positional signaling within the retina and for polarity transforms in general. Evidence is presented elsewhere that the reprogramming is highly specific and determined by the axial relations in the retinal fragments. Thus, the system provides an anatomically defined source of axial signals that can be independently perturbed. Moreover, by examining their abilities to reprogram normal fragments, it may now be possible to analyze the axial relations and specified state of experimental eyes submitted to procedures that preclude the recovery of normal visual function.

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Photosynthate and Nitrogen Requirements for Seed Production by Various Crops

Abstract. *Seed biochemical composition was the basis for segregating 24 crops into four distinct groups. Nitrogen requirements of pulses and soybeans were so great that sustained seed growth demanded continued nitrogen translocation from vegetative tissues. This translocation must eventually induce senescence in these tissues, restrict the duration of the seed-fill period, and limit seed yield.*

Seeds of crop species vary a great deal in their chemical composition and these differences significantly influence their utility to man. The formulations of livestock feed or the diets of humans, for example, are based to a great extent on the relative proportions of protein, carbohydrate, and lipid of the various available grains. Recently, attempts have been made through crop breeding to alter the chemical composition of seeds and thereby enhance their nutritional and economic value.

However, the impact of altering the chemical composition of seeds on the photosynthate and nitrogen relationships within the crop plant and, consequently, on crop productivity have rarely been considered. It has long been known that the caloric values of protein, carbohydrate, and lipid are quite different. Assuming the leaves of a crop produce photosynthate at a fairly uniform rate and hence yield a constant caloric output, it necessarily follows that changes only in the chemical constituents of seeds must alter biomass yield. An objective of this analysis was to compare the biomass yield per unit of photosynthate of seeds having different relative amounts of protein, carbohydrates, and lipid. In addition, altering the protein content of seeds also changes the amount of nitrogen required in the production of seed biomass. Since nitrogen fertilization is recognized as a critical factor in crop production, changes in nitrogen demand resulting from alterations of seed composition may require a reevaluation of management techniques for crop nitrogen supplies. Therefore, a second objective of this analysis was to examine the nitrogen requirements of seeds with varying protein contents.

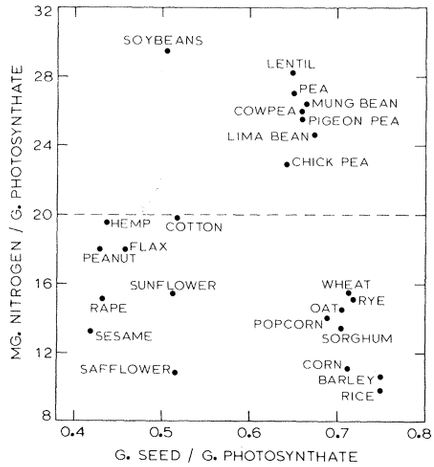

Fig. 1. Plot of milligrams of nitrogen required and grams of seed biomass yielded per gram of available photosynthate for the 24 crop species analyzed. The dashed line represents the nitrogen requirement when the nitrogen supply rate is 5 g ha^{-1} day^{-1} and the available photosynthate rate is 250 kg ha^{-1} day^{-1}.

The relative amounts of protein, carbohydrates, lipid, and ash (on the basis of fresh weight) of 24 crop seeds were used in this analysis (*1*). All data were first converted to dry weight and a wide range in the relative composition of seeds was obtained (see Table 1). The ranges for the relative amount of protein, carbohydrate, and lipid were 8 to 38 percent, 19 to 88 percent, and 1 to 54 percent, respectively. While the data for a given species may be unrepresentative of some genotypes within the species, the range in these data allows evaluation of the seed biomass production and nitrogen requirements of cultivars with differing chemical compositions.

The relative seed compositions were first used to calculate the photosynthate requirements for biomass production. The results of an exhaustive examination of the biochemical pathways for the production of proteins, carbohydrates, and lipids from