We do not know if the dependence of the symbiotic amoebas on their symbionts involves any genetic interactions between the genomes of the hosts and symbionts. We have attempted to detect plasmid DNA's, which, if present in the symbionts, might play a role in the establishment of stable symbiosis, as by conferring resistance against the amoebas' digestive enzymes to the symbionts. Our recent results obtained by CsCl density-gradient centrifugation and gel electrophoresis have shown the presence of one plasmid group that migrates slower than the bands representing the chromosomal DNA's in 0.7 percent agarose gel (*8*). The molecular weights of these plasmids have not yet been determined.

There are other examples in which intracellular infective agents or symbionts are known to cause changes in cellular phenotypic characters in free-living cells (*9*) as well as in multicellular organisms (*10*). However, the change in a phenotypic trait reported here, the acquisition of temperature sensitivity, is unique in that it is brought about by newly acquired endosymbionts that are indispensable to the host's survival. Furthermore, an apparent integration of the symbionts into the cell has occurred within a relatively short period of time; that is, in fewer than 200 host cell generations. This contrasts with the conventional belief that the establishment of stable symbiosis and the possible integration of symbiotic partners occurs over a very long period of time (*11*). Once they are integrated into the cell as obligate components, such as the required endosymbionts in the dependent amoebas, it is difficult to decide whether such components should be regarded as cell organelles or foreign bodies. A further study of the amoeba-bacteria symbiosis might provide insight into the origin of eukaryotic cell organelles and contribute to our understanding of the mechanism for nonchromosomal inheritance.

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Origin of the Retina from Both Sides of the Embryonic Brain: A Contribution to the Problem of Crossing at the Optic Chiasma

Abstract. *Cells originating from one of the first two blastomeres of the frog embryo were labeled either by injecting them with horseradish peroxidase or by changing the ploidy of one blastomere. Both methods show the labeled cells confined to the same side of the brain as the labeled blastomere except for cells that have moved from the opposite side into the ventral diencephalon and ventral part of the retina. Reciprocal movement of cells from each side of the prospective forebrain into the prospective retina on the opposite side starts before the neural tube closes and results in the formation of an incipient optic chiasma which may provide the pathway for optic axons to grow from the retina to the opposite side of the brain.*

The origin of the optic vesicles as a pair of outgrowths from the sides of the embryonic forebrain has been described in all vertebrate species that have been examined (*1*). Fate maps of the prospective eye region in the neural plate of amphibian embryos also show a bilaterally symmetrical pair of prospective eye-forming zones (*2*), although the two prospective eye-forming zones appear to be connected across the midline in the newt (*3*). Experiments in which cyclopia resulted from the action of various agents on the embryos, apparently because of failure of segregation of a single median eye rudiment, have provided indirect evidence that eyes may arise from a single median anlage from which cells segregate to form the two optic primordia during the closure of the neural tube (*4*). We now provide what we believe to be the first direct evidence showing that each neural retina originates from both sides of the prospective forebrain in the frog.

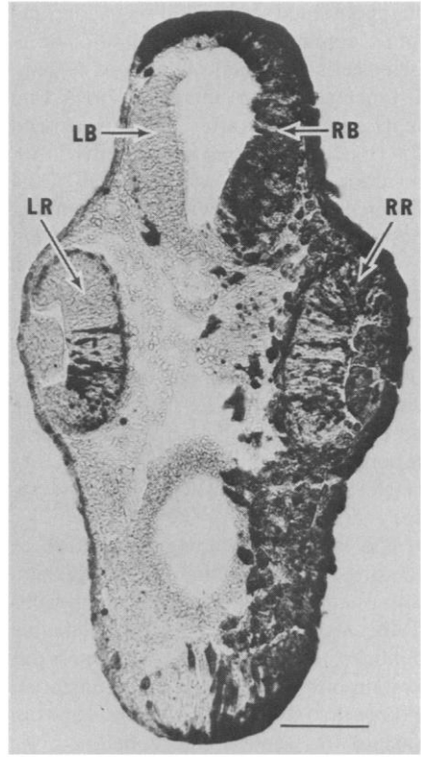

Fig. 1. Coronal section through *Xenopus* at stage 37 showing the localization of the dark reaction product for horseradish peroxidase, a label which was injected into the right blastomere at the two-cell stage. The label is seen in the right side of the brain (*RB*), right retina (*RR*), and ventral half of the left retina (*LR*), while the dorsal half of the left retina is unlabeled. Note the absence of label from the left side of the brain (*LB*), and the reduced labeling of the ventral half of the right retina. The bar is 100 μm.

Two methods of marking cells were used: by inducing a change in ploidy as a result of temperature shock to the fertilized egg, and by injecting horseradish peroxidase (HRP) into one blastomere at the two-cell stage.

Eggs were expressed from mature female *Xenopus laevis* and immediately fertilized in vitro with fresh macerated testes. Twenty minutes after fertilization, the eggs were heated to a temperature of 35.5°C for 6 minutes and then quickly cooled to 20°C. Similar treatment has been shown to result in heteroploidy and, in a small percentage of cases, to produce chromosome mosaics in which different parts of the animal are composed of cells with different chromosome numbers (*5*, *6*). Eggs were placed in individual petri dishes in a 5 percent Steinberg solution. The cleavage pattern was observed and recorded for each embryo to the 16-cell stage. At the neurula stage, embryos were selected in which

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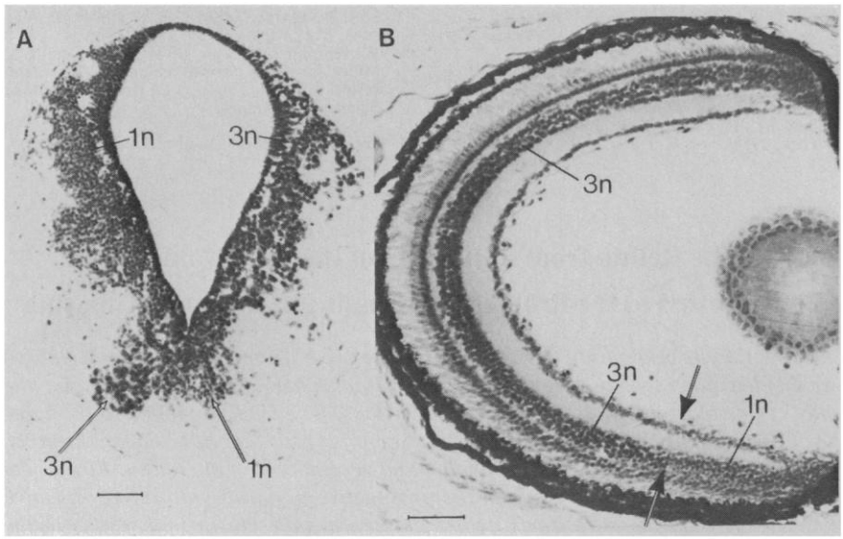

Fig. 2. (A) Coronal section through the diencephalon of a left-right mosaic *Xenopus* at stage 47. The midline formed the boundary between haploid (*1n*) and triploid (*3n*) cells except for bilateral translocation of cells across the midline in the ventral diencephalon (arrows). Bar is 200 μm. (B) Coronal section through the left eye temporal to the optic disk of a left-right mosaic *Xenopus* at stage 53 in which the right side of the animal was haploid and the left side triploid. The retina is triploid (*3n*) except for a segment of haploid cells (*1n*) at the temporo-inferior (postero-ventral) retinal margin. Arrows show the boundary between triploid and haploid cells. Bar is 200 μm.

mosaicism was clearly evident by the left-right asymmetry of the neural folds and by differences in the size of the cells between the left and the right sides of the neural plate. In the tail bud stage, the left-right chromosome mosaics show a marked difference in the sizes of the eyes. Such animals formed about 10 percent of the original number of embryos, and these selected animals were killed at various stages from stage 20 to 52 (*7*). They were processed by conventional histological methods for light microscopy, and the distribution of the mosaicism was determined in serial sections. A total of 23 embryos were analyzed.

In preparation for injection of HRP, some eggs were dejellied mechanically. After the first, second, or third cleavage, an injection of HRP (type VI, Sigma, 20 percent in Steinberg solution; 2 to 4 nl, about 3 percent of the volume of the blastomere) was given into a selected blastomere through a glass micropipette with a tip diameter of about 10 μm. These embryos were killed at stages 17 to 38 and were then prepared according to the method of Graham and Karnovsky (*8*) for the histochemical localization of HRP; diaminobenzidine tetrahydrochloride was used as the chromogen. Control embryos which had received a sham injection of Steinberg solution or had received no injections were processed in parallel with the experimental embryos.

Of the 39 embryos originally injected with HRP, seven died shortly afterward, mainly because of failure of the injected blastomeres to divide. The same proportion of sham-injected embryos died. Analysis was confined to the 32 embryos that continued to develop normally, and maps were made of the distribution of labeled cells in the central nervous system. Uninjected control embryos showed no reaction product after being processed for HRP. In embryos killed shortly after injection, the HRP was confined to the injected blastomere with no evidence of diffusion out of the injected cell. Recent evidence indicating a weight limit of 1900 daltons for molecules passing through intercellular junctional channels (*9*) virtually eliminates the possibility of passage of HRP (44,000 daltons) through intercellular junctions. We have elsewhere described the distribution of labeled cells in embryos after injection of HRP into single blastomeres at 2-, 4-, 8-, and 16-cell stages (*10*).

The significant finding in relation to the origins of the retina is that injection into one blastomere (either left or right) at the two-cell stage resulted in labeling confined to all structures in the nervous system on the same side as the injection except for some cells on the opposite side of the ventral diencephalon, optic stalk, and ventral half of the retina (Fig. 1). Translocation of labeled cells to the side opposite the injection was seen at stage 17, the earliest stage examined.

The mosaic animals could be analyzed because of differences in cell size and numbers related to ploidy. The polyploid cells are much larger than normal and have larger nuclei; more than two nucleoli are frequently visible (*11*). Haploid cells are smaller than normal and have smaller nuclei and a single nucleolus. The number of neurons is inversely proportional to their ploidy; polyploid cell populations are diminished in cell number, whereas haploid cell populations are increased in cell number (*6*). These differences in the numbers of cells and their sizes on the two sides made it easy to recognize the boundaries between populations of neurons on the basis of different ploidy. In left-right chromosome mosaics, polyploid cells were confined to one side of the midline while diploid or haploid cells were confined to the other side. No mixing of cells occurred at the midline between such left-right chromosome mosaics except for a reciprocal transposition of cells across the midline (polyploid cells to the haploid side and vice versa) in the ventral diencephalon just rostral to the hypothalamus (Fig. 2A). These transposed cells could be traced into the optic stalk and into the retina at the junction with the optic stalk. After differentiation of retinal layers and retinal cell types in the stage-52 tadpole, these transposed cells, recognizable by the marked difference in the size of their nuclei, were seen in all cellular layers of the retina (Fig. 2B). The region of the retina containing the transposed cells, reconstructed from serial sections, occupied a wedge-shaped segment in the temporo-inferior (caudo-ventral) quadrant of the retina, with its apex at the optic disk and its base at the retinal margin (Fig. 2B). This will be called the translocated segment of the retina.

We have demonstrated that cells move into the presumptive retina from the opposite side of the presumptive forebrain as early as stage 17 after HRP is injected into one blastomere at the two-cell stage, which shows that transposition of cells must have started before the beginning of neural tube closing. However, the time of initial translocation, the precise origin of the translocated cells, and the mechanism of their movement are not known. Because of dilution of the HRP with successive cell divisions, the method could not be used for studying the eventual location of the descendants of the transposed cells after embryonic stages. However, the translocated cells were easily recognizable at later stages of development in the left-right chromosome mosaic animals (Fig. 2).

The wedge shape of the translocated segment, with its apex at the optic disk and its base at the retinal margin (where addition of new retinal cells normally occurs) indicates that the translocated segment originated from founder cells situated close to the optic disk and that the

segment grew in the normal way, by the addition of cells at the retinal margin (*12*). Therefore, the translocated segment may be considered to be a polyclone derived from founder cells that had moved into the retinal rudiment from the opposite side of the presumptive forebrain. Wedge-shaped segments of pigmented retinal cells have also been observed in allophenic mice (*13*), in mosaic pigmented retina of axolotls (*14*), and in *Xenopus* in which marked cells have been grafted into the retina at embryonic stages (*15*). In those cases, too, the wedge shape of the marked polyclone was thought to show that the founder cells were initially close to the optic nerve head and that the clone grew by addition of cells at the retinal periphery. To our knowledge, however, no previous evidence has been published of reciprocal translocation of prospective retinal cells across the midline or of any contribution of cells to the retina from the opposite side of the embryo. We suggest that the translocated cells establish an incipient chiasma before the outgrowth of nerve fibers from the retina. If a similar translocation occurs in the mammalian embryo, the question arises whether the anomaly of crossing of optic axons at the chiasma found in albino mammals (*16*) may be due to an anomaly of the translocated retinal segment.

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Eye-Specific Termination Bands in Tecta of Three-Eyed Frogs

Abstract. *An extra eye primordium was implanted into the forebrain region of embryonic* Rana pipiens. *During development both normal and supernumerary optic tracts terminated within a single, previously uninnervated tectal lobe. Autoradiographic tracing of either the normal or supernumerary eye's projection revealed distinct, eye-specific bands of radioactivity running rostrocaudally through the dually innervated tectum. Interactions among axons of retinal ganglion cells, possibly mediated through tectal neurons, must be invoked to explain this stereotyped disruption of the normally continuous retinal termination pattern.*

During development, ganglion cell axons from the vertebrate retina terminate in a highly ordered pattern that maps the retina, and thus visual space, within the tectal lobes of the midbrain. The stereotypy of this projection and the ability of ganglion cells in lower vertebrates to reestablish the pattern during axonal regeneration suggest that highly specific pre- to postsynaptic cell interactions may be involved (*1*).

This theory of rigid retinal ganglion cell to tectal cell chemoaffinities has recently been challenged. Expansion and compression of the visual field projection following retinal or tectal ablations indicate a more plastic system (*2–5*). In a modification of the chemoaffinity hypothesis, it is proposed that graded affinities between cells of one part of the retinal field for one part of the tectal field serve to orient the projection along topographic axes. Competition between retinal ganglion cell axons could then organize the presynaptic terminals over the available postsynaptic space (*4*, *6*, *7*).

We present here anatomical results in a preparation designed to test the importance of optic fiber to optic fiber interactions during the development of the anuran retinotectal map. The three-eyed *Rana pipiens* used in these experiments have two complete retinal projections innervating a single previously uninnervated (that is, naive) tectal lobe from early development.

The animals were produced by implanting either a right or left eye primordium in the forebrain region of embryos with minimal dorsoventral rotation of the transplant and without disturbing the two normal optic evaginations. All operations were performed between Shumway stages 17 to 19, before retinal axons penetrate the brain but after the retinal axes have been determined (*8*). In

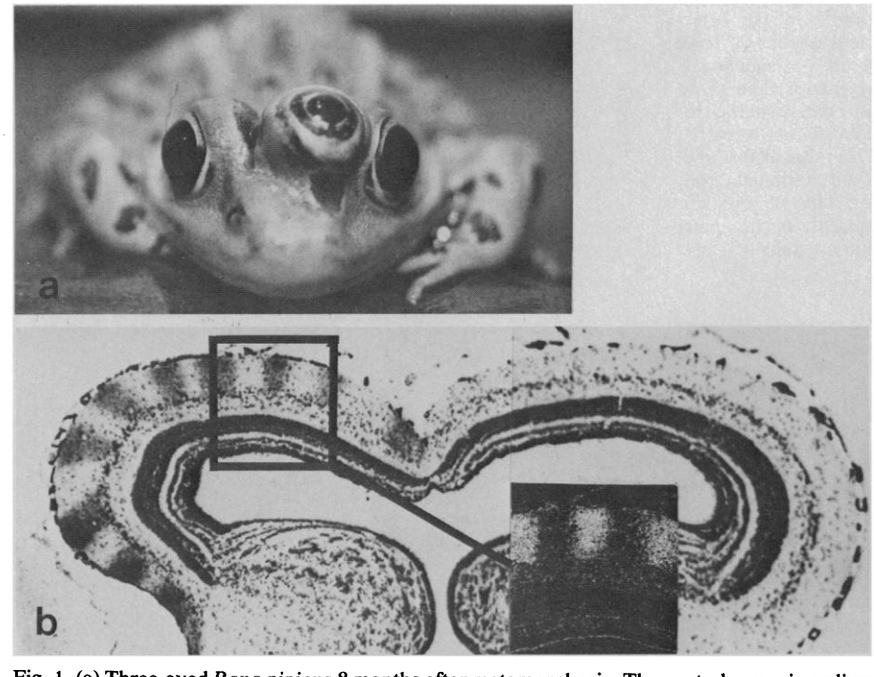

Fig. 1. (a) Three-eyed *Rana pipiens* 8 months after metamorphosis. The central eye primordium was implanted at Shumway stage 17 from a similarly staged donor. The supernumerary eye has externally normal dimensions, but lacks a pupillary response. (b) Autoradiographic distributions of grain densities in the optic tectum of a 3-month postmetamorphic three-eyed frog after injection of 10 μCi of [^{3}H]proline into the vitreous body of the normal eye. (Inset) Dark-field enlargment showing the pronounced segregation of labeled and unlabeled regions of the tectal neuropil.

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