Cell patterning in pigment-chimeric eyes of *Xenopus*: Local cues control the decision to become germinal cells

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ABSTRACT Between 2.5 and 4 days of development, cell proliferation in the *Xenopus* eye becomes confined to a narrow region of germinal cells at the front rim of the eye cup. Continued growth of the eye (which lasts until well beyond metamorphosis) is by the continued proliferation of cells in this germinal zone. To determine what factor(s) promotes cell division in this region of the eye long after it ceases at the back of the eye (near the optic nerve), we have transplanted small groups of eye cells from pigment-deficient embryos into the eyes of albino hosts, transposing cells from the mitotically quiescent back of the eye to the germinal zone and vice versa. Regardless of their position of origin in the donor eye, only implants into the host germinal zone behaved like germinal cells—as assayed in the living growing eye by the addition of black tissue to the pigment retinal epithelium. Conversely when donor germinal cells were implanted into the back of the host eye, they ceased dividing once they became integrated into the eye and remained as a tiny black spot on the back of the host eye. This suggests that local environmental cues, rather than intrinsic cellular determinants, specify the fates of eye cells ensuring that cells on the eye rim will continue to function as germinal cells while others will withdraw from the cell cycle.

In an attempt to elucidate this, we have transplanted small groups of embryonic eye cells from pigmented donor embryos into albino host eyes (9, 12), transposing cells between the emerging germinal zone and the mitotically quiescent back of the eye and vice versa. Transplants that adopted a germinal cell fate could be recognized readily in the growing eye by the persistent and orderly addition of new black tissue to the distal rim of the pigmented retinal epithelium (PRE), which formed an elongating black sector in the larval eye. Our findings indicate that, when the germinal zone is forming (at stages 36–38), pigmented germinal cells and PRE cells in the process of withdrawing from mitotic division are interchangeable and that, regardless of whether the graft is taken from the germinal or non-germinal region of the donor eye, only the cells implanted into the germinal zone of the host eye become germinal cells. This suggests that local environmental cues, rather than intrinsic determinants, specify nongerminall versus germinal cell fates in the developing eye. A preliminary account of this work has been presented (13).

METHODS

The 399 pigmentation chimeras described in this report were prepared by embryonic microsurgery in 16 separate operating sessions in San Diego between January 1983 and May 1986. Embryos of the African clawed frog were obtained by choricion gonadotropin-induced amplexus of adult mating pairs, reared from egg laying through the surgeries to maturity in 20% (vol/vol) saline [15% Holtfreter's and 5% Steinberg's saline], staged according to the normal tables (14, 15), anesthetized in Fuqual (tricaine) for surgery (diluted 1:5000) and for subsequent photographic sessions (diluted 1:2500–1:5000), maintained post-operatively in individual Falcon Petri dishes for 5–10 days, and then raised individually in 5-inch finger bowls on nettle powder. For surgical host embryos, we used spawnings of partially inbred strains of the periodical albino mutant in *Xenopus laevis* (ap/ap). This autosomal recessive mutation (16, 17) is autonomously expressed in the PRE of the eye (9). For pigmented donor embryos, we used spawnings of pigmented wild-type *X. laevis* breeders imported from Africa, spawnings of the *Xenopus borealis* marker strain (18), and F1 hybrids obtained by mating an albino male with an *X. borealis* female. Essentially four experiments were done and are designated as series I–IV in Fig. 1. Except in series I, which was drawn from a larger study comparing germinal cell fates at different angular positions around the germinal zone of the eye,

Abbreviation: PRE, pigmented retinal epithelium.

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*We use the term fate in the simple descriptive sense to refer to what the transplant did, whether it grew and contributed new black cells in an orderly fashion to the PRE of the growing larval eye. The term carries no implications about the developmental potential or covert determination of cells in the transplant.
and the thereafter, larger grafts from wax grooved were from donor embryos laevis stage-matched to, and X.

Individual donor embryos ranged from stage 36 to stage 38, and individual host embryos ranged from stage 32 to stage 38.

The range of embryonic stage of individual donors (stages 30–38) and hosts (stages 26–38) was somewhat broader in series I than was the range of donor and host stages in series II–IV. It is worth stressing, however, that we found no differences between series I cases prepared from older embryos—about one-third of the series I sample was strictly stage-matched with series II–IV—and those prepared from embryos at slightly earlier developmental stages. Additionally, the series I sample contained 16 dorsal (series IA) and 6 ventral (series IB) transplants in which the pigmented X. laevis donor embryos were of the tetraploid strain bred by Reinschmidt and Tompkins (12); these chimeras showed pigmentation patterns similar to chimeras prepared from wild-type or X. borealis grafts.

Pairs of one pigmented donor and one albino host embryo were dejellied mechanically, anesthetized, and aligned in grooved wax Petri dishes at a magnification of ×37.5 under fiberoptic illumination. A small patch of albino tissue was excised from the host eye rudiment and discarded; a corresponding patch of eye tissue was excised from the pigmented donor embryo and introduced into the host wound. Most grafts contained 4–8 pigmented cells (as judged by direct visual inspection at surgery) and correspondingly sized patch of underlying neural cells. In a few individuals, slightly larger grafts (containing about 12–24 pigmented cells) were introduced. Chimerae were removed from anesthesia after 10–30 min. (For further details see ref. 20.)

Beginning on the first post-operative day, and at regular intervals thereafter, individual chimerae were reanesthetized and the black/white pattern of the chimeric eye was photographed (×37.5) using Polaroid 667 film and a Zeiss dissecting microscope (see Fig. 2).

Approximately 25% of the attempted transplants were frank surgical failures. Most of these cases failed to show any pigmented cells during the 5-day post-operative period; in a few others, pigmented cells evident on the first post-operative day had disappeared by the second post-operative day. Although select cases of both types of surgical failure were reared (and photographed regularly) through late larval stages, they never showed pigmented cells and are not included in the sample of 399 cases described (Results).

Representative cases (totaling about half the sample) were immersion-fixed at a succession of later tadpole stages (larval stages 50–66), for analysis of sectioned material. Some of these chimeric eyes were fixed in buffered glutaraldehyde/paraformaldehyde, post-fixed in potassium dichromate and osmium, and used to prepare 1-μm plastic sections stained with methylene blue; others were fixed in Carnoy’s fluid, dehydrated through alcohols, and used to prepare 6- to 8-μm paraffin sections stained with quinaerine HCl (19).

RESULTS

In the first group of experiments, a small patch of pigmented cells from the nascent germinal zone on the front of the donor eye was introduced into a corresponding germinal zone site on the front of the host albino eye. Two variants of series I were carried out (Fig. 1). In 165 series IA cases, a dorsal site (12 or 1 o’clock on a right eye) on the ring-like germinal zone was chosen for both the donor tissue and the host eye; in 51 series IB cases, a ventral and slightly anterior (5 o’clock on a right eye) site was chosen for both the donor and host. In 50 of these 216 chimerae, the pigmented transplant either missed the host germinal zone altogether (24, dorsal; 5, ventral) or was displaced from the host germinal zone during the first 3 days after the surgery (17, dorsal; 4, ventral). In all of these chimerae, the transplant stopped growing and remained as a small black spot on the back of the host PRE—the host eye continued to grow by the addition of albino tissue to the front of the eye. In the great majority of other cases (n = 166; 124, dorsal; 42, ventral), one or more of the transplanted PRE cells continued to divide and adopted a germinal cell fate (Figs. 2 and 3 a and b). The addition of cells to the PRE margin from the transplants was orderly and sustained over many weeks of larval growth, and in time resulted in a more or less continuous black sector in the chimeric eye. These sectors radiate out from the back of the eye, around the front of the eyeball, and often extend through the germinal zone to include a segment of the iris (Figs. 2 and 3 b). Consistent with earlier findings on the growth of the eye in Xenopus (9, 10), growth dorsally began to attenuate at mid-late larval stages, and, in some individuals (Fig. 3 a), the pigmented cells eventually withdrew from the germinal zone. In a number of other cases, the descendants of the transplant persisted within the germinal zone (and continued to add cells to PRE) throughout the life of the larva.

In a second series of experiments, small patches of pigmented PRE cells were transplanted from the mitotically quiescent zone at the back of the donor eye into the same position at the back of the albino host eye (Fig. 1). In each case, the site chosen for both the transplanted cells and the implant into the host eye was in the dorsal quadrant of the eye, just proximal to the equator. In 37 of the 40 series II cases, the black patch healed into place on the back of the host eye. After a short period (lasting no more than a few days) of growth during which black cells were added to the transplant resulting in a modest increase in its size (Fig. 4 a), it remained as a small black spot at the back of the host eye, which changed little in either size or shape throughout the life of the animal. In the remaining 3 cases, the pigmented cells
of the transplant failed to heal into the host eye and remained as a small black nodule of ectopic eye tissue deep within the host orbit.

In the third series of experiments, germinal cells from the front of the donor eye were introduced into a dorsal (post-equatorial) site in the mitotically quiescent PRE on the back of the host eye. Of 43 series III chimerae, the graft failed to integrate into the eye in only 3 chimerae. In the remaining 40 cases, the pigmented transplant healed into the host PRE; after a short period of growth during the healing-in period, it ceased to grow; and, as albino tissue was added to the front of the growing eye, it remained as a small black spot in the PRE on the back of the host eye. Two such cases are shown in Fig. 5.

Finally, in a fourth series of experiments, pigmented PRE cells from the dorsal part of the eye, in the mitotically quiescent region behind the equator of the eye, were transplanted into the host germinal zone at either a dorsal (series IVA, n = 47) or ventral (series IVB, n = 53) implantation site. In 20 chimerae, the transplanted cells either missed the host germinal zone altogether (n = 10: 5 cases at the dorsal implantation site; 5 cases at the ventral site) or were displaced from it during the healing-in period (n = 10: 5 cases at the dorsal site; 5 cases at the ventral site). When this occurred, the transplant did not grow after the initial healing period and remained as a small black spot on the back of the growing larval eye. In 5 other series IV cases (2 IVA; 3 IVB), the only pigmented derivatives of the transplant we observed were a few cells in the iris or a nodule of ectopic pigment cells deep within the eyeball. In the remaining 75 cases, the transplant adopted a germinal cell fate, either in the dorsal or ventral part of the eye, and gave rise to black cells that populated a black sector of the PRE in a manner indistinguishable from that described above for our germinal cell transplants (Figs. 6 and 7). In 35 series IVA chimerae, the dorsal implant formed an elongating black sector but, as is common for dorsal germinal cells, in 10 cases eventually ceased growth (Fig. 6b). In 25 series IVA chimerae (Fig. 6a) and in 40 series IVB chimerae (Fig. 6b), pigmented cells continued to be generated in germinal zone during the life of the animals.

A detailed histological analysis of the chimeric material is beyond the scope of the present report, but here it may be mentioned that whenever the transplant appeared (on external view) to have become successfully integrated into the PRE on the back of the host eye, the sectioned material showed a small, coherent patch of black cells within the otherwise albino PRE layer near the optic nerve head at the back of the host retina; occasionally stray black cells were
seen outside the PRE, within the underlying neural retina. Similarly, after transplants into the host germinal zone, there
was always a well-integrated sector of black cells in the PRE, and, in the majority of these chimeric eyes, black cells were present in the iris, as well as in the PRE, and continued to proliferate within the germinal zone.

**DISCUSSION**

The aim of the present study was to examine the factors in the developing eye that restrict mitotic growth to a ring-like zone of germinal cells at the margin of the retinal epithelia. We have found that when pigmented germinal cells are transplanted into the mitotically quiescent PRE at the back of the eye, they quickly heal into place and cease to divide; as the host eye grows by adding (albino) tissue at the retinal margin, the transplant remains as a tiny black spot near the optic nerve head. By contrast, PRE cells from the back of the eye, when transplanted into the host germinal zone, often adopt a germinal cell fate, supporting the active growth of a coherent sector territory of pigmented cells similar to those seen after germinal-cell-into-germinal-zone transplants. This suggests that local cues in the germinal and nongerminal environments within the eye specify the fates of the cells so that some continue to divide while others become mitotically quiescent.

Our observations refer specifically to the growth of the pigment epithelium, although many of our grafts carried a double marker (pigmented X. borealis) so that the analysis can later be extended to the neural retina (18, 19). In addition, our observations are confined to the period during which the germinal zone is emerging in the developing eye (stages 36–38), and occasional dividing cells sometimes persist in the mitotically quiescent back of the eye. In this sense our study is an analysis of cell fates rather than of mitotic reactivation per se. Moreover, the adoption of germinal cell fates, leading
FIG. 7. Excerpts from the photorecords of two series IVB chimerae. (×17.) (a) Case SI 3339 featured a small transplant from the right eye of a stage 38 *X. borealis* × albino *X. laevis* donor embryo into the right eye of a stage 36 albino host. Like many series IB chimerae, germinal descendants of this transplant expanded their angular territory on the host germinal zone over time; this case is somewhat idiosyncratic in that the expansion trapped a white germinal cell (twc) that went on to form a clonal white cell file (wcf) in the older larval eye. Rotation of views, lateral (L) through anterior (A) to medial (M). (b) Case SI 2407 featured a small transplant from the left eye of a stage 37 *X. borealis* × albino *X. laevis* donor embryo into the right eye of a stage 35/6 albino host. Typical of a few individuals in series IB and IVB, this transplant formed a narrow black sector along the course of the ventral fissure (vf).

to a coherent black sector in the PRE, is not merely a stimulus to general (disorderly) growth but an orderly process of asymmetric cell division and distal cell accretion to the PRE margin (20).

It is of interest that mammalian PRE cells can incorporate 3H-thymidine in response to injury and that dissociated chicken PRE cells can reenter cell division in vitro and grow to confluence (21, 22). In our experiments transplants into the mitotically quiescent back of the eye often showed some cell division and growth during the "healing-in" period. This was usually short lived, but, in a few cases, integration of the transplant into the host eye was delayed for several days, and in these the phase of cell division and growth could be quite prolonged. It would appear that integration into the PRE leads to mitotic arrest in much the same way as contact inhibition in vitro. However, it is difficult to explain the fate of germinal cells on the simple basis that they lie at the rim of the PRE and, therefore, cannot attain confluence. The pigmented germinal zone is, in fact, several cells thick and is not a free edge. While it lies at the rim of the PRE, a monolayer of pigmented cells extends beyond the germinal zone into the iris, and pigmented germinal cells may be able to contribute terminally differentiated cells to the iris as well as the PRE (12). Indeed, in a majority of the cases in which PRE cells were transposed from the back of the eye into the germinal zone, the pigmented germinal cells derived from the grafts went on to populate the iris as well as the PRE (Figs. 6a and 7).

In principle, the partitioning of the embryonic eye into a germinal and a nongerminial zone could be due to some form of "mitotic clock" or to other intrinsic factors that program the cells to be "back-of-the-eye cells" or "front-of-the-eye cells" long before the germinal zone emerges. Our findings would seem to exclude such mechanisms and point, rather, to purely local cues, within the microenvironment of the transplanted cells, which determine whether the cells will behave as germinal cells (if they are at the retinal margin) or promptly withdraw from the mitotic cycle (if they are placed elsewhere). Such cues might be part of a more widespread positional signaling mechanism—e.g., a proximodistal gradient with a threshold response (23, 24)—or an entirely local stimulus such as the presence of a specific mitogen only in the vicinity of the germinal zone.

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