Specification of first quartet micromeres in *Ilyanassa* involves inherited factors and position with respect to the inducing D macromere

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SUMMARY

In the embryos of the gastropod *Ilyanassa obsoleta*, the development of several ectodermal structures requires an inductive interaction between the micromeres and the D macromere. The first quartet micromeres (1a, 1b, 1c and 1d) contribute to the head of the larva and descendants of 1a and 1c normally develop the eyes. The eyes do not develop if 1a and 1c are removed at the eight-cell stage. However, regulative eye development may occur if the precursors of 1a and 1c are removed at the two- or four-cell stage. One purpose of this study was to demonstrate which cells of the cleavage-stage embryo have the potential to develop an eye. The results of blastomere deletion experiments suggest that only the first quartet micromeres have this ability. In addition, the 1b micromere was found to be equivalent to 1a and 1c, but 1d was found to have a poorer eye-forming ability. A second purpose of this study was to examine how eye development is normally restricted to the 1a and 1c micromeres. Cell transplantation experiments demonstrate that the proximity of a first quartet micromere relative to the inducing D macromere is important for determining whether or not it will go on to develop an eye. The 1b micromere may not develop an eye during normal development because it is too far from the D macromere. However, the eye-forming ability of the 1d micromere is not influenced by its close position to the D macromere, but is restricted by its polar lobe lineage.

Key words: *Ilyanassa obsoleta*, Spiral cleavage, Induction, Cell transplantation

INTRODUCTION

In embryos that undergo spiral cleavage, the D blastomere plays an important role in the organization of the embryo and larva (reviewed in Freeman and Lundelius, 1992). In embryos of polar lobe-forming species, the D blastomere is specified as the polar lobe shunts material from the vegetal pole of the egg into one cell during early cleavage. Early developmental stages of the polar lobe-forming gastropod *Ilyanassa obsoleta* are shown in Fig. 1. The vegetal polar lobe is shunted to the CD blastomere at the two-cell stage, and the D blastomere at the four-cell stage. As a consequence of inheriting the polar lobe material, the D blastomere is larger than the other three blastomeres and can be used as a reference for identifying all cleavage stage cells. The next three cleavages divide the embryo into three quartets of small micromeres at the animal pole and four larger macromeres at the vegetal pole. The sixth cleavage produces the 4d micromere, or mesentoblast, which gives rise to much of the mesoderm and endoderm (Conklin, 1897; Render, 1997) and the other three fourth quartet micromeres, which contribute to the endoderm of the larva. Micromeres from the D lineage form at slightly different times and are slightly different in size compared to their counterparts from the other three quadrants (Clement, 1952). The derivatives of the four quartets of micromeres continue to divide at the animal pole of the embryo, and during gastrulation this cap of cells spreads over the macromeres by epiboly. A veliger larva develops after 6-7 days. All of the ectoderm, including the velum, eyes, statocysts, shell, foot and operculum, derives from the micromeres of the first three quartets (Cather, 1967; Clement, 1967, 1986a,b; Render, 1991, 1997; McCain, 1992). The development of the micromeres to form these specialized ectodermal structures depends upon an inductive signal from the D macromere. When the polar lobe is removed, the macromeres continue to produce quartets of micromeres, but no micromeres take on the cleavage pattern that is characteristic of micromeres from the D quadrant (Crampton, 1896; Clement, 1952). The resulting larva is radially symmetrical and it does not develop the heart and intestine, which normally derive from the 4d mesentoblast, nor does it develop the eyes, statocysts, foot, operculum, shell, or a bilateral velum which normally derive from the ectodermal micromeres (Clement, 1952; Atkinson, 1971). Therefore, the polar lobe material is required for the specification of the D quadrant, the organization of the bilateral larva, and the induction of the micromeres to form specialized ectodermal structures. By removing the D blastomere at different times in *Ilyanassa*, Clement (1962) found that the 3D macromere has induced the micromeres at a time between the formation of the third quartet of micromeres and the 4d mesentoblast (24-28 cells). It is not known when the inductive signaling begins, nor is the molecular nature of the inductive signal known.
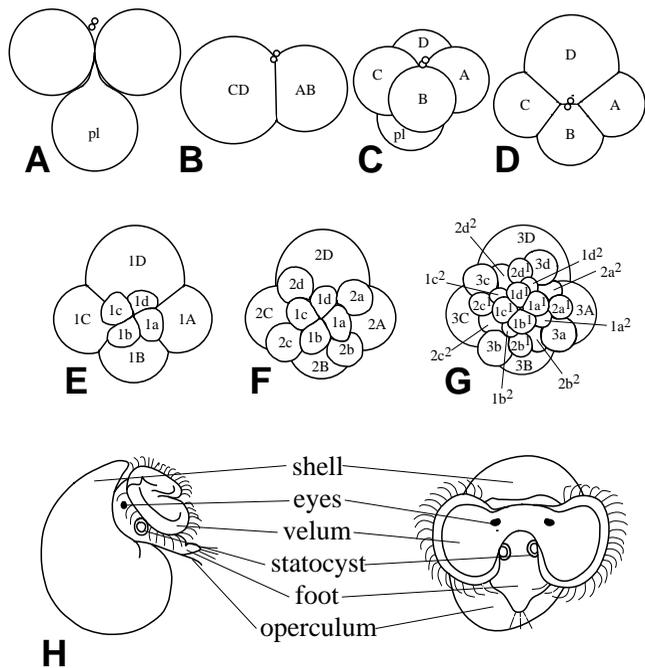
Fig. 1. Normal development of *Ilyanassa obsoleta*. The polar bodies mark the animal pole in (A-D). Embryos in (A-C) are shown in a side view; embryos in (D-G) in an animal pole view. (A) At first cleavage the polar lobe (pl) forms at the vegetal pole and it has a thin connection to the rest of the embryo. (B) At the two-cell stage the polar lobe has been inherited by one cell, which is designated as the CD blastomere. (C) At second cleavage the polar lobe forms again in the CD blastomere and it is inherited by the D cell. (D) The four-cell stage embryo is divided into A, B, C and D quadrants, which correspond to the left, ventral, right and dorsal parts of the larva, respectively. (E) The third cleavage results in an eight-cell stage embryo with the first quartet of micromeres at the animal pole and the four larger macromeres at the vegetal pole. (F) The fourth cleavage produces a twelve-cell stage embryo as the macromeres divide to produce the second quartet of micromeres, and a 16-cell stage embryo (not shown) when the first quartet micromeres divide unequally. (G) During the fifth cleavage the third quartet of micromeres forms and the second quartet micromeres divide, resulting in a 24-cell stage embryo. Three of the first quartet micromeres ($1a^1$, $1b^1$ and $1c^1$) then divide to produce a 27-cell stage embryo (not shown). (H) A veliger larva forms after 7 days of development (side and front view).

The first quartet micromeres (1a, 1b, 1c and 1d) contribute to the head of the veliger larva (Clement, 1967; Render, 1991). The head consists of a bi-lobed, ciliated velum that is used for swimming and feeding. Each velar lobe has one eye that contains pigment cells, photoreceptor cells, corneal cells, and an acellular lens, and each eye is closely associated with the cerebral ganglia (Gibson, 1984). The fate maps of the first quartet micromeres, summarized in Fig. 2, demonstrate that each of these cells contributes to a specific region of the velum, in addition to contributing to the apical plate region between the eyes (Render, 1991). Descendants of the 1a micromere make the left eye and part of the left velar lobe, and the 1c micromere gives rise to the right eye and most of the right velar lobe. The micromeres 1b and 1d contribute to regions close to the eyes, but their descendants never participate in eye development; 1b contributes to ventral portions of both velar lobes, and the descendants of 1d overlap with the descendants

of 1a and 1b in the left velar lobe. Micromeres from the second and third quartets also contribute to the posterior face of the velum, underneath the first quartet derivatives (Render, 1997).

When all four first quartet micromeres are removed from the embryos of the polar lobe-forming gastropod *Bithynia*, the resulting larva is normal in all respects except that it does not develop a head (van Dam and Verdonk, 1982). This result indicates that these four cells are necessary for the development of head structures. In *Ilyanassa* the embryo does not regulate following the removal of individual first quartet micromeres (Clement, 1967). The deletion of each of these cells results in head defects, which correspond to the fate map of the deleted cell (Fig. 2). Eyes never develop following the removal of the eye precursor cells 1a and 1c, suggesting that these two micromeres may be the only cells which have the potential for eye development. Regulative eye development occasionally occurs when the same experiments are done in the embryos of *Bithynia*. When 1a or 1c is removed in this species, the left or right eye develops after a delay of 1 day in an average of 31% of the cases (van Dam and Verdonk, 1982). Regulative eye development does not take place when the 1a or 1c micromeres are removed in *Ilyanassa* even when the larvae are raised several days after the time when the eyes normally form (Sweet and Boyer, 1990). However, regulative eye development has been demonstrated in the embryos of *Ilyanassa* under more extreme experimental conditions where blastomeres are isolated at the two- or four-cell stage. Clement (1956) removed the AB blastomere at the two-cell stage to isolate CD half embryos, and he removed the B and C blastomeres at the four-cell stage to isolate AD half embryos. The resulting CD and AD half larvae formed two eyes in 14% and 18% of the cases, even though the embryos contained only one eye precursor cell (1c or 1a, respectively). McCain and Cather (1989) extended these experiments, creating BD half embryos by removing the A and C blastomeres at the four-cell stage. Even though these embryos lacked both of the normal eye precursor cells, 27% of the larvae developed at least one eye. Also, Clement (1956) removed the A, B and C blastomeres at the four-cell stage to isolate D quarter embryos and found that some of the resulting larvae developed one eye (no quantitative data presented). Thus, there are other cells in the *Ilyanassa* embryo besides 1a and 1c that have the potential to develop an eye.

The purpose of this study is to identify the cells that have the regulative potential to develop eyes in embryos of *Ilyanassa* and to examine how this potential is restricted to the 1a and 1c micromeres during normal development. All first quartet micromeres were removed to determine whether micromeres from the second and third quartets have the potential to develop eyes. To determine the eye-forming potential of each first quartet micromere, three of the four first quartet micromeres were removed at the eight-cell stage to test the potential of the remaining micromere. Micromere potential was also tested in half embryos which have been shown to have greater regulative abilities. In these experiments, two blastomeres were removed at the four-cell stage, and then either both or one first quartet micromere was removed after the next cleavage. The results suggest that all four first quartet micromeres, and no other cells, have the potential for eye development, though the 1d micromere has less eye-forming potential in comparison to 1a, 1b and 1c. A transplantation technique was then developed to examine how eye-forming potential is normally restricted. The results support the hypothesis that 1a, 1b and 1c are equivalent
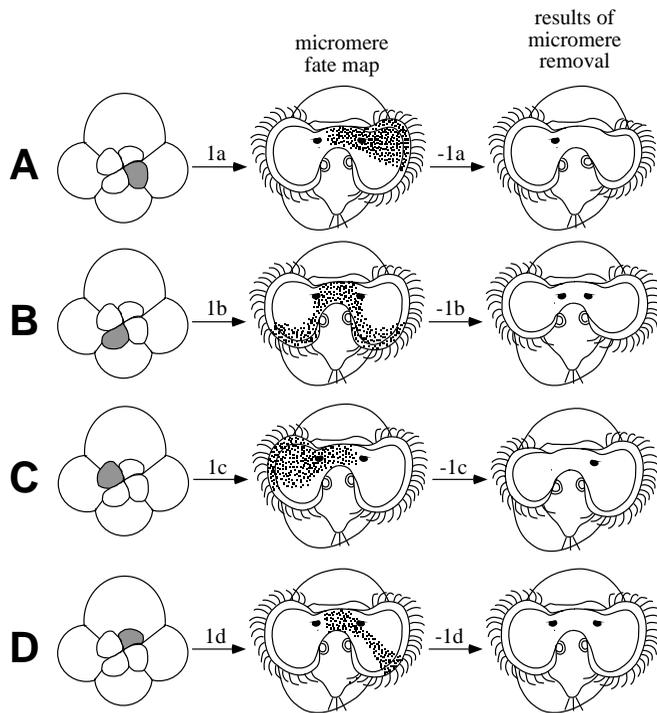
Fig. 2. Fate maps for the first quartet micromeres (Render, 1991) and the results of removing individual first quartet micromeres (Clement, 1967). (A) The 1a micromere forms the left eye, part of the left velar lobe and the region between the eyes (apical plate). When 1a is removed, the left eye never develops and the left velar lobe is small. (B) The 1b micromere contributes to the ventral regions of both velar lobes and the apical plate. When 1b is removed the eyes are close together and both velar lobes are reduced in size. (C) The descendants of the 1c micromere make the right eye, part of the right velar lobe and the apical plate. Following the removal of 1c the right eye is missing and the right velar lobe is small. (D) The descendants of the 1d micromere contribute to a small region of the left velar lobe and the apical plate. No consistent defects result following the removal of 1d.

in their eye-forming potential and that the position of these micromeres with respect to the inducing D macromere plays an important role in determining which first quartet micromeres will develop an eye. Polar lobe removal restores the eye-forming potential of the 1d micromere, indicating that the polar lobe normally restricts 1d from developing an eye.

MATERIALS AND METHODS

Biological material

Adult snails were obtained from Ward's Scientific (Rochester, NY) and the Marine Biological Laboratory (Woods Hole, MA) and maintained in artificial sea water (Instant Ocean, Aquarium Systems, Mentor, Ohio) in a recirculating aquarium at room temperature on a 16:8 hours light:dark cycle. Handling of the adults and embryos is described by Collier (1981). Egg capsules were removed from the sides of the tank and opened by snipping off the basal tip with scissors. The embryos were carefully removed from the egg capsule and washed several times in Millipore-filtered artificial sea water (Jamarin, Osaka, Japan) with an antibiotic (100 µg/ml streptomycin).

Blastomere deletions

Blastomeres were killed by pricking them with a hand-pulled glass

needle. After about 1 minute, each lysed cell was removed as one piece from the remainder of the embryo. Unoperated embryos from the same capsule were used as controls. Experimental embryos were examined during the next few cleavages to ensure that the remaining cells were dividing properly. Controls and individual experimental embryos were maintained in separate wells in multi-well glass plates in Millipore-filtered Jamarin sea water with an antibiotic (JSW). The embryos were examined and transferred to fresh JSW every day or every other day. After 7-9 days the resulting larvae were examined live to identify larval structures. Cilia were prevented from beating with a solution of 100 mM sodium azide in 80% JSW. Muscles were relaxed using a saturated solution of chloretone in JSW, which was then diluted 1:1 in JSW. The sodium azide mixture and the chloretone mixture were combined 2:1 to treat velar cilia and muscles at the same time. Larvae resulting from half embryos were scored for the presence of eyes, velar tissue, external shell, internal shell, statocysts, operculum, foot and gut pigment. Regulation often occurred, resulting in the development of more of these structures than expected. However, these half embryos sometimes developed poorly where expected structures were missing in the larvae. Individuals were discarded if they developed less than three of these structures. Eyes were identified as dense aggregates of melanin-containing cells and were scored even if they were malformed. The lens could frequently be seen, but was not identified in every case. Long velar cilia was used as a marker for velum differentiation. External shell, internal birefringent masses, statocysts and operculum were identified by their birefringence when viewed with polarized light and by their morphology. Statocysts consist of a spherical statolith enclosed in a capsule. External shell was identified by the presence of birefringence on the outside surface of the larva. Internal birefringent masses probably represent malformed shell and/or statocysts (Clement, 1962; Cather, 1967; McCain, 1992). The operculum was identified by an external birefringent film, which was in close proximity to the foot. The foot was identified by the presence of long sensory bristles. Diffuse black pigment on the inside or outside of the larva was scored as gut pigment. Larvae resulting from blastomere deletions were anesthetized, fixed in 4% paraformaldehyde, mounted in Euparal (Clement and Cather, 1957) and photographed.

Micromere transplantation

Donor embryos were treated with a solution of rhodamine isothiocyanate (RITC) to label cell membranes. The treatment was modified from the method used by Ettensohn and McClay (1988). 1 mg RITC was dissolved in 5 µl dimethyl sulfoxide. This solution was then diluted in 1 ml of JSW, vortexed and filtered (0.22 µm pore size syringe filter). The embryos were placed in 20 µl stock RITC solution in 1 ml JSW for 30 minutes in the dark. After the embryos were labeled, the normal orientation of the micromere to be transplanted was marked by placing carbon particles on the apical surface of the cell. To increase their adhesion to the embryo, the carbon particles were pretreated with a 1 mg/ml solution of poly-L-lysine in water, followed by a rinse in JSW. The donor embryos were then transferred to the operating dish with the host embryos.

Transplantations were performed in a 35 mm plastic Petri dish coated with 2% agar in JSW using a hand-pulled glass needle and an eyebrow hair mounted in the tip of a pulled Pasteur pipette. All micromanipulations to the host and donor embryos for a given transplantation were completed within a 10-minute time interval. Host embryos were prepared by killing and removing one micromere to provide a space for the donor micromere. The donor micromere was isolated by killing and removing all surrounding cells in the RITC-labeled donor embryo. The donor micromere was then transported to the host embryo, patted into position in the appropriate orientation according to the position of the carbon marks, and placed in a hole made in the agar. After 2-3 hours each experimental embryo was examined to ensure the transplanted micromere was adhering to the rest of the embryo and that cleavage was progressing normally. Experimentals were discarded if the descendants of the transplanted micromere were not integrated into the host embryo.

One set of controls included untreated embryos from the same egg capsule as the host embryos. A second set of controls included complete embryos that had been treated identically to the donor embryos, including treatment with carbon particles and the RITC solution. Each experimental embryo was raised in a separate well in a multi-well glass plate. The controls and experimentals were examined and transferred to fresh JSW every day or every other day. At 7-9 days the experimental larvae were anesthetized as described above and examined with epifluorescence microscopy to view the descendants of the transplanted cell. The larvae were photographed live using TriX film at 1600 ASA. The film was developed with Diafine developer (Acufine, Inc., Chicago, IL).

Polar lobe removal

One set of transplantation experiments required donor embryos lacking the cytoplasmic determinants that define the D lineage, such that the resulting '1d' micromere could be transplanted to the position of an eye precursor cell. To obtain these embryos, the polar lobe was removed at first cleavage according to Clement (1952), with some modifications. First, the position of the prospective D cell was labeled with poly-L-lysine-treated carbon particles as soon as the CD blastomere could be identified. A fine glass thread was then pressed against the thin region that connects the polar lobe to the rest of the embryo. This was repeated several times over a span of a few minutes until the polar lobe fell away from the embryo. The '1d' micromere was then isolated at the eight-cell stage as described above.

Scanning electron microscopy

Scanning electron microscopy was used to examine eight-cell stage embryos following micromere removal and the early development of embryos following micromere transplantation. Embryos were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) overnight at 4°C. After rinsing in buffer, the embryos were post-fixed in 1% osmium tetroxide in buffer for 1 hour. The embryos were rinsed in buffer and dehydrated through an ethanol series to 100% ethanol and then through a series of amyl acetate in ethanol to 100% amyl acetate. They were critical-point-dried (Samdri-790) with carbon dioxide as the transition fluid, and sputter-coated (Ladd) with a gold palladium alloy. The embryos were examined with a Philips 515 scanning electron microscope with 10 KV high voltage and photographed with Polaroid I55 P/N film.

RESULTS

Embryos lacking the first quartet of micromeres never develop eyes or velar cilia

The first quartet of micromeres normally contributes to the head of the veliger larva and the 1a and 1c micromeres normally develop the eyes (Render, 1991). All four first quartet micromeres were removed (−1abcd) to determine whether the second and third quartet micromeres can develop an eye. In many respects, the experimental larvae (Fig. 3C) developed like controls (Fig. 3A,B). The body axes of the larvae could be distinguished, and for the most part, each larva developed an external shell, foot, operculum, two bilateral statocysts, and a complete gut. Eyes did not develop in any of these

embryos (16 cases; Table 1), suggesting that this potential is restricted to the first quartet micromeres. These embryos also did not develop other head structures. The larvae never had velar lobes or long velar cilia, though they often had a stump of tissue in the place where the head would normally be. This stump may be the everted stomodeum and/or malformed velar tissue derived from the second and third quartet micromeres.

Individual first quartet micromeres rarely regulate to form the eyes in the absence of the other three first quartet micromeres

The potential of each first quartet micromere to develop an eye was examined by removing three of the four first quartet micromeres at the eight-cell stage. In these embryos, only one first quartet micromere remains to contribute to the head. Other larval structures generally developed normally, but there were some minor, though inconsistent, irregularities. The results of this assay are summarized in Table 1 and Fig. 4. For the most part, the development of the head reflected the fate map of the remaining first quartet micromere. When 1a was the only remaining first quartet micromere (−1bcd), the larvae usually developed the left velar lobe (50%) and the left eye (62%; Fig. 4A). However, in addition to forming a velar lobe on the left side, a small velar lobe sometimes formed on the right side (27%). The right velar lobe was usually bordered by long velar cilia, but it rarely included the right eye (2 cases). In this experimental group there were also larvae that developed one velar lobe with an eye, but the lobe was rotated with respect to the rest of the body such that the left/right position was unclear. There were no larvae that had formed only the right velar lobe and the right eye. These results suggest that in the absence of the other three first quartet micromeres, the 1a micromere develops according to its normal fate to form a left velar lobe and left eye.

When 1b was the only remaining first quartet micromere (−1acd), it almost always developed a small velum without any eyes (95%; Fig. 4B). The velum was either bilaterally symmetrical or the right velar lobe was slightly larger than the left velar lobe. In one case the 1b micromere developed only the right velar lobe and the right eye. Thus like 1a, the 1b micromere usually developed according to its normal fate in the absence of the other first quartet micromeres, but it also demonstrated a small ability to regulate to develop an eye.

When the potential of the 1c micromere was tested (−1abd), it often developed according to its usual fate to form only the right velar lobe and right eye (31%; Fig. 4C). In addition to
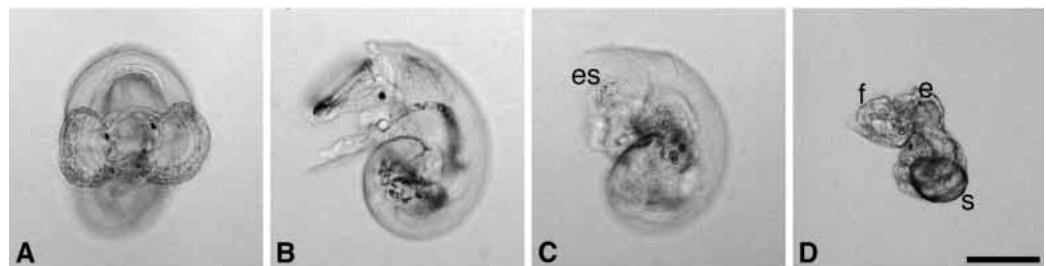

Fig. 3. Embryos lacking the first quartet do not develop heads. (A) Front view of a control larva. (B) Side view of a control larva. (C) Larva resulting from the removal of all four first quartet micromeres. There are no eyes or other recognizable head structures. A large stump of tissue, the everted stomodeum (es), is in the position where the head should be. (D) Example of a larva that develops from a BD half embryo. This half larva has a small shell (s), a foot (f), two statocysts, an operculum and an eye (e). Bar, 100 µm.

Table 1. Development of head structures in embryos from which three or four first quartet micromeres were removed

Experiment	−1abcd	−1bcd	−1acd	−1abd	−1abc
Remaining micromere	−	1a	1b	1c	1d
Total	16	26	21	16	14
Eyes					
None	16 (100)	3 (12)	20 (95)	4 (25)	14 (100)
At least one eye	0	23 (88)	1(5)	12 (75)	0
Left	−	16 (62)	−	−	−
Right	−	−	1 (5)	8 (50)	−
One (position unclear)	−	5 (19)	−	4 (25)	−
Two	−	2 (8)	−	−	−
Velum					
None	16 (100)	1 (4)	−	5 (31)	14 (100)
At least one lobe	0	25 (96)	21 (100)	11 (69)	0
Left lobe	−	13 (50)	−	-	−
Right lobe	−	−	1 (5)	5 (31)	−
One lobe (position unclear)	−	5 (19)	−	3 (19)	−
Two lobes	−	7 (27)	20 (95)	3 (19)	−

Values are the number of embryos that develop head structures, with percentages in parentheses.

developing head structures on the right side, 19% of the larvae also formed a small velar lobe on the left side. In some cases a single velar lobe and eye formed, but the left/right position was unclear (19%). However, 1c never formed head structures that were clearly on the left side only (mostly 1a derivatives), and the 1c micromere never regulated to develop the left eye.

When the potential of 1d was tested (−1abc), the resulting larvae never developed an eye or any other identifiable head structures (Fig. 4D). No velar lobes or long velar cilia formed even though the fate map shows that 1d contributes to the left velar lobe (Render, 1991). However, there was often a ciliated stump of tissue in the position where the velum would normally be. This stump may have been a part of the stomodeum, which was often everted, or it could have derived from 1d or micromeres from the second and third quartets.

1b and 1d can regulate to form eyes in half embryos

Micromere potential was also tested in half embryos because they have been shown to have enhanced regulative abilities (Clement, 1956; McCain and Cather, 1989). Two blastomeres (either A and B, B and C, or A and C) were removed at the four-cell stage to isolate the half embryos (CD, AD and BD half embryos, respectively). The D blastomere was included in all cases because it is necessary for the induction of the micromeres to form the specialized ectodermal derivatives, including the eyes (Clement, 1956, 1962). After the third cleavage either zero, one or two first quartet micromeres were removed. Each type of experiment is named according to which quadrants remain and which micromeres were removed. For example, an embryo that contains the B and D quadrants but lacks the 1d micromere is named BD−1d.

All combinations of half embryos used in this study are shown in Fig. 5, and their abilities to form head structures are summarized in Table 2. Because the body axes are difficult to distinguish in these half larvae, the position of the ectodermal structures (left or right) was not scored. An example of a half larva is shown in Fig. 3D. The external shells of the half larvae were typically small, if present, and the half larvae often formed an internal birefringent mass. There were either zero, one or two recognizable statocysts per half larva. The foot and operculum often formed, but they were sometimes bifurcated.

Even though embryos never developed eyes or velar cilia following the removal of all four first quartet micromeres (as described above), it is possible that micromeres from the second or third quartets have the ability to form these head structures under the regulative conditions of the half embryos. To test this possibility, half embryos were isolated and both first quartet micromeres were removed after the third cleavage (AD−1ad, BD−1bd, CD−1cd). The resulting half larvae never formed eyes (19 cases). Thus, under these conditions favoring regulation, the first quartet micromeres appear to be the only micromeres which have the ability to develop eyes. In addition, even though micromeres from the second and third quartets
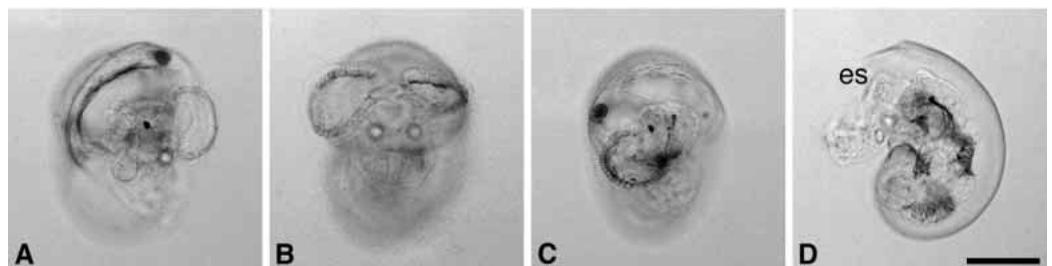

Fig. 4. Individual first quartet micromeres develop according to their normal fate in the absence of the other three first quartet micromeres. (A) When 1a is the only remaining first quartet micromere (−1bcd) it usually develops the left velar lobe and left eye. (B) When the potential of 1b is tested (−1acd) it usually forms a small velum with no eyes. (C) When the 1c micromere is tested (−1abd), it often develops according to its usual fate to form a small velar lobe on the right side which includes one eye. (D) When the 1d micromere is the remaining micromere (−1abc), no identifiable head structures develop, though these embryos usually have a stump of tissue, which includes an everted stomodeum (es), where the head would normally be. Bar, 100 μm.

Table 2. Development of head structures in half embryos

Experiment	Remaining micromere	Total*	Total examined	Eye‡	Velar cilia
AD	1a, 1d	28	25	23 (92)	20 (80)
AD−1a	1d	24	10	1 (10)	0
AD−1d	1a	31	28	26 (93)	17 (68)
AD−1ad	−	5	5	0	0
BD	1b, 1d	63	56	30 (54)	48 (86)
BD−1b	1d	19	10	4 (40)	5 (50)
BD−1d	1b	25	24	19 (79)	20 (83)
BD−1bd	−	7	5	0	0
CD	1c, 1d	36	32	32 (100)	19 (59)
CD−1c	1d	22	14	4 (29)	3 (21)
CD−1d	1c	28	21	17 (81)	14 (67)
CD−1cd	−	9	9	0	0

Values are shown with percentages in parentheses.

*The total number of half larvae before discarding those with poor development (see Materials and methods).

‡The number of half larvae that developed at least one eye. Of 113 control half embryos (AD, BD, CD), two BD half embryos went on to develop two eyes (2%). Of 107 half embryos which possess one micromere (MD−1m), one AD−1d embryo developed two eyes (1%).

normally contribute to other parts of the head (Render, 1997), they never made recognizable velar structures under these experimental conditions.

To test the ability of the 1a eye precursor to develop an eye in half embryos, AD half embryos were isolated and the 1d micromere was removed (AD−1d). When 1a was tested in isolation, it developed an eye in 93% of the cases. To test the other eye precursor cell, 1c, CD half embryos were isolated and the 1d micromere was removed (CD−1d). Under these conditions the 1c micromere developed an eye in 81% of the cases. To test the ability of the 1b micromere to regulate to develop an eye, BD half embryos were isolated and the 1d micromere was removed (BD−1d). The 1b micromere developed an eye in 79% of the cases. The frequency of eye development by 1b is not significantly different from the frequency of eye development by 1a and 1c as assessed by a chi square test ($P=41$, $n=19$). Thus 1b appears to have the same ability to develop an eye in this assay compared to the normal eye precursor cells. Three combinations of experiments were used to test the 1d micromere for its ability to regulate to develop an eye (AD−1a, BD−1b, CD−1c). On average, the 1d micromere regulated to develop an eye in 26% of the cases. The difference in the frequency of eye development by 1d compared to the frequency of eye development by the eye precursor cells is statistically significant ($P<0.0001$, $n=34$). The 1d micromere may have a low eye-forming potential because it is the closest first quartet micromere to the inducing D macromere, or because it is lineally related to the D macromere and the polar lobe.

Controls for transplantation experiments

The experiments described above demonstrate that each first quartet micromere has the potential to develop an eye, though 1d appears to have less eye-forming potential compared to 1a, 1b and 1c. Transplantation experiments were used to examine the role of cell position in the further restriction of the potential of the first quartet micromeres. The transplantation procedure is shown in Fig. 6. To provide the position for a donor micromere, one of the host micromeres is killed and removed. Fig. 7A,B shows an eight-cell stage embryo that was fixed
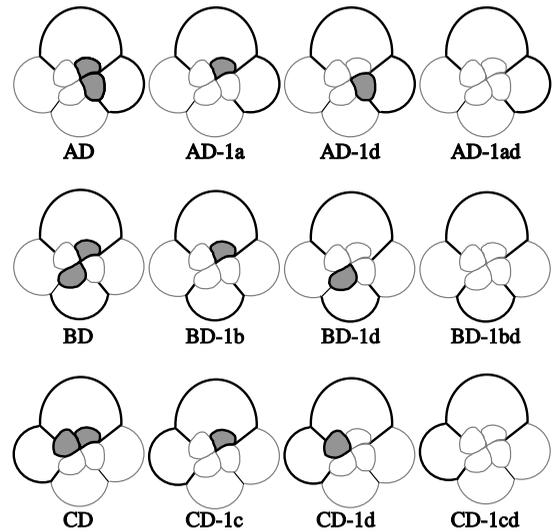

Fig. 5. All combinations of half embryos used to test the regulative potential of the micromeres. Half embryos were isolated by removing two cells (gray outline) at the four-cell stage. To serve as controls, no further manipulations were done to some half embryos (AD, BD and CD). In other half embryos one first quartet micromere was removed (gray outline) after the next cleavage, to test the potential of the remaining first quartet micromere (shaded). In others, both first quartet micromeres (gray outline) were removed to test the potential of the second and third quartet micromeres. Each type of half embryo is named according to which quadrants are present (AD, BD or CD) and which first quartet micromeres are removed (−1a, −1b, −1c, −1d, etc.).

immediately after this procedure. There is no visible damage to the surface of the embryo and there is no cell debris left behind. When a micromere is transplanted into the position in the appropriate orientation, it adheres to the host embryo and it cleaves in the appropriate direction (Fig. 7C, arrow).

The first set of transplantations consists of homotopic controls where the precursor to the left eye, 1a, was transplanted back to its normal position in a different embryo at the same stage. By virtue of their rhodamine label, the descendants of the transplanted 1a micromere were found in the region between the eyes (apical plate) and in the left velar lobe of the larva, which is expected from the fate map (data not shown, though the distribution of the labeled cells was nearly identical to the pattern shown in Fig. 8A′). This indicates that the transplanted micromere and its descendents continued to divide and contribute to head structures. However, there was often a small amount of fluorescence in other regions of the larva, including the right velar lobe and, less commonly, in the mantle edge and the foot. The unexpected patterns of fluorescence suggest that some of the label was lost from the descendants of the transplanted cell and perhaps distributed nonspecifically to these regions by virtue of morphogenetic movements. It should be emphasized, however, that very little cell mixing occurs during the normal development of these embryos (Verdonk and van den Biggelaar, 1983). Also, according to the fate map of the first quartet micromeres, each of these cells consistently gives rise to a specific region of the head, though some mixing occurs in the apical plate region (Render, 1991). In all 27 cases in which 1a was transplanted back to its normal position, the donor 1a micromere made normal velar
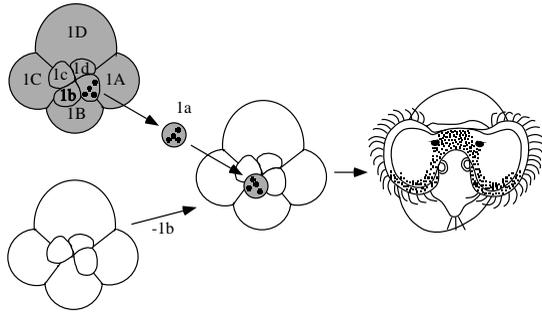
Fig. 6. Transplantation procedure. In this example, the 1a micromere is transplanted to the 1b position. The donor embryo is labeled with RITC (shaded) and the apical surface of the 1a micromere is marked with an arrowhead pattern of carbon particles, which points to the future position of the $1a^2$ derivative. The donor micromere is then transferred to an unlabeled host in which a position has been made by removing the 1b micromere. Using the carbon marks, it is oriented into the appropriate position where the $1a^2$ daughter will lie in the $1b^2$ position. After 7 days, epifluorescence microscopy is used to find the position of the descendants of the transplanted micromere.
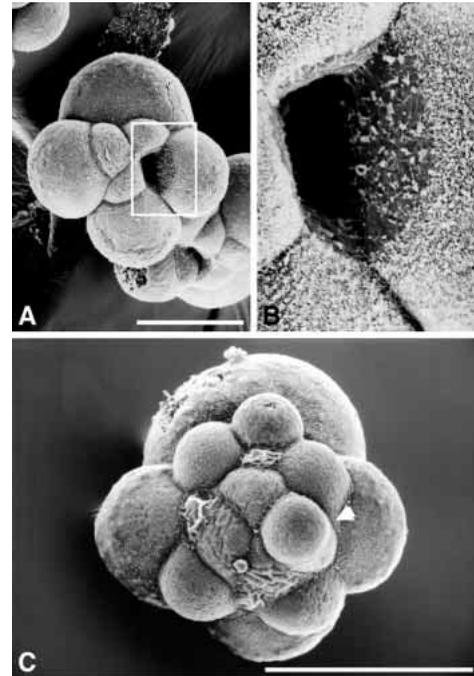

Fig. 7. Scanning electron micrographs of early embryos used during the transplantation procedure. (A) An eight-cell stage embryo fixed within 10 minutes following the removal of the 1a micromere.
(B) Enlargement of the boxed area shown in A. There are fewer microvilli in the area formerly occupied by the 1a micromere, and there is a hole which extends within the embryo. (C) A micromere (arrow) was transplanted to the position of 1a at the eight-cell stage and the embryo was fixed as the third quartet of micromeres was beginning to form (end of 16-cell stage). The transplanted micromere has divided to form $1m^1$ and $1m^2$ derivatives. The $1m^2$ derivatives in the B, C and D quadrants have collapsed and appear wrinkled. Bars, 100 μm.

structures. Of these, 13 larvae developed the left eye (48%; Table 3). In several cases the fluorescent label was not directly associated with the left eye, but it surrounded the eye. The removal of the 1a micromere always results in the absence of the left eye (Clement, 1967; Sweet and Boyer, 1990), so if the left eye develops following the transplantation of a cell to the 1a position, then the left eye must have developed from the descendants of transplanted cell, even though this cannot be unambiguously confirmed by epifluorescence microscopy.

1b is nearly equivalent to 1a in its potential to develop an eye

The 1b micromere normally contributes to the apical plate and the ventral portions of both velar lobes, but it does not develop an eye (Render, 1991). The results of the experiments described above demonstrate that 1b has the ability to develop an eye in

Table 3. Eye development and internal birefringent mass formation by transplanted micromeres

Donor micromere	Host position	Total	Eye	Birefringent mass
1a	1a	27	13 (48)	–
1b	1a	26	8 (31)	–
1d	1a	45	1 (2)	–
1a	1b	23	1 (4)	–
1a	1d	26	14 (54)	–
1b	1d	25	13 (52)	–
'1d'	1a	21	10 (48)	–
2a	1a	8	0	1 (13)
2b	1a	7	0	2 (29)
2c	1a	20	0	7 (35)
1a	3c	21	4 (19)	–

Values are given with percentages in parentheses.

The difference in the frequency of eye development by the 1b micromere in the 1a position and that of the homotopic control (1a) is not statistically significant by a chi-square test (*P*=0.20). The difference in the frequency of eye development by 1d in the position of 1a and that of the homotopic control is statistically significant (*P*<0.0001). The difference in the frequency of eye development by '1d', which lacks the polar lobe, and that of the homotopic control is not statistically significant (*P*=0.066).

half embryos. To test the eye-forming potential of 1b under more normal conditions, in which all the cells of the embryo are present, the 1b micromere was transplanted to the position of the 1a micromere. The descendants of the transplanted 1b micromere were in the apical plate and left velar lobe, as predicted from the fate map of the 1a micromere, and the donor 1b micromere developed the left eye in 8/26 cases (31%; Fig. 8A,A'). The difference between the frequency of eye development by 1b and the homotopic control 1a is not statistically significant by a chi square test (*P*=0.20). This demonstrates that the 1b micromere can regulate to take on the fate of the 1a micromere, and that it can develop an eye at nearly the same frequency as the 1a micromere under similar conditions.

1d rarely develops an eye in the position of 1a

The 1d micromere normally contributes to the apical plate and the left velar lobe, forming a wedge of tissue between the descendants of the 1a micromere and the 1b micromere (Render, 1991). As suggested by the results of half embryo experiments, 1d has the ability to develop an eye, but its potential appears to be restricted compared to 1a, 1b and 1c. The eye-forming potential of the 1d cell was tested again by transplanting it to the position of the 1a micromere. The transplanted 1d micromere contributed to the apical plate and left velar lobe in all cases (Fig. 8B,B'), but it developed the left eye in only 2% of the larvae

(1/45). This demonstrates further that the potential of 1d is not equivalent to 1a and 1b.

Close proximity to the D macromere is important for the specification of 1a and 1b to make an eye

The 1b micromere may not become specified to develop an eye under normal conditions because it is too far from the D macromere at the time of the induction. To test this, and to test whether the eye precursor cell retains its ability to develop an eye when moved to a distant position relative to the inducing D macromere, the 1a micromere was transplanted to the position of 1b. The descendants of the transplanted 1a micromere came to lie in the same regions as expected from the fate map of the 1b micromere (Fig. 8C,C′). In 22 cases, only the left and right eyes formed; however, an ectopic third eye developed next to the endogenous left eye in one case. The overall result indicates that in the position of the 1b micromere, 1a takes on the fate of 1b and becomes restricted from developing an eye. This supports the idea that the position of the 1b micromere is not conducive for eye development and that it may be too far from the inducing D macromere.

To further test whether distance from the inducing D macromere is important for determining whether or not a micromere will develop an eye, 1a or 1b was transplanted to the position closest to the inducing D macromere, the position of the 1d micromere. The descendants of the transplanted 1a and 1b micromeres developed an ectopic third eye in 54% and 52% of the cases (Fig. 8D,D′). The ectopic eye from the donor micromere developed either in the apical plate or in the left velar lobe adjacent to the host left eye and it appeared to be morphologically normal (Fig. 9).

1d inherits factors from the polar lobe, which restrict it from developing an eye

Experiments described above indicate that the 1d micromere has a reduced eye-forming potential compared to the other first quartet micromeres. This is not a consequence of the close proximity between 1d and the inducing D macromere because other first quartet micromeres may develop an eye when placed in the position of 1d. Rather, this decreased potential may be a consequence of the lineage of 1d. The 1d micromere may be different from the other first quartet micromeres because it is a descendant of the D blastomere, which inherits the polar lobe
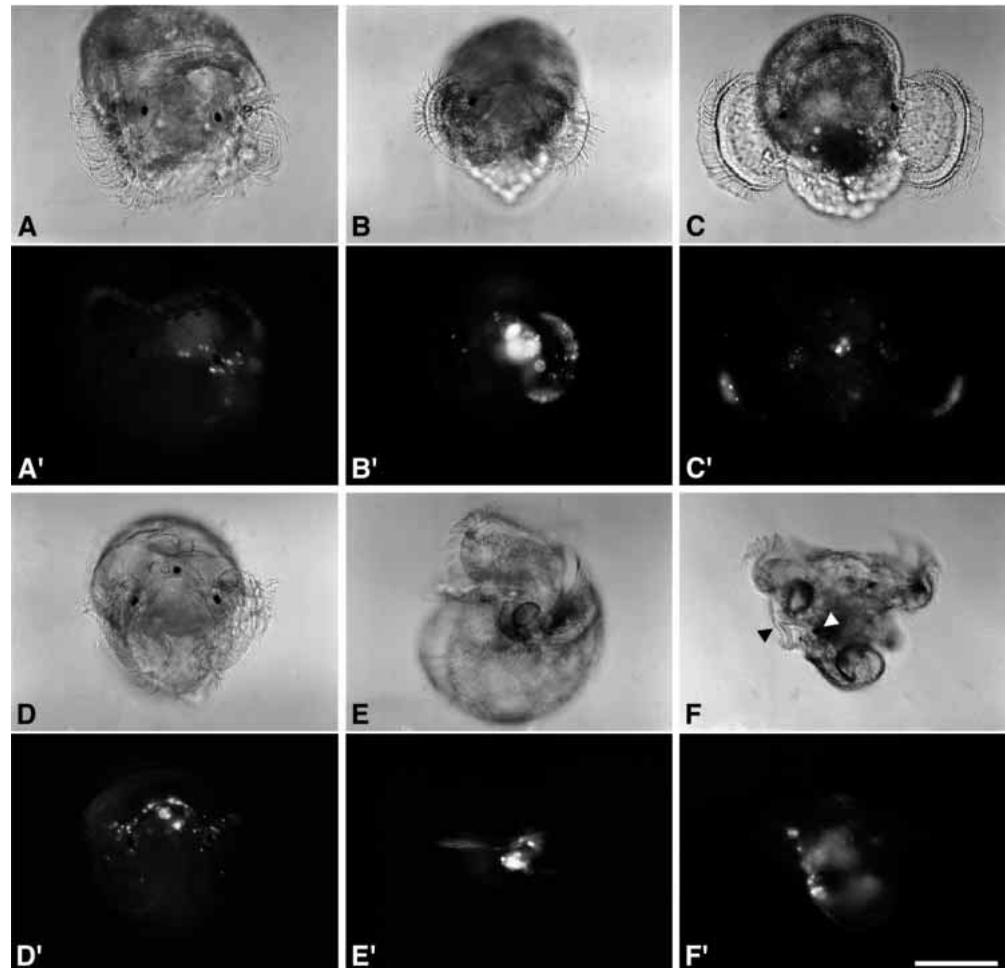

Fig. 8. Head development following the transplantation of micromeres to different regions in the embryo. Light micrograph images of the resulting veligers (A-F) and corresponding fluorescence micrographs (A′-F′). (A,A′) Following the transplantation of the 1b micromere to the position of the 1a micromere, the descendants of the transplanted cell are in the left velar lobe and the central head region (apical plate). The left eye has developed from the transplanted 1b micromere. (B,B′) Following the transplantation of the 1d micromere to the position of 1a, the descendants of the transplanted 1d micromere contribute to the apical plate and left velar lobe, but they rarely develop the left eye. (C,C′) Following the transplantation of the 1a micromere to the position of 1b, the fluorescent descendants of 1a are symmetrically distributed in the apical plate and in both velar lobes. The resulting veliger larva usually develops only the left and right eyes; the transplanted 1a micromere does not form an ectopic eye. (D,D′) Following the transplantation of the 1b micromere to the position of 1d, the transplanted cell often formed an ectopic third eye in the apical plate region or adjacent to the endogenous left eye. The descendants of the transplanted 1b micromere lie in the apical plate and in the dorsal regions of both velar lobes. (E,E′) Following the transplantation of the 2c micromere to the position of 1a, the left eye is absent and an ectopic mass of birefringent material forms behind the left velar lobe. The fluorescent descendants of the transplanted 2c micromere are behind the left velar lobe, surrounding the ectopic birefringent mass. (F,F′) In this example of the transplantation of the 1a micromere to the position of 3c the descendants of the transplanted cell are in the right side of the embryo, and an ectopic band of velar cilia (black arrowhead) and an ectopic eye (white arrowhead) have formed between the host shell and velum. Bar, 100 μm.
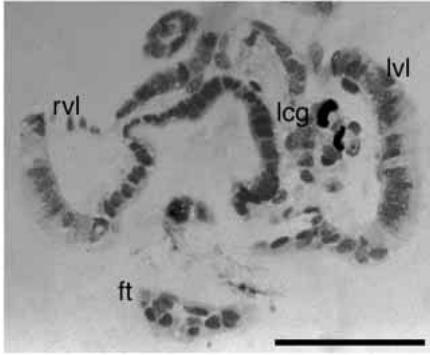
Fig. 9. Section through the head of a larva resulting from the transplantation of 1b to the position of 1d. (A) This section passes through the left and right velar lobes (lvl, rvl) and the foot (ft) of the larva. Two eyes are present in the left velar lobe; one eye was made by the host tissue (derivatives of 1a) and the ectopic eye was made by the transplanted 1b micromere. One eye appears to be small due to the level of the section. Both eyes are cup shaped, have a lens, and are associated with the cells of the left cerebral ganglion (lcg). Bar, 100 μm.

at second cleavage. To directly test whether the polar lobe plays a role in restricting the potential of the 1d micromere, the polar lobe was removed at first cleavage and the eye-forming potential of the resulting '1d' micromere was examined by transplanting it to the position of 1a. A labeling technique was first used to demonstrate that the donor micromere was indeed the '1d' micromere (Fig. 10). At the two-cell stage, the most clockwise half of the CD blastomere when viewed from the animal pole will become the D blastomere. When it was clear which cell was inheriting the polar lobe at first cleavage (CD), the prospective D cell was labeled with carbon particles. The particles always resided on the D blastomere at the four-cell stage, indicating that this technique can be used to label reliably the prospective D blastomere just after first cleavage (Fig. 10A). This labeling technique was used in combination with polar lobe removal to create and identify a 'D' blastomere (Fig. 10B). When the polar lobe was removed at first cleavage, the carbon particles always resided on one of the two vegetal cross-furrow blastomeres. The labeled blastomere is the 'D' blastomere, which would have inherited the polar lobe. In normal embryos, the 1d micromere is slightly smaller than the other first quartet micromeres; however in lobeless embryos all first quartet micromeres are the same size (not shown; Clement, 1952). In this study the '1d' micromere was identified as the first quartet micromere daughter of the labeled 'D' cell.

The '1d' micromere was transplanted to the position of 1a in a host embryo with a normal D quadrant. Under these conditions, the '1d' micromere developed the left eye in 48% of the cases (10/21). The results demonstrate that the '1d' micromere has the same ability to develop an eye as the 1a or 1b micromeres and that the eye-forming potential of 1d is normally restricted by the polar lobe.

Second quartet micromeres do not have the potential to develop an eye

In normal development, the second quartet micromeres contribute to the statocysts and shell (Cather, 1967; Clement, 1986a; McCain, 1992; Render, 1997). These organs are made partly of calcium carbonate and they can be identified by their

birefringence when viewed with polarized light. To test whether second quartet micromeres have the ability to develop an eye, the 2a, 2b or 2c micromeres were transplanted to the position of 1a. The descendants of the transplanted cell were usually in the area expected from the fate map of the 1a micromere (apical plate and left velar lobe), but they were also found to a greater extent in the ectodermal tissue behind the left velar lobe, including the mantle edge which secretes shell material (Fig. 8E,E′). The transplanted second quartet micromeres never developed an eye (35 cases total) and the left velar lobe was often misshapen in the regions containing the descendants of the second quartet cells. Small birefringent masses commonly formed from the descendants of the transplanted cell within or behind the left velar lobe (29%; 10/35).

Eyes may develop in regions other than the head

To test whether an eye can form in an area other than the normal head region, the 1a micromere was transplanted to the position of 3c. The 3c micromere is very close to the inducing D macromere and relatively far from the first quartet micromeres. This micromere normally contributes to the right half of the foot, the right statocyst, the dorsal mantle edge, and the posterior region of the right velar lobe (Render, 1997). In most cases the larvae that form following this transplantation are similar to the larvae that develop following the removal of the 3c micromere (Clement, 1986b). The right half of the foot was absent or reduced in size; there was no right statocyst; and the shell was often small and malformed, or there was only a large internal birefringent mass. There were often defects in the right velar lobe, sometimes including the absence of the right eye. Because of the greater extent of larval defects and because of problems in identifying individual descendants of the labeled micromere (described above), it was difficult to determine whether the transplanted 1a micromere made head structures unless ectopic structures had developed far from the host structures in a region containing the fluorescent label, or if there were supernumerary
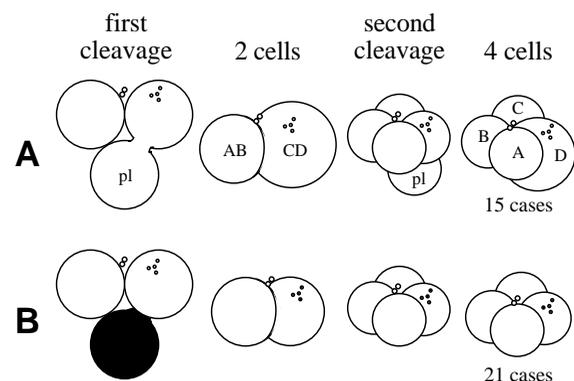

Fig. 10. Marking experiments to demonstrate the identity of the 'D' quadrant in the absence of the polar lobe. (A) As the polar lobe (pl) was being resorbed into one cell (CD) at first cleavage, the prospective D blastomere was marked with carbon particles. The marks always reside on the D blastomere at the four-cell stage. (B) The prospective D blastomere was marked at first cleavage and the polar lobe was removed immediately (shaded). At the four-cell stage the marks always lie on one vegetal cross-furrow blastomere (the 'D' blastomere).

structures which were associated with the fluorescent label. The descendants of the transplanted cell were most often in the right ventral region of the larva (Fig. 8F,F′), where many descendants of the 3c micromere normally exist. In this region, an ectopic eye had developed in 19% of the cases (4/21).

DISCUSSION

In the embryos of the gastropod *Ilyanassa obsoleta*, the development of the micromeres of the first, second and third quartets to form specialized ectodermal structures requires an inductive signal from the D macromere (Clement, 1962). The eyes normally develop from descendants of two of the first quartet micromeres, 1a and 1c (Render, 1991), and the removal of these two cells at the eight-cell stage always results in larvae that lack eyes (Clement, 1967). Because the removal of these micromeres results in such specific and consistent defects, which correspond to their normal fate, it has been suggested that these two cells are the only cells with the potential to develop eyes. However, studies with half and quarter embryos isolated at the two- or four-cell stage demonstrate that regulative eye development may take place in the absence of the normal eye precursor cells, indicating that other cells in the embryo have the potential to develop eyes (Clement, 1956; McCain and Cather, 1989). In this study, blastomere deletion and micromere transplantation experiments were used to determine which micromeres have this eye-forming potential and to examine how eye development is restricted only to the descendants of 1a and 1c during normal development.

The first quartet micromeres are the only cells with the potential to develop the eyes

Three experiments were done to test whether second or third quartet micromeres have the ability to regulate to develop the eyes. First, all four first quartet micromeres were removed at the eight-cell stage. This experiment has also been done in the polar lobe-forming embryos of *Bithynia* (van Dam and Verdonk, 1982). In both species, the eyes never develop in the absence of the cells of the first quartet. To further test the potential of the second and third quartets of micromeres, half embryos were isolated at the four-cell stage and the two first quartet micromeres were removed at the equivalent of the eight-cell stage. The remaining cells in the embryo were not able to form eyes even under these conditions, which often result in regulation (Clement, 1956; McCain and Cather, 1989). In a third test, second quartet micromeres were transplanted to the position of 1a. Eyes never developed from the descendants of the transplanted cell; instead these cells produced small masses of birefringent material, suggesting that these cells may be specialized to produce structures containing calcium carbonate, including the shell and statocysts. Overall, the results strongly support the idea that the potential for eye development is restricted to the micromeres of the first quartet. However, it remains possible that the second and third quartet micromeres may develop an eye under greater regulative conditions.

The velum is normally composed of descendants of the first, second and third quartets of micromeres (Render, 1991, 1997). However, when the four first quartet micromeres were removed in this study, the resulting larvae never developed velar lobes with long velar cilia. This was also the case when the first quartet micromeres were removed in half embryos. These results suggests that, under normal conditions, the descendants of the

first quartet micromeres may recruit descendants of the second and third quartet micromeres in the formation of the velum.

Each first quartet micromere has the potential to develop an eye

Two sets of blastomere deletion experiments were done to test the eye-forming potential of each first quartet micromere. In one test, three first quartet micromeres were removed such that only one first quartet micromere remained to contribute to the head. Under these conditions, the remaining 1a, 1b and 1c micromeres each formed the structures that they would normally form in the veliger larva. For the most part, the results are consistent with the fate maps of these cells (Render, 1991) and they are complementary to the results of their removal (Clement, 1967). There were very few cases of regulative eye development under these conditions. However, some embryos developed parts of the velum which are not a normal fate of the remaining micromere. This could be explained by regulative development of the descendants of the remaining first quartet micromere or the additional velar tissue may have developed from descendants of the second and third quartet micromeres. Following the removal of the 1a, 1b and 1c micromeres, the remaining 1d micromere never regulated to develop an eye. Moreover, 1d did not develop any velar cilia or recognizable velar tissue, which is its normal fate (Render, 1991), suggesting that 1d is different from the other first quartet micromeres.

A second and more informative test of the eye-forming potential of the first quartet micromeres takes advantage of the regulative properties of half embryos. Unlike embryos from which only a few micromeres are removed, regulative eye development commonly occurs following the removal of one half or three quarters of the embryo at the two- or four-cell stage, as long as the inductive D blastomere remains (Clement, 1956; McCain and Cather, 1989). When tested individually in half embryos, 1a, 1b and 1c each developed an eye at nearly the same frequency (AD−1d, BD−1d, CD−1d). Therefore, the 1b micromere has the same ability to develop an eye compared to the normal eye precursor cells. Further evidence in support of this comes from the results of transplanting 1b to the position of 1a. In the position of this eye precursor cell, 1b developed an eye at nearly the same frequency as the homotopic control. The developmental abilities of the 1d micromere in half embryos (AD−1a, BD−1b, CD−1c) were very different compared to what was found for 1d when it was the remaining first quartet micromere in an otherwise whole embryo (−1abc). In the half embryos, the 1d micromere did develop an eye; however, the frequency of eye development by 1d was significantly lower than that of the other first quartet micromeres.

The polar lobe restricts the potential of the 1d micromere

The blastomere deletion experiments described above demonstrate that the eye-forming potential of the 1d micromere is restricted compared to the potential of the other first quartet micromeres. To determine whether this difference is due to the close position of 1d relative to the D macromere, the 1d micromere was transplanted to the position of the 1a eye precursor cell. The transplanted 1d micromere rarely developed an eye in this position, suggesting that 1d may be different from the other first quartet micromeres by virtue of its lineage rather than by its position in the embryo. To directly

test this possibility, the polar lobe was removed at first cleavage and the resulting '1d' micromere was transplanted to the position of the 1a micromere. Under these conditions the '1d' micromere developed an eye at a similar frequency compared to the other first quartet micromeres. This strongly suggests that 1d normally does not develop an eye because its potential is restricted by its polar lobe lineage.

Position with respect to the D macromere is important for eye development

The 1b micromere also does not contribute to an eye in normal development, even though some of its descendants are close to the regions where the left and right eyes form (Render, 1991). However, unlike the 1d micromere, 1b has the same potential to develop an eye compared to the normal eye precursors. When 1a was transplanted to the 1b position, it rarely developed an eye, suggesting that the 1b position is not conducive for eye development. When 1a or 1b was transplanted to the position of 1d, which is very close to the D macromere, an ectopic third eye often formed, suggesting that close proximity of the micromere to the inducing cell is important for eye development. An eye also developed from the 1a micromere when it was transplanted to the position of 3c, which is relatively far from the other first quartet micromeres, but close to the D macromere. Thus, the proximity of the transplanted cell to the D macromere is also more important for eye development than its position relative to the other first quartet micromeres.

Model

Based on the results of experiments presented here and elsewhere, Fig. 11 summarizes a model for some of the early aspects of the specification of the eyes in the embryos of *Ilyanassa*. The potential to develop an eye is first restricted to the first quartet micromeres when they form at the eight-cell stage. There are several ways to explain how this potential might be mediated. In the embryos of the nematode *Caenorhabditus elegans*, maternal transcription factors are segregated to specific cells during cleavage and these factors are involved in the specification of these cells (reviewed by Bowerman, 1998). The eye-forming potential of first quartet micromeres in the embryos of *Ilyanassa* may be a result of a similar mechanism where positive-acting transcription factors are segregated only to the first quartet micromeres, or negative factors are segregated out of these cells. Another possibility is that eye-forming potential is mediated by the activation or inactivation of globally distributed factors within the first quartet micromeres. Such a mechanism has been hypothesized to explain the autonomous specification of the micromeres in sea urchin embryos (Davidson, 1989). Eye-forming potential could also be a result of inductive signals from the sister macromeres, for example 1A could induce 1a to have the potential to develop an eye. If such an inductive interaction takes place, it would occur soon after the formation of the first quartet micromeres because the second quartet micromeres never develop eyes when they are placed in the position of 1a at the eight-cell stage. Such inductive signaling occurs in four-cell stage embryos of *C. elegans*, where the P2 blastomere induces its sister cell EMS to develop the endoderm (Goldstein, 1992, 1993).

At the same time as the first quartet micromeres gain their eye-forming potential, the potential of 1d is suppressed by its polar lobe lineage. The polar lobe has also been shown to have

an inhibitory influence over the fate of the 1d micromere in the embryos of the polychaete annelid *Sabellaria cementarium* (Render, 1983). It is possible that the polar lobe contains negative factors that are ultimately inherited by 1d as it forms at the third cleavage. Alternatively, the polar lobe may cause positive factors to be segregated out of the 1d micromere. Besides restricting the eye-forming potential of 1d, the polar lobe also causes the 1d micromere to be smaller and have a unique cleavage pattern compared to the other first quartet micromeres (Crampton, 1896; Clement, 1956). Thus, it is possible that the polar lobe restricts the eye-forming potential of the 1d micromere by affecting its size and division. Whatever the nature of the inhibitory influence over eye development by 1d, the suppression by the polar lobe is not complete because 1d has the ability to develop an eye in half and quarter embryos.

In addition to suppressing the potential of the 1d micromere in *Ilyanassa*, the polar lobe gives the D macromere the ability to induce the micromeres. The molecular nature of the inductive interaction is not yet known. However, as a consequence of the induction, the potential of the first quartet micromeres to develop an eye is further restricted. It is possible that 1a and 1c contact the D macromere at the time of the induction whereas 1b does not. Alternatively, 1a and 1c may have a greater area of contact with the D macromere compared to 1b. As a consequence of being closer to the D macromere, or having more contact with it, 1a and 1c may receive a sufficient amount of signal to proceed further down the pathway toward eye development. The 1b micromere may not receive the appropriate amount of inductive signal and go on to develop other head structures. This may be similar to what occurs in the four-cell stage *C. elegans* embryo, where the ABa and ABp blastomeres are initially equivalent in their potential, but because only ABp contacts the inducing P2 blastomere, it responds by producing intestinal valve cells whereas ABa does not (reviewed by Bowerman, 1998).

Model's explanation of experimental embryology

When only a few micromeres are removed from the cleavage-stage embryo the remaining cells do not compensate for the loss, and the defects in the resulting larvae correspond to the fate maps of the cells that were removed (i.e. there is little regulation;
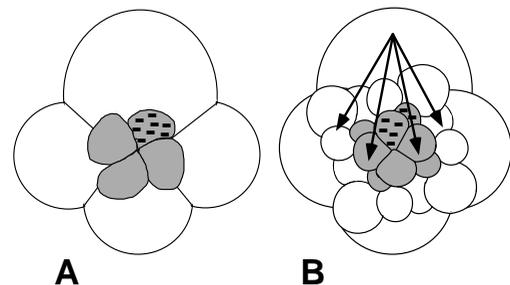

A   **B**

Fig. 11. A model depicting some of the early events in the specification of the first quartet micromeres to make the eyes. (A) The potential to develop an eye (shaded) is restricted to the first quartet micromeres as they form at the eight-cell stage. At the same time, the eye-forming potential of 1d is suppressed by its polar lobe lineage (−). (B) Between the 24- and 28-cell stage the D macromere signals all micromeres (arrows). The competent first quartet micromeres that are closest to the D macromere (1a and 1c derivatives) are induced to develop an eye, whereas the furthest first quartet micromere (1b) is not.

Cather, 1967; Clement, 1967, 1986a,b; McCain, 1992). Removal of large parts of the embryo at earlier stages often results in greater regulative development, but this regulation only occurs if the inducing D macromere is present (Clement, 1956; McCain and Cather, 1989). The difference in the degree of regulation between embryos from which only a few micromeres have been removed and half or quarter embryos could be due to micromere position relative to the D macromere. In embryos from which a few micromeres are removed, there may be minimal changes in cell position such that the remaining micromeres receive the same inductive signals as they normally would and develop according to their usual fate. Thus, the model presented here for the specification of the first quartet micromeres in the embryos of *Ilyanassa* can explain the lack of regulative eye development following the removal of either 1a or 1c (Clement, 1967) only if the 1b micromere does not move into these positions. Following micromere removal in the gastropods *Lymnaea* and *Ilyanassa*, the site formerly occupied by a first quartet micromere is filled in by a second quartet micromere rather than by another first quartet micromere (Arnolds et al., 1983; H.C.S., unpublished observations). Isolating half embryos involves removing two cells at the four-cell stage. With only half of the cells present throughout cleavage, there must be many changes in cell positions and cell contacts compared to normal embryos. The regulative development of half embryos may be a result of these changes in micromere position relative to the D macromere. As cells come to lie in different positions compared to normal, they may become induced to contribute to different structures. In BD half embryos, the 1b micromere may be closer to the D macromere compared to normal, and in this position it may receive a greater amount of signal than it would normally and as a consequence it may go on to regulate to develop an eye. It is also possible that the 1b micromere receives a greater concentration of inductive signal in BD half embryos by different means. During the inductive event, all cells in the embryo may act as a sink for the inductive molecule. When large parts of the embryo are removed, as in the half embryos, there are fewer cells to sequester the inductive molecule, and as a consequence, the 1b micromere may receive more inductive signal than it normally would and respond by developing an eye. Similarly, regulative eye development by the 1d micromere in half and quarter embryos may take place if the inductive signal becomes concentrated enough to overcome the inhibitory influence of the polar lobe.

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