Early onset of phenotype and cell patterning in the embryonic zebrafish retina

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Summary

The regular arrangement of retinal cone cells in a mosaic pattern is a common feature of teleosts. In the zebrafish, Brachydanio rerio, the retinal cone mosaic comprises parallel rows consisting of a repeating motif of four cone types. In order to elucidate the temporal and spatial aspects of the genesis of the cone mosaic in the developing retina, we generated a monoclonal antibody that specifically binds to the double cone photoreceptor of the adult. We first saw staining in the developing retina with this antibody, FRet 43, at 48 hours postfertilization, the time at which the first photoreceptor cells undergo their final mitotic division. We then injected embryonic fish with the thymidine analog, 5-bromo-2'-deoxyuridine (BrdU), confirming with a double-labeling experiment that the onset of FRet 43 antigenicity occurs within three hours of the cellular division that generates the double cone photoreceptors. Then we stained tangential sections of the 54-hour embryonic retina with FRet 43, further showing that cells devoid of staining alternate with stained pairs of cells in a pattern that is consistent with the arrangement of photoreceptors in the adult cone mosaic. These results indicate that a marker of the double cone phenotype is expressed at approximately the same time as cellular birthday and that the mosaic patterning is present within 6 hours of this expression.

Key words: retinal development, double cones, cone mosaic, photoreceptors, cell patterning.

Introduction

In the retinas of many teleosts, the cone photoreceptors are arranged in a simple, repeating pattern known as the cone mosaic (Lyall, 1957; Engstrom, 1963a). The zebrafish retina (Brachydanio rerio) is no exception. Engstrom (1960) was the first to describe the cone mosaic in this species as a row pattern consisting of an invariant array of four cone types (the short single, the long single, and the unequal double cones); this mosaic was found to extend throughout the photoreceptor layer.

Using radioactive thymidine to mark actively dividing cells, Nawrocki (1985) had previously shown that zebrafish photoreceptors first become postmitotic at 48 h (48 h = 48 hours postfertilization). Kljavin (1987) then used electron microscopy to examine the subsequent differentiation of these postmitotic photoreceptors, demonstrating that outer segments of both rod and cone photoreceptors first appear in a localized patch on the ventral aspect of the retina at 60 h. In Branchek and BreMillers' light microscopy studies (1984), however, the cone mosaic was not apparent until 8 days postfertilization and some of the photoreceptor cell types were not identifiable until 12 days. From Branchek and BreMillers' results, one might hypothesize that the nascent cone cells exist as a homogenous population of unspecified cell types until very late in the development of the retina. Alternatively, one might propose that specific cone types are present in a cryptic form prior to the onset of their late overt morphological differentiation. To determine which of these alternative schemes is correct, we developed a monoclonal antibody, FRet 43, that specifically binds to the double cone photoreceptor. We hoped that the temporal and spatial pattern of the onset of FRet 43 antigenicity would reveal whether a diverse population of cone cells exists during early stages of development.

FRet 43 staining confirmed that the double cones are indeed present prior to morphological differentiation. The time of onset of antigenicity at 48 h was indistinguishable from the time at which Nawrocki (1985) had shown that the first photoreceptor cells undergo their last mitotic division. We further examined the temporal relationship between cellular birthday and onset of double cone specificity by staining retinas of embryonic fish that had previously been injected with the thymidine analog, BrdU, with both anti-BrdU and FRet 43 antibodies. These double-label experiments showed that a marker for the double cone cell phenotype is expressed within three hours of the final cell division.

Because of its early expression, the FRet 43 antigen also provided us with a marker to investigate photoreceptor pattern formation. The arrangement of stained and unstained cells demonstrated that a pattern consist-
ent with the adult mosaic is present in the 54-h embryo. These results suggest that photoreceptor cell types differentiate and form the mosaic pattern very early in development, only shortly after their cellular birthdays.

Materials and methods

Generation of hybridomas

Male Balb/c mice were immunized at multiple intraperitoneal and subcutaneous sites with a preparation of whole adult retinal cells that had been fixed in 2% paraformaldehyde for 30 minutes at room temperature and resuspended in PBS and Freund's adjuvant. Each immunization consisted of retinal cells obtained from approximately fifteen adult wild-type zebrafish. Each mouse received at least nine immunizations over the course of approximately one year.

The cell fusion and cloning of hybridomas were completed by the methods described by Ciment and Weston (1982) and modified by Trevorrow et al. (1990). An immunohistochemical screen was performed on eye-containing transverse sections of lightly fixed adult zebrafish heads.

Antibody typing and subtyping were performed utilizing an immunodiffusion kit for mouse monoclonal antibodies (Miles).

Animals

In the wild-type zebrafish, the cellular details of the photoreceptor layer are obscured by the pigment epithelial processes that extend into this layer of the retina. Therefore, all animals used were homozygous mutant at one or more of the loci affecting pigment expression as previously described by Streisinger et al. (1986). In the double-labeling experiments, alb-1 zebrafish (Brachydanio rerio) were used; in all other experiments, we used animals that were homozygous mutant at three pigment pattern loci: gol-1, alb-1, and spa-1. These fish provide a convenient tool for histological studies of the retina as they lack all but the reflective pigmentation. In particular, antibody staining of photoreceptor cells is not obscured by the closely associated pigment epithelium.

Embryos were obtained from spontaneous spawnings from animals used were homozygous mutant at one or more of the loci affecting pigment expression as previously described by Streisinger et al. (1986). Embryos were sectioned by the method described by Hanneman et al. (1988). Vibratome sections of adult eyes were obtained in the following manner. Eyes were dissected from the heads of adults. The corneas were opacified and the lens extracted. The resulting eye cup was then gently aspirated to remove the vitreous fluid and then filled with a warmed solution containing 17% gelatin in 10% Hank's saline. The eyec was then mounted in the gelatin solution; 30- to 60-μm sections were cut and transferred onto gelatin-coated slides and allowed to dry.

Tissue for the cryostat sections was prepared by immersing either whole embryos or adult heads in a 4°C fixative solution for 12 to 20 hours. This fixative solution was composed of 1% paraformaldehyde in 0.1 M phosphate buffer at a pH of 7.2-7.4. After fixation, the tissue was washed and immersed in 30% sucrose at 4°C until permeated. The adult heads were then frozen directly onto the mounting platform for sectioning, whereas embryos were aligned and embedded in Tissue Tek O.C.T. compound prior to freezing. Sections of 16 μm were cut and transferred to gelatin-coated slides and allowed to dry.

Immunohistology

Sample preparation for sections labeled with FRet 43

Tissue for the cryostat sections was prepared by immersing either whole embryos or adult heads in a 4°C fixative solution for 12 to 20 hours. This fixative solution was composed of 1% paraformaldehyde in 0.1 M phosphate buffer at a pH of 7.2-7.4. After fixation, the tissue was washed and immersed in 30% sucrose at 4°C until permeated. The adult heads were then frozen directly onto the mounting platform for sectioning, whereas embryos were aligned and embedded in Tissue Tek O.C.T. compound prior to freezing. Sections of 16 μm were cut and transferred to gelatin-coated slides and allowed to dry.

Tissue for the vibratome sections was subjected to the same fixation protocol as described above, washed, and immersed in 0.3 M sucrose at 4°C. Embryos were sectioned by the method described by Hanneman et al. (1988). Vibratome sections of adult eyes were obtained in the following manner. Eyes were removed and the cornea was opened to extract the lens. The resulting eye cup was then gently aspirated to remove the vitreous fluid and then filled with a warmed solution containing 17% gelatin in 10% Hank's saline. The eye was then mounted in the gelatin solution; 30- to 60-μm sections were cut and transferred onto a gelatin-coated slide.

Immunological labeling was performed as described by Hanneman et al. (1988).

Sample preparation for sections labeled with both FRet 43 and anti-BrdU antibody

Heads were removed from the fish and were fixed for 3 hours at 4°C in fixative (4% paraformaldehyde, 4% sucrose, and 0.15 mM CaCl₂ in 0.1 M phosphate buffer, pH 7.3). The tissue was washed in several changes of phosphate buffer and embedded in a solution of 5% sucrose and 1.5% agar, warmed to 40°C. The embedded heads were sunk in 30% sucrose overnight at 4°C.

Sections of 20 μm were incubated overnight with FRet 43 antibody at 4°C. After washing in PBS, a biotinylated secondary antibody was added for 30 min. Sections were again washed in PBS and then incubated in an avidin–biotinylated complex for 60 min. After several washes, the sections were incubated in a solution containing 0.05% diaminobenzidine (DAB) and 1% DMSO in 0.05 M phosphate buffer, pH 7.4, for 10 min. Hydrogen peroxide was added to the DAB solution to give a final concentration of 0.003%. The sections were monitored visually. After the brown reaction product
was evident, the reaction was stopped by washing thoroughly with PBS. The sections were then incubated with an anti-BrdU antibody. After treatment with a peroxidase-conjugated secondary antibody, DAB was again used as a chromogen. In this instance, the reaction was run in the presence of nickel and cobalt and the resulting deposit was black.

Results

**FRet 43 antigenic distribution**

The monoclonal antibodies that we generated against zebrafish retinal cells stained adult retinal histological sections in a variety of patterns. Of these, the FRet 43 antibody specifically stained the double cone pair in the photoreceptor cell layer (Fig. 1) and was chosen for further study. It was hoped that FRet 43 could be used to delineate the onset of the double cone phenotype, and by marking this specific set of cells, permit the analysis of the origin of the embryonic retinal mosaic. An immunodiffusion test showed that this monoclonal antibody is an IgG1.

Light microscopic examination (Fig. 1) suggested that the antigen to which FRet 43 binds is membrane-associated. The staining in the inner segmental and nuclear regions conformed to the cell surface whereas the outer segmental staining was darker and uniform throughout, perhaps because of immunoreactivity associated with the membranous disk components. In addition, the staining extended from the pedicles to the tip of the outer segments so that the entire surface of the double cone pair became evident.

Many of the anatomical features of the double cone pair that are not readily apparent in unstained retinal preparations become clear in the FRet 43-labeled light micrographs. For example, the FRet 43-stained preparation clearly reveals lateral fin projections at the level of the double cone nuclei, a feature that Borwein and Hollenberg (1973) had previously shown in EM studies to be present in the double cone photoreceptors of the 'four-eyed' fish, *Anableps anableps* L. Fig. 1 also shows a characteristic sinuous line of darker staining between the cone pair at the level of the cone inner segments and cone nuclei. This line of staining marks the juxtaposed membranes of the two members of the double cone pair.

The schematic in Fig. 1 summarizes the morphological details of the zebrafish double cone pair. As can be seen in this drawing, the double cone pair comprises a

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**Fig. 1. Double cone morphology.** The photomicrograph shows a vibratome section through an intact zebrafish retina immunolabeled with FRet 43 antibody. The immunopositive, double cone pairs are oriented so that the outer segments are at the top of the photomicrograph and the pedicles, which extend into a synaptic region, are seen midway between the top and bottom. The arrows point to the lateral fin projections at the base of the nuclear region. Above this, a dark line of staining demarcates the juxtaposed plasma membranes of the double cone pair. The schematic depicts the morphological features just described. The short member of the double cone pair is represented at the forefront of the drawing with the long member to the rear on the right. Scale bar: 10 μm.
long member and a short member. Close inspection of the photomicrograph in Fig. 1 confirms that the individual members of the double cone pair are both asymmetrically positioned and unequal in size.

We note that FRet 43 staining in the adult retina was not confined to the double cone cells as the antibody also sometimes labeled another cell type located in the inner nuclear layer (INL) (Fig. 2). In those cases in which the FRet 43 staining was robust, these FRet 43-positive inner nuclear layer cells were found in a spatially patterned array (data not shown). Each cell had a pronounced sclerally oriented process (Fig. 2) as well as a thin projection that extended vitreally into the inner plexiform layer (IPL). These observations convinced us that these sparsely scattered, yet regularly spaced, FRet 43-immunopositive cells represented a subpopulation of bipolar neurons. These putative bipolar cells were located close to the outer surface of the inner nuclear layer and displayed stereotypic axonal projections, which branched and terminated at characteristic strata within the vitreal half of the IPL (Fig. 2).

Although a complete survey of FRet 43 immunoreactivity has not been undertaken, this antibody has also been noted to stain noncellular material in the adult ear as well as scattered cells in the adult hypophysis (data not shown). It should be noted that FRet 43-immunopositive retinal bipolar cells have never been observed in the embryo. However, FRet 43 antigenicity has been seen in two other embryonic tissue types: the hypophysis and the pineal (data not shown). Shared antigenicity between photoreceptor cells and pinealocytes has previously been demonstrated for a number of proteins in other species (Mirshahi et al. 1984; van Veen et al. 1986; Vigh-Teichmann et al. 1980).

The adult cone mosaic

Engstrom (1960) concluded from light microscopic studies that the zebrafish cone mosaic should be

![Fig. 2. A transverse section through the adult retina, showing FRet 43-immunopositive cells in the inner nuclear layer. Shown are the thin outer plexiform layer (arrowhead), the inner nuclear layer (the cellular region located in the top half of the photomicrograph), the inner plexiform layer (the noncellular area in the middle of the photomicrograph), and the ganglion cell layer (the cells located in the bottom half of the photomicrograph). The stained cells have the morphological characteristics of bipolar cells; processes are seen to project bidirectionally into both plexiform layers. As can be seen, the putative axonal projections branch at a characteristic stratum in the inner plexiform layer. Scale bar: 10 μm.](image-url)
depicted as a 'regular cone arrangement, in which rows of double cones alternate with rows of single cones.' Yet if a row is defined by the close apposition of cell types, then electron micrography reveals that the rows of cones present in the outer lamina of the zebrafish neural retina are actually perpendicular to those described by Engstrom (Fig. 3A). Single rows, as defined here, contain all the cone types and the space between the rows is occupied by the rod myoids. Within the row, the short single cones (characterized here by the darkly staining endoplasmic reticula that occupy the interiors of the inner segments) alternate with a cellular motif consisting of five other photoreceptors (characterized here by lobular nuclei). In turn, the motif comprises a central long single cone flanked on each side by a double cone pair. The positions of the cone types within
a row have been further defined in Fig. 3B. Here, it can be seen that the long single cones are always bound by the long members of the double cone pair and the short single cones by the short members. The pattern revealed in Figs 3A and 3B is schematically represented in the drawing contained in Fig. 3C.

Fig. 3D is a photomicrograph of a whole mount of the photoreceptor layer that displays the double cone pedicles stained with FRet 43 antibody. At the top of the photo, one observes the bent, flattened photoreceptor cells. At the bottom, the double cone pedicles are aligned in rows of doubled configurations. This extension of the cone mosaic into the synaptic layer has previously been described in a number of other teleosts (Engstrom, 1963b; Wagner, 1978).

Photoreceptors: cellular birthdays and onset of FRet 43 antigenicity

Using radioactive thymidine to label actively dividing cells, Nawrocki (1985) had previously shown that the first postmitotic zebrafish photoreceptor cells arise at approximately 48 h. Using FRet-43 staining as a marker for double cone differentiation, we performed a number of experiments to determine the temporal relation-

Fig. 4. Onset of FRet 43 antigenicity in the embryonic zebrafish. A, D and E are photomicrographs of transverse, cryostat sections through the zebrafish embryo at progressive time points. In these photomicrographs, ventral is to the right, dorsal is to the left, and medial is at the top. As can be seen in A, which is a section of the 48-h embryo, the first FRet 43-immunopositive cells are located in a discrete patch on the ventral aspect of the eye of this particular fish. Although there are cases in which the first stained cells are located more centrally, the appearance of an initial ventrally located patch of FRet 43-immunopositive cells is the commonly observed pattern. In B, the stained cells in A have been magnified to show the longitudinal line of staining that bisects some of the stained configurations (arrows). This suggests that the double cones may be paired at the time of their final mitotic division. In C, which also is a photomicrograph of the 48-h embryo, the eye has been sectioned tangentially, allowing one to observe the arrangement of stained and unstained cells within the patch. Although the stained configurations occasionally suggest a semblance of rows, a definitive pattern is not recognizable at this time. In D, which is a section through the 54-h embryo, the stained patch appears at a more central location than in A and has increased in area. By day 4, as is shown in E, the stained patch encompasses all but the most peripheral margins of the retina. A, D and E also reveal the progressive maturation of the layers of the neural retina. Scale bar: (A), (C), (D) and (E), 10 µm.
Fig. 5. Embryonic zebrafish retina that has been double-labeled with anti-BrdU and FRet 43 antibodies. Fish were injected with 5-bromo-2'-deoxyuridine (BrdU) at 51 h and killed at 54 h. Actively dividing cells are black or grey and FRet 43-positive cells are brown. At 51 h, only the cells in the peripheral margins and the scleral-most layer of the retina (i.e. the photoreceptor layer) are undergoing cell division. By 54 h, those scleral-most cells in the central and ventral regions of the retina have formed a monolayer of columnar cells, which will later mature into the multilayer of distinctly shaped photoreceptor cells of the adult. A discrete ventral patch of these sclerally located cells stains strongly with the FRet 43 antibody. Those scleral cells that are unstained by FRet 43 appear to stain with higher than background levels of BrdU, indicating that this specific marker for the double cone cell appears within 3 hours of final mitosis. In addition to the ventrally located patch, the scleral-most cells in the central regions are beginning to stain with FRet 43. An occasional cell appears to stain with both FRet 43 and anti-BrdU antibodies (arrowheads). Scale bar: 20 μm.
ship between cellular birthday and the specification of double cone identity.

First, we screened fourteen 44-h embryos for FRet 43 antigenicity. Of these, only one fish stained with the FRet 43 antibody in the photoreceptor cell layer (data not shown). We attributed the presence of FRet 43 staining in this particular fish to variation in the rate of development, concluding that FRet 43 staining was not actually present four hours prior to cellular birthday. By 48 h, all 38 embryos that were screened contained FRet 43-immunopositive photoreceptor cells. In the embryonic eye shown in Fig. 4A, FRet 43-immunoreactive cells appear in a discrete, ventrally located patch on the scleral surface of the neural retina. This location is where Kjavin (1987) observed the first outgrowth of photoreceptor outer segments at 60 h, i.e. 12 hours after we demonstrated labeling. In our study, some peripheral-to-central variability in the specific location of this patch of initially stained cells has been seen (see Fig. 4D).

In the higher magnification of the stained area, shown in Fig. 4B, the immunopositive configurations were characterized by a longitudinal line of darker staining (see arrows), which suggested that each configuration represented a double cone pair. In Fig. 4C, which is a tangential section through the patch, the FRet 43-immunoreactive cells are interspersed in a somewhat disordered fashion among those cells that lack staining; a semblance of a row pattern may or may not be present at 48 h. By 54 h, the area containing FRet 43-positive cells has increased (see Fig. 4D). By 96 h, a time at which the zebrafish first actively feeds using visual cues (Clark, 1981), only the peripheral margins of the photoreceptor cell layer remain unstained (Fig. 4E).

These data suggested to us that the onset of FRet 43 antigenicity closely followed the spatial and temporal pattern of photoreceptor birthdays as seen in Nawrocki's birthday studies (unpublished data). This suggested to us that the double cone phenotype may appear as early as the final mitotic division. To test this hypothesis, we injected the yolk sacs of 51-h embryos with a thymidine analog, BrdU, and then killed the animals 3 hours later. The animals were then double-labeled with both anti-BrdU and FRet 43 antibodies.

In Fig. 5, BrdU-positive cells are black or grey, whereas Fret-43 positive cells are brown. At 51 h, most retinal cells in the inner layers of the retina are postmitotic; cell division is confined to the photoreceptor layer and the peripheral regions of the retina. A discrete patch of F Ret-43 positive cells can be seen on the ventral aspect of the embryonic eye. Those photoreceptors found in regions not stained by FRet 43 show some level of BrdU staining. In those regions in the central retina in which FRET-43 staining is just beginning to be apparent, cells appear to stain with anti-BrdU at higher than background levels. These data show that this marker for the double cone cell phenotype is expressed within 3 hours of the final cell division.

Embryonic photoreceptor cell patterning
Examination of the 54-h embryo strongly suggested to us that cell patterning had occurred in the photoreceptor layer by this time. As previously seen in the 48-h embryo, close inspection of transverse sections of the 54-h retina revealed that most (if not all) stained configurations were characterized by a longitudinal line of darker staining in the midregion (Fig. 6A). In tangential sections of the 54-h embryo (Figs 6B and 6C), a darker line of staining bisected many of the stained configurations (see the short arrows and inset in Fig. 6C), confirming the doubled nature of these embryonic cell types.

By far the most striking feature observed in the stained 54-h retina, though, was the appearance of order in the arrangement of the stained and unstained cells (Figs 6B and 6C). Not only were the stained configurations isolated on all sides by unstained cells, but it also appeared that the FRet 43-immunopositive cells were arranged in rows. In addition, the lines that bisected the individual stained configurations (Fig. 6C), and which presumably marked the position of the apposing membranes of the double cone pair, were all approximately parallel. In the adult, the juxtaposed membranes of the double cone pair are perpendicular to the row. Assuming that this is also true in the embryo, the long arrows in Figs 6B and 6C would define a row of cone cells.

As can be seen in Fig. 6C, rows of cells containing stained configurations alternate with rows with no staining; and the stained configurations within a row alternate with unstained cells. This pattern of staining is consistent with the adult photoreceptor mosaic. The cells in the unstained rows may represent developing rods, previously shown to be present by Nawrocki et al. (1985) at 6–8 days and by Kjavin (1987) at 60 hours. The cells contained in the rows with stained configurations are arranged in a manner that is consistent with the adult mosaic in which the double cone pair alternates with one of the two single cone types (see Fig. 4). This evidence strongly suggests that the adult mosaic is cryptically present at a time when the photoreceptors are morphologically indistinguishable from one another.

Discussion

We have developed a monoclonal antibody, FRet 43, that is specific for the double cone phenotype in the photoreceptor layer of the zebrafish retina. We have used this antibody to explore the relationship between cellular birthday, onset of cellular phenotype, and mosaic pattern formation. Using morphological criteria, Branchek and BreMiller (1984) were unable to definitively identify the double cone phenotype until day 12 postfertilization and could not distinguish a mosaic pattern until day 8. Using our marker, we could identify the double cone phenotype at 48 h, and by 54 h, these FRet 43-positive cells were arranged in a pattern consistent with that of the adult mosaic. We then injected 51-h zebrafish with the thymidine analog, BrdU, and by killing at 54-h, were able to show with a
Fig. 6. Patterning in the 54-h embryo as revealed by FRet 43-antibody staining. (A) A photomicrograph of a transverse vibratome section through the eye of a 54-h embryo. Longitudinal lines that bisect many of the stained configurations (see arrows) suggest that the double cones are paired by 54h. B and C, which are tangential sections of the 54-h retina, confirm this observation as many of the stained profiles also are bisected by a line of darker staining (see short arrows). The magnification of the stained configuration that is shown in the insert (C) clearly shows the darkened bisecting line that defines the stained shapes as double cones. In addition, B reveals that the arrangement of stained and unstained configurations is no longer random. Rows containing stained cells (see long arrows) alternate with rows without stained cells. Also, in the rows containing stained cells, the stained configurations alternate with unstained cells in an arrangement that is consistent with the adult pattern (see text). C, which is a photomicrograph of the same section as shown in B at a different level of focus, shows that the bisecting lines are parallel. Assuming that the rows correspond to those of the adult, the bisecting lines will be perpendicular to the rows. Scale bar: (A) through (C), 10 μm.

double-labeling experiment that the FRet 43 marker appears within three hours of the birthday of double cone cells.

A number of other investigators (Araki, 1984; Adler et al. 1984; Adler, 1986; Araki et al. 1987) have looked at the onset of phenotypic expression of photoreceptor characteristics in postmitotic, dissociated retinal cells. They have shown that these cells are able to express at least some of their photoreceptor characteristics even when dissociated and grown in vitro. The present results, which show the very early expression of a photoreceptor-specific marker, are consistent with these findings. Recent retinal lineage studies done both in the rat (Turner and Cepko, 1987) and the frog (Wetts and Fraser, 1988; Holt et al. 1988) show that retinal progenitor cells can give rise to a multitude of clone types, suggesting that the earliest a cell type can be specified is during its final cell cycle. If retinal cell fate is indeed determined by environmental factors as suggested by Turner and Cepko (1987), one might surmise from the present study that the factors inducing the expression of the FRet 43 marker must be present within three hours of the S phase of the cells' final cell division.

However, this cell-specific expression may not imply that these cells are committed to their fate. Adler and Hatlee (1989) examined the plasticity of dissociated chick embryonic retinal cells following their final mitotic division. They injected radioactive thymidine in vivo, and later dissociated the cells. They found that the ratio of photoreceptors to nonphotoreceptors in the population of dissociated, unlabeled (i.e. postmitotic)
cells depended on the time of dissociation. Their results suggest that nonphotoreceptor retinal cells are committed only after an extended exposure to the in vivo environment. An immunocytochemical study by McLoon and Barnes (1989), however, shows that a marker for the chick ganglion cell phenotype, type, and double cone-specific FRet 43 marker, is expressed as soon as these cells become postmitotic.

Our results, along with those of McLoon and Barnes (1989), might at first seem to contradict those of Adler and Hatlee (1989). However, at least two explanations can be proffered that would reconcile these apparently contradictory findings. First, retinal cells that are derived environmental information. An immunocytochemical study by McLoon and Barnes (1989), however, shows that a marker for the chick ganglion cell phenotype, like the double cone-specific FRet 43 marker, is expressed as soon as these cells become postmitotic.

Second, there is nothing to suggest that all cells within the retina utilize the same mechanisms to arrive at cellular identity. Photoreceptors and ganglion cells may be determined early, whereas the cells in the inner nuclear layer may require a longer exposure to the microenvironment, perhaps even using the ganglion cells and photoreceptors as a template from which to derive environmental information.

Our double cone marker, FRet 43, has permitted us to study pattern formation in the photoreceptor layer of the embryonic retina. By 54 h, rows containing both stained and unstained cells alternate with rows containing only unstained cells. It is likely, from comparison with the adult pattern, that the cells present in the unstained rows are rod photoreceptors. Rods have been shown to be present in the one-cell thick photoreceptor lamina of the 60-h retina (Kljavin, 1987). Our study also clearly suggests that each of the stained configurations within the rows containing immunopositive cells comprise the two members of the double cone pair. The stained configurations alternate systematically with unstained cells, suggesting that the unstained cells within these rows are the two single cone types. These results are compatible with EM studies of the guppy retina (Kunz et al. 1983) in which double cones can be distinguished prior to the initiation of outgrowth of the outer segments. Using EM techniques, however, these researchers were unable to identify a cone mosaic until after the outgrowth of the outer segments. But we observe a pattern in the 54-h embryo that is consistent with the adult mosaic. This is six hours before the observed outgrowth of outer segments (Kljavin, 1987), and many days before the mosaic was first detected previously in the zebrafish (Branchez and BreMiller, 1984).

This rapid sequence of events in the zebrafish retina, in which cell-specific markers and pattern arises within a few short hours of cellular birthday, is not dissimilar to that described in the Drosophila retina (Ready et al. 1976; Tomlinson, 1985; and Tomlinson and Ready, 1987). In Drosophila, cell–cell interactions appear to play an important role in the developing retina (for a review, see Ready, 1989); proteins expressed in some photoreceptor types appear to be required for the differentiation of other, neighboring photoreceptors (Reinke and Zipursky, 1988; Tomlinson et al. 1988). If other photoreceptor-specific markers in the zebrafish are developed, it can then be investigated whether similar mechanisms, in particular cell interactions that generate patterning, exist in this vertebrate retina.

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