

Reversal of developmental competence in inverted amphibian eggs

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SUMMARY

Inverted amphibian embryos were employed for an analysis of pattern formation in early embryogenesis. Axolotl (*Ambystoma*) and *Xenopus* eggs were inverted prior to the first cleavage division and permitted to develop upside down to the early gastrulation stage. In both cases the cleavage patterns of the animal and vegetal hemispheres were reversed. By gastrulation, however, developmental arrest began, and no inverted embryos developed beyond neurulation. The state of competence of the animal and vegetal hemisphere cells of inverted embryos was examined in a series of tissue transplantations, usually into genetically marked (albino) hosts. In all cases the developmental competence of the original animal and vegetal hemisphere cells of inverted embryos had been reversed. For example, the egg's original vegetal hemisphere developed into various neural structures.

Those observations should eventually be useful in formulating models to account for the manner in which various regions of the amphibian egg cytoplasm generate early embryonic patterns.

INTRODUCTION

Pattern formation in early amphibian embryogenesis proceeds through an ordered set of events. The first event (in monospermic anuran eggs) is sperm penetration, which plays a key role in establishing the plane of bilateral symmetry of the egg (Ancel & Vintemberger, 1948). Next, the cleavage furrow pattern is set up, probably in response to the position of the cleavage nuclei (Chung & Malacinski, 1982). The regional development of competence to respond to inductive interactions follows, and the inductive events driven by the primary embryonic organizer tissue occur after that. Finally, the developmental fate of various regions of the embryo becomes fixed (determined) and differentiation begins. Tissue-specific protein-synthesis patterns are expressed (Mohun, Tilly, Mohun & Slack, 1980) which presumably reflect the developmental program established by those cascading effects of symmetrization, competence and induction.

The possible role cytoplasmic localizations play in regulating those events of

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early embryogenesis has been analysed in either rotated (off axis) or inverted eggs for almost a century. Born (1885), Schultz (1894), Penners & Schleip (1928*a,b*), Motomura (1935), and Pasteels (1938, 1939) provided general descriptions of the development of inverted eggs. Alternations in pigmentation, cleavage pattern, or site of involution were observed. Conflicting data and the emergence from those studies of widely divergent interpretations have, however, obscured the potential impact of those earlier findings.

The investigation described in this report was designed to re-examine pattern formation in inverted eggs. Our previous studies revealed that early pattern formation in inverted axolotl and *Xenopus* eggs is completely reversed. The location of the first cleavage furrow is switched to the original vegetal hemisphere, and the small/large blastomere pattern of the animal/vegetal hemisphere is reversed (Malacinski & Chung, 1981; Chung & Malacinski, 1982). Inverted embryos, however, fail to develop beyond gastrulation and neurulation. Since substantial alterations in the pattern of early embryogenesis occurred, a specific question which pertains to one of the earlier steps of pattern formation was asked: Is the developmental competence of the original animal (prospective ectoderm/mesoderm) and vegetal (prospective endoderm) hemispheres reversed in inverted eggs which display a reversal of the animal/vegetal cleavage pattern? Regions of inverted embryos were grafted to control (normal orientation) embryos. The participation of the graft in various developmental pathways was traced through organogenesis. Since the pattern of differentiation of the graft usually resembled the surrounding host tissue, it was concluded that regional developmental competence was reversed in inverted eggs.

MATERIALS AND METHODS

Ambystoma and *Xenopus* eggs were collected, demembrated, and inverted as described previously (Malacinski & Chung, 1981). By maintaining the eggs in 10% Ficoll in individual wells, they remained inverted throughout the period from first cleavage to early gastrulation. The hemisphere of the egg which faced gravity is referred to as the 'G' hemisphere. In control (uninverted) eggs, 'G' corresponds to the vegetal (lightly pigmented) hemisphere. The hemisphere that opposed gravity is referred to as the 'OpG' hemisphere. Normally, that corresponds to the animal (darkly pigmented) hemisphere. In inverted eggs the hemispherical orientations are reversed.

The grafting procedures have been described previously (e.g., Malacinski, Brothers & Chung, 1977). Briefly, just prior to surgery, the vitelline membrane was removed from both donor and host embryos. Host tissue was removed from the appropriate location and discarded. The graft was inserted and permitted to heal. The SEM procedures followed earlier protocols (e.g., Youn, Keller & Malacinski, 1980).

RESULTS

Previous results from our laboratory have demonstrated that when axolotl (*Ambystoma mexicanum*) and *Xenopus* eggs are inverted prior to the first cleavage division, their animal/vegetal hemisphere cleavage patterns reverse completely (Fig. 1 and Malacinski & Chung, 1981; Chung & Malacinski, 1982).

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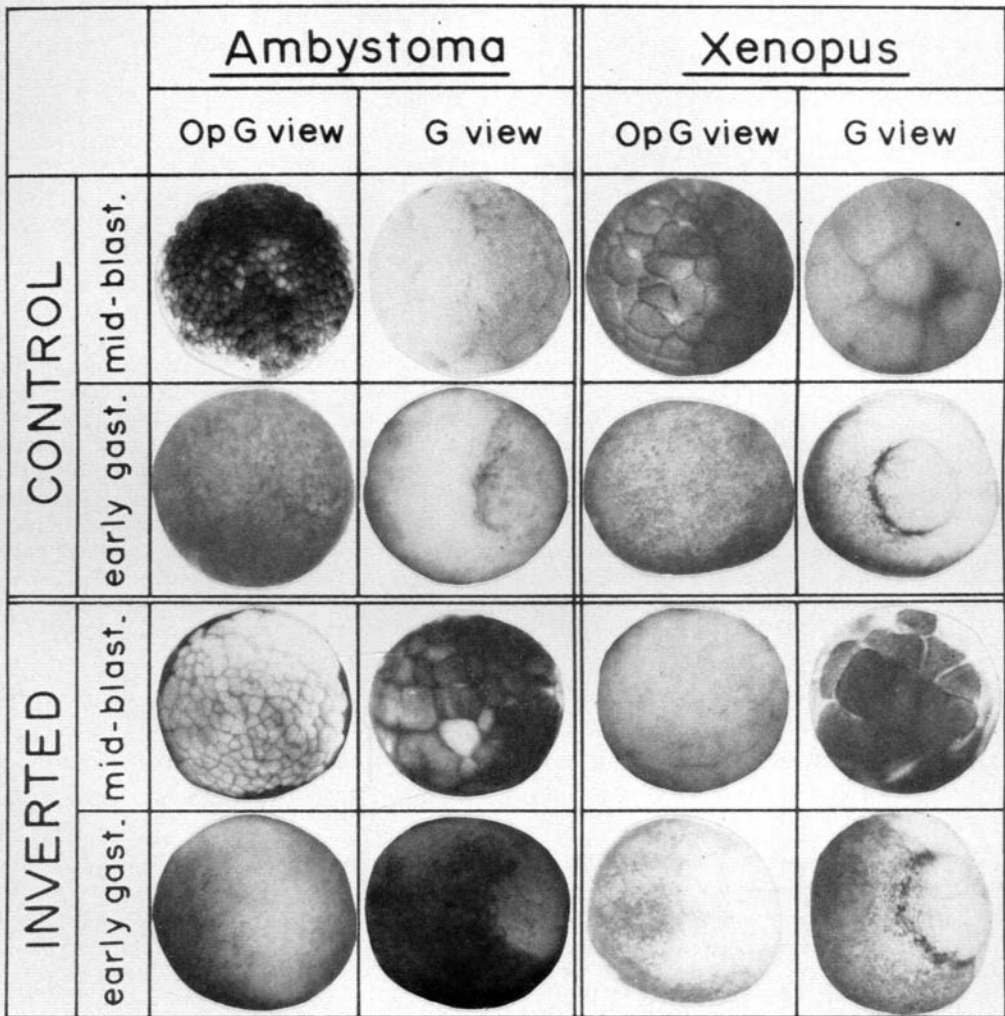


Fig. 1. Cleavage patterns displayed by control (normal orientation) and inverted axolotl (*Ambystoma mexicanum*) and *Xenopus* embryos. Fertile eggs were inverted prior to first cleavage, according to previously published methods (Malacinski & Chung, 1981). The hemisphere which opposed gravity (OpG side) always displayed smaller sized blastomeres than the hemisphere which faced gravity (G side).

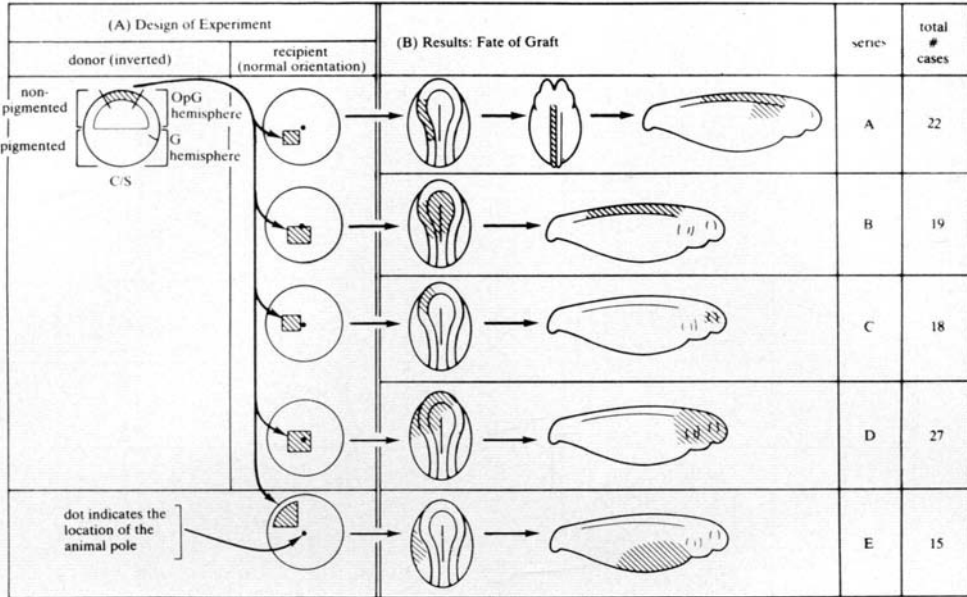
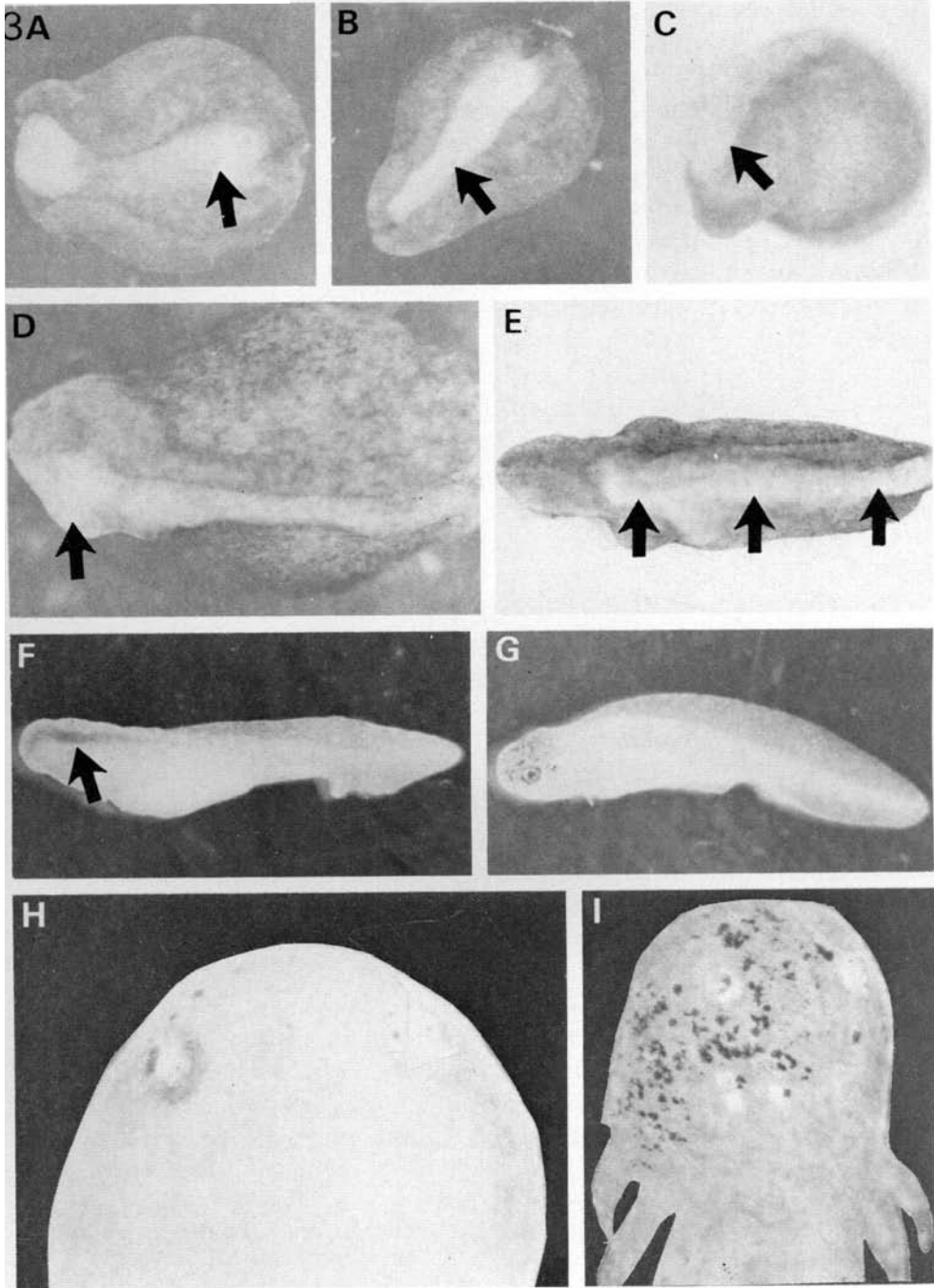


Fig. 2. (A) Design of the grafting experiments that tested the competence of the original vegetal hemisphere of inverted embryos. Eggs were inverted prior to first cleavage and permitted to develop in the inverted state to early gastrulation. The 'prospective neural ectoderm' was then grafted in various places on the animal hemisphere of normal (uninverted) recipient embryos. The recipients are oriented in the diagram with their dorsal sides facing the bottom of the illustration. The graft actually replaced the normal host tissue, which was discarded. Because of the pigmentation differences between donor and host tissues the fate of the graft could be easily followed. (B) Typical results. Regardless of where the donor tissue was placed on the animal hemisphere of a normal host it participated in the normal ectoderm/mesoderm differentiation pathways specified by the surrounding host tissue.

Although the cleavage pattern is reversed, development beyond the gastrulation stage invariably ceases prior to the onset of primary embryonic induction (i.e., neurulation). Several hundred inverted eggs have been carefully examined and none has been observed to complete neurulation. Although the dorsal lip had

Fig. 3. Photographs of axolotl embryos which carried the grafts diagrammed in Fig. 2. The lightly pigmented graft (arrows) could easily be recognized at early stages in the darkly pigmented neural ectoderm of the host: (A) Graft on one side of the neural plate. (B) Graft in the entire neural plate region. (C) Graft in the neural fold region. The appearance of typical grafted regions at later stages is shown for series A in (D) and for series B in (E). An albino host onto which part of the neural plate (series B) was grafted is shown in (F). When the optic vesicle primordium and surrounding tissue was grafted on an albino host (series C) a pigmented eye and a pigmented set of neural crest cells developed (G). An early larval stage albino recipient containing an optic primordium graft (series C) is displayed in (H). An early albino larva which exhibits pigmented neural crest cells as well as a pigmented eye from a series C graft is shown in (I).



often formed in a seemingly normal fashion, involution was usually incomplete and embryogenesis failed to proceed further (Malacinski & Chung, 1982).

To ascertain the extent to which competence of various regions of an inverted embryo had been altered, the series of grafts described in Figs 2, 7 and 9 were performed. In the first set (Fig. 2), the region of the inverted embryo (early gastrula) that resembled prospective neural ectoderm was grafted into place on a control (normal orientation) embryo. That procedure provides a test of the developmental competency of the donor tissue. By employing pigment markers the developmental fate of the graft was easily followed. Axolotl embryos were employed most extensively since they provide several advantageous features: (1) their comparatively large size facilitates the grafting of relatively small and well-characterized areas of the embryo; (2) two types of genetically pigmentless eggs (white, *d/d* and albino, *a/a*) are available for use as recipients of genetically darkly pigmented donor tissue; and (3) their entire cleavage pattern, beginning with the formation of the first furrow on the original vegetal hemisphere, is reversed in inverted eggs (Malacinski & Chung, 1981). *Xenopus* eggs were also employed to discover whether the competence of various regions was altered in a manner similar to axolotl embryos. Genetically albino *Xenopus* eggs were occasionally used as recipients of darkly pigmented presumptive ectoderm tissue.

The diagram in Fig. 2 indicates the area of the original vegetal hemisphere of an inverted embryo that was grafted into various locations on normal recipients. As indicated in Fig. 1, the OpG (original vegetal) hemisphere of inverted eggs is lightly pigmented. Yet, compared to the darkly pigmented G (original animal) hemisphere, it contains the smaller sized blastomeres. When grafted onto normal recipient embryos in the darkly pigmented hemisphere the light patch of grafted tissue was easily followed through early organogenesis (e.g., Fig. 3 A–C). Virtually all of the approximately 100 grafts which were performed healed properly and developed normally. Each case is therefore included in the data given in Fig. 2. The complete range of developmental competencies that could be analysed with pigment markers were observed. A neural tube (Fig. 3 D–F), eye (Fig. 3 G), pigmented cells of presumed neural crest origin (Fig. 3 H, I) and large regions of the head (Fig. 4 A, B) were all detected among one or another of the recipient embryos. Even ciliated epidermal cells differentiated from the grafted cells (Fig. 4 C, D). Similar experiments were performed with inverted *Xenopus* embryos. Fig. 5 contains photographs which display several of the

Fig. 4. In series D (Fig. 2), prospective axolotl head parts (arrows) were grafted onto wild type hosts. Late tail-bud recipient embryo displays grafted head ectoderm is shown in (A). At a later stage the graft was also visible (B). A scanning electron micrograph at 72 \times (C) reveals that the grafted region (enclosed by dotted lines) differentiated cilia. The higher magnification view (720 \times) of the area enclosed in the box displays well-developed cilia (D). They are virtually identical to the cilia observed on the flank epidermis of an ungrafted (control) region of the same embryo (E). Magnification bars for (C) = 0.2 mm; (D) and (E) = 20 μ m.

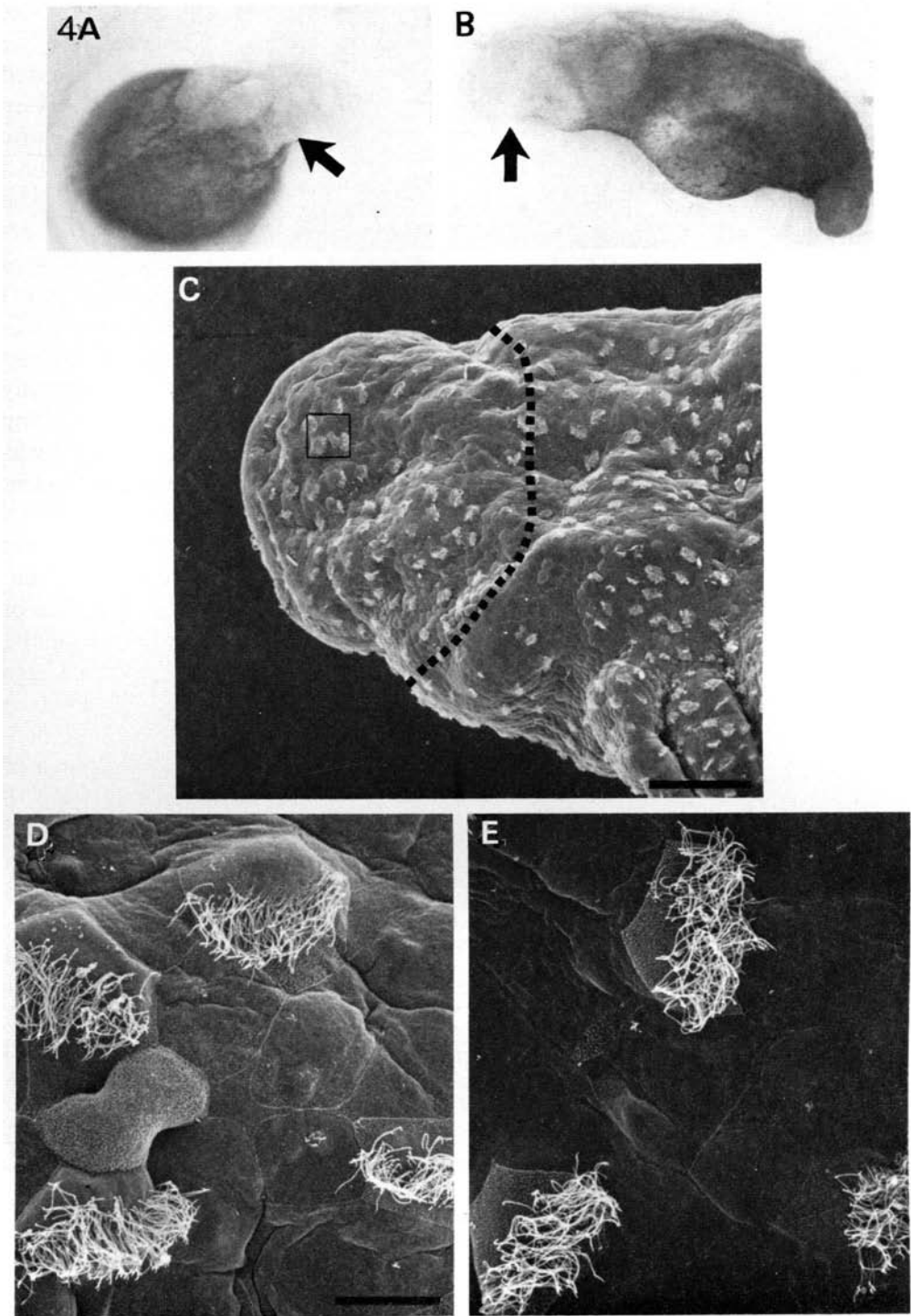


Fig. 4

grafts described in Fig. 2. The whole range of ectodermal differentiations, including neural tube (Fig. 5C), epidermis (Fig. 5D), eye (Fig. 5E), and pigment cells (Fig. 5F) was observed. As was the case for the axolotl grafts, ciliated epidermal cells were observed to differentiate in the grafted tissue (Fig. 6).

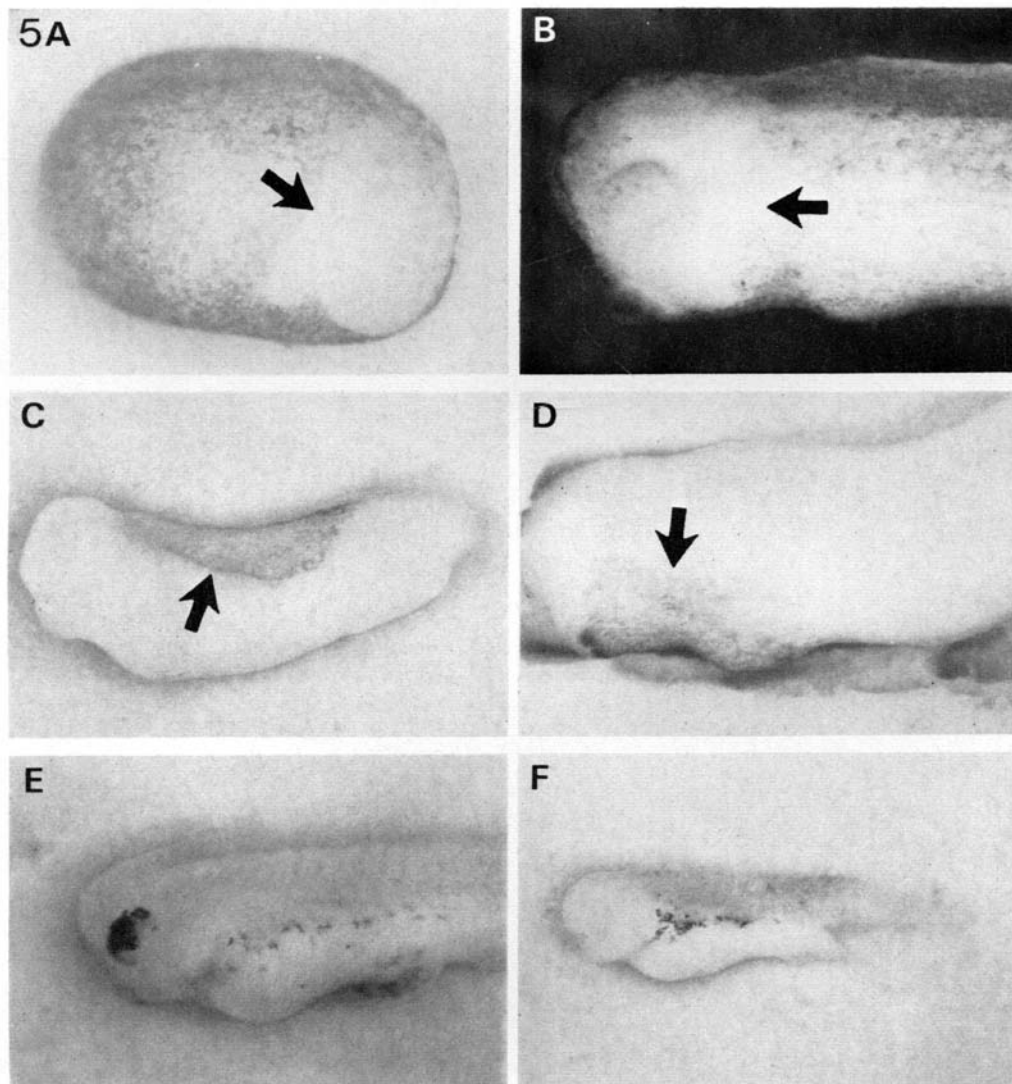


Fig. 5. Photographs of grafted *Xenopus* embryos. The lightly pigmented graft (arrow) could be easily recognized on a wild-type host. The grafts in (A) and (B) are from series D (Fig. 2). Grafts of wild-type tissue (arrows) onto albino hosts are even more distinct, and are shown in (C)—series B; and (D)—series E. A series B graft is shown in (E), and a series C graft, which displays distinct pigmented cells, is shown in (F).

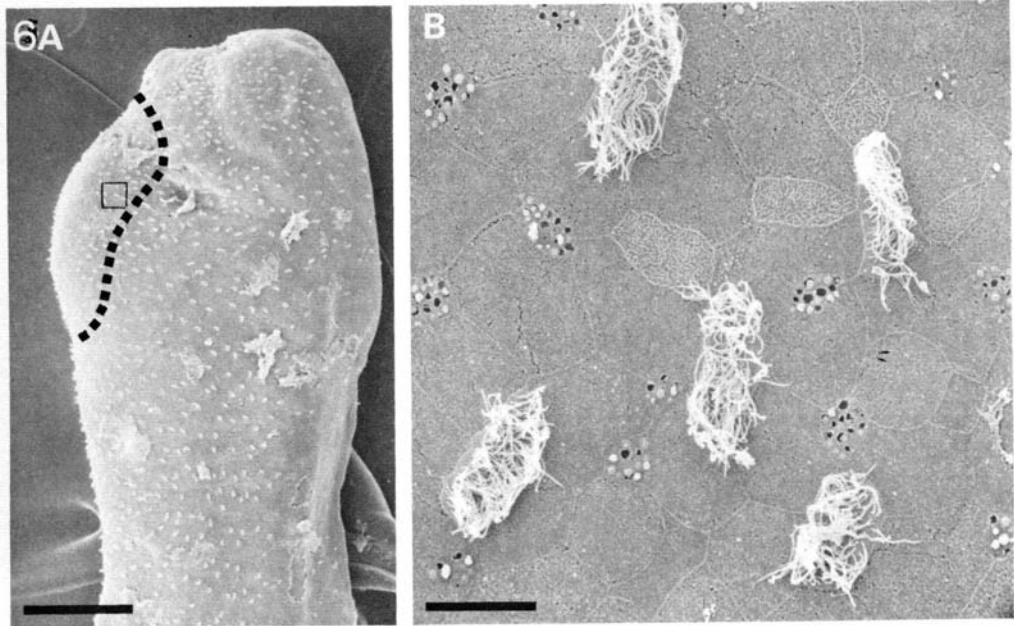


Fig. 6. Scanning electron micrograph (A) reveals that the grafted *Xenopus* region (enclosed by dots) differentiated cilia ($36\times$). A higher magnification view ($700\times$) of the area enclosed in the box in (A) is shown in (B). Bar in (A) = 0.4 mm ; bar in (B) = $20\text{ }\mu\text{m}$.

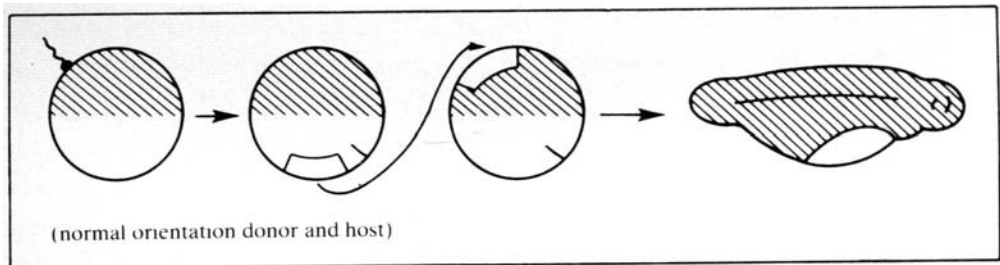


Fig. 7. Diagram of the control experiment which was designed to establish whether vegetal hemisphere (endodermal) cells would display a differentiation pattern (e.g., appearance of cilia) similar to the host ectodermal cells.

Clearly, the OpG (original vegetal) hemisphere displayed all of the developmental competencies usually associated with the animal hemisphere of control (normal orientation) embryos.

Reversal of developmental competence of the original vegetal hemisphere of inverted eggs can be contrasted with the ability of normal orientation vegetal hemisphere cells to participate in fate reversal. Vegetal hemisphere cells from control (normal orientation) embryos were grafted into the animal hemisphere

region of normal orientation recipient embryos (Fig. 7). Nine grafts were made and all healed properly. Each, however, failed to develop cilia although the surrounding cells of animal hemisphere origin clearly did (Fig. 8). It can be concluded, therefore, that the reversal of developmental competence discovered in the series of grafts illustrated in Fig. 2 is associated with the

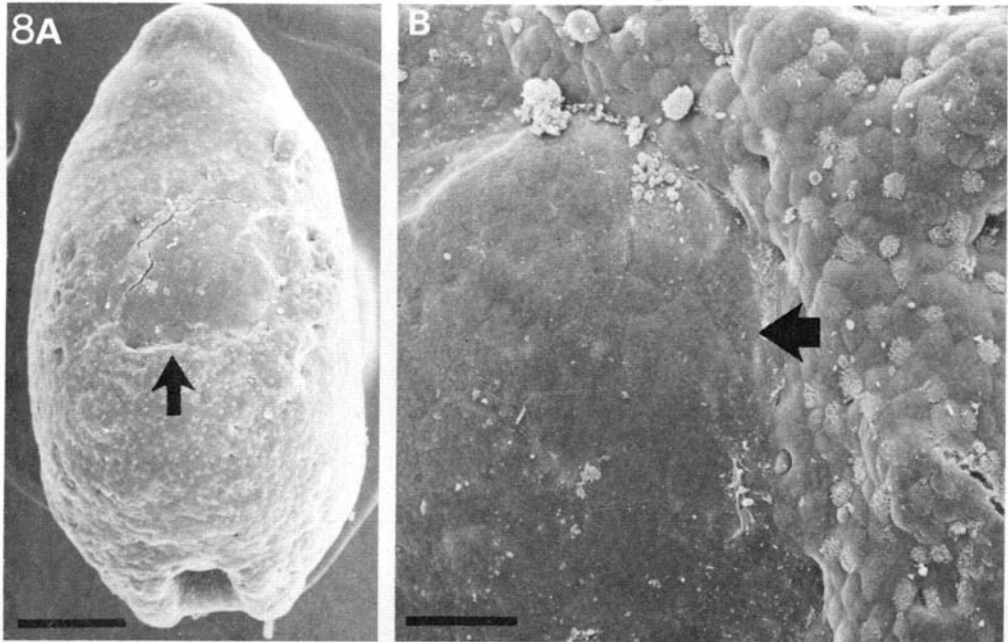


Fig. 8. Endodermal tissue (arrows) from a normal orientation (control) axolotl embryo was grafted to the animal hemisphere (prospective epidermis) region of a control embryo. The grafted area healed properly, but failed to develop cilia, in contrast to the cells in the surrounding area. (A) ventral view at 36 \times . Bar = 0.4 mm. (B) higher magnification (180 \times) view of the graft. Bar = 80 μ m.

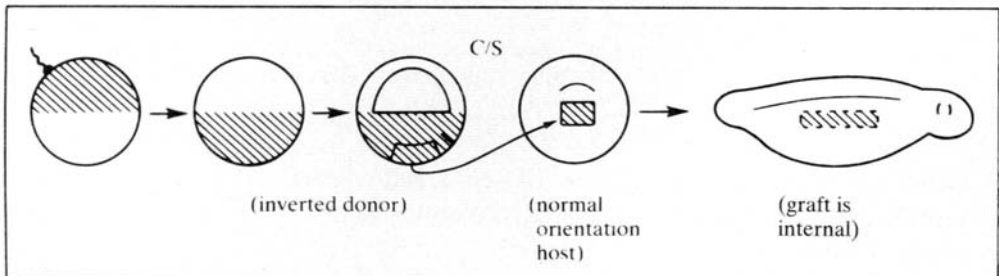


Fig. 9. Design of the grafting experiment for testing the competence of the original animal hemisphere of inverted embryos. The pigment which was localized in the G hemisphere served as a marker for the donor cells, which were grafted into the unpigmented endodermal region of a control (normal orientation) embryo.

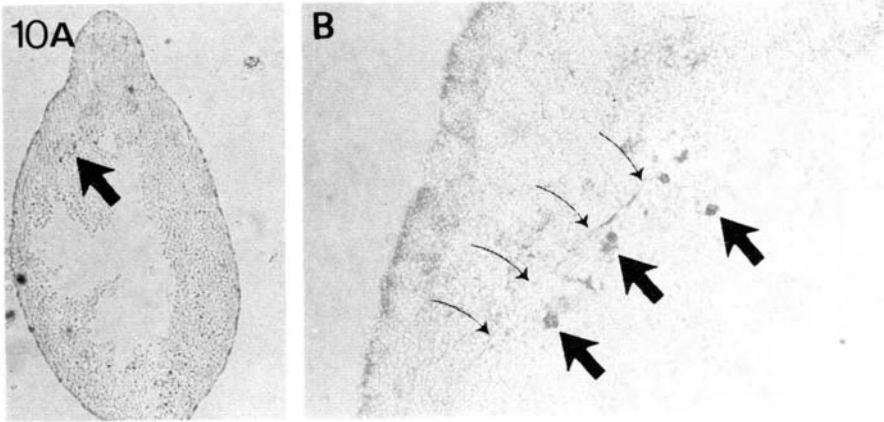


Fig. 10. Histological sections (unstained) of an axolotl host (normal orientation) embryo onto which a patch of G (original animal) hemisphere from an inverted early-gastrula-stage embryo was grafted (Fig. 9). Low-magnification view ($16\times$) is shown in (A). The arrow points to pigmented cells which mark the grafted tissue. Higher magnification view ($160\times$) is shown in (B). Large arrows indicate the melanin granules. Thin arrows reveal the line of separation between the lateral mesoderm and the endoderm.

inversion routine. Endodermal cells do not normally have the capacity to change their developmental competence after the early gastrula stage.

Whether G (original animal) hemisphere cells display a reversal of developmental competence was also examined. The manipulation illustrated in Fig. 9 was performed on eight sets of embryos. Original animal hemisphere cells are darkly pigmented so their fate could be conveniently followed. A patch of tissue was placed adjacent to the prospective ventral lip area of an early gastrula (normal orientation) host. Involution of the grafted tissue occurred. At the tail-bud stage the host embryo was fixed, embedded and sectioned (without staining). The pigmented cells could be clearly recognized (Fig. 10) as being associated with the developing gut. Reversal of the developmental competence of original animal hemisphere cells of inverted embryos had apparently occurred.

DISCUSSION

Of all the features of early embryogenesis (e.g., induction, differentiation, determination, etc.) that have been analysed in various types of experimental systems, the capacity of tissue to respond to stimuli (competence) has received the least attention. In the case of the amphibian embryo ectodermal derivatives appear to be more dependant upon inductive stimuli than endodermal derivatives. The classical viewpoint states that differentiation of several ectodermal derivatives cannot occur without prior stimulation by inducing factors

provided by the mesoderm (reviewed in Nakamura & Toivonen (1978)). More than just the inductive stimulus is, however, required. In addition, the responding tissue must be prepared to receive the inductive stimulus. The ability to respond to a specific inducing stimulus has been termed 'competence' (Waddington, 1932, 1936). Competence of specific areas of the amphibian embryo appears to develop progressively. At earlier (e.g., pre-gastrular) stages, the developmental competence of the prospective ectoderm is relatively diverse. By the end of neurulation it is, however, substantially diminished (reviewed by Holtfreter & Hamburger, 1955).

For the present studies the development of competence of the ectoderm was chosen as a model system. The differentiation patterns displayed by the ectoderm are highly diverse and more easily recognized than those of the endoderm or mesoderm. In addition, the differentiation of the ectoderm is generally considered to be more dependant on prior tissue interactions than are either of the other two germ layers.

The data generated by the operations illustrated in Fig. 2 demonstrate that the competence of the OpG hemisphere tissue of inverted eggs is reversed. That reversal was presaged by the reversal of the original animal/vegetal cleavage pattern (Fig. 1; Malacinski & Chung, 1981). In contrast to a previous report (Stanisstreet, Jumah & Kurais, 1980), the capacity to differentiate into ciliated cells was clearly reversed. Those workers, however, examined isolated blastomeres of inverted embryos whereas the present studies dealt with whole embryos. In addition to cilia pattern, the present studies reveal that the ability to form pigmented cells and optic vesicles was also reversed. The reason why inverted embryos do not develop beyond gastrulation, despite the reversal of the regional capacities for differentiation remains unknown. Perhaps the mechanics of gastrulation are defective in inverted embryos. The abnormal gastrulation displayed by inverted embryos could result from incomplete or distorted tissue interactions, even though the acquisition of developmental competence does indeed occur.

To develop models that explain early amphibian pattern formation, it is desirable to have an experimental system which responds to external perturbations. The amphibian egg system described in this report provides just that. Since competence reverses in inverted eggs, one feature of any model must be that morphogenetic information for competence is *not* anchored to the egg cortex, but rather is located in the internal egg cytoplasm. Preliminary studies in our laboratory (Radice, Neff & Malacinski, 1981; Chung & Malacinski, 1982) have revealed that upon inversion some of the major cytoplasmic components (e.g., yolk) shift from the original vegetal hemisphere to the G (original animal) hemisphere. Curiously, the shift is not complete and varies among species of egg. Axolotl eggs display a relatively complete shift, while *Xenopus* eggs exhibit a much less pronounced shift of internal cytoplasmic components. Future studies should extend those observations. More complete knowledge of which cytoplasmic

components shift in inverted eggs and where they are normally localized in control embryos may lead eventually to the establishment of the cause/effect relationships of early pattern formation.

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