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Cellular Interactions in Early C. elegans Embryos

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Summary

In normal development both the anterior and posterior blastomeres in a 2-cell C. elegans embryo produce some descendants that become muscles. We show that cellular interactions appear to be necessary in order for the anterior blastomere to produce these muscles. The anterior blastomere does not produce any muscle descendants after either the posterior blastomere or one of the daughters of the posterior blastomere is removed from the egg. Moreover, we demonstrate that a daughter of the anterior blastomere that normally does not produce muscles appears capable of generating muscles when interchanged with its sister, a cell that normally does produce muscles. Embryos develop normally after these blastomeres are interchanged, suggesting that cellular interactions play a major role in determining the fates of some cells in early embryogenesis.

Introduction

Cellular interactions have been shown to play a major role in determining cell fate in almost all organisms studied, and are important for at least some aspects of postembryonic development in C. elegans (Sulston and White, 1980; Kimble and White, 1981). However no evidence of cell–cell interactions has been found in early nematode embryos, which have remained a classic example of "mosaic" or "cell-autonomous" development (Boveri, 1910; zur Strassen, 1959; Laufer et al., 1980). Because the complete cell lineage of C. elegans has been established and shown to be essentially invariant, it is now possible to examine in detail the question of whether cell determination in nematode embryogenesis requires cell–cell interactions.

Much of the experimental work on C. elegans embryos beyond about the 28-cell stage has involved the use of a laser microbeam to ablate specific cells (Sulston et al., 1983). In many cases the ablated cells detach from the embryo, and probably can be considered to be removed completely. Adjacent cells tend to occupy the space left by the ablated cell, and thus can be placed in contact with new neighbors. In general, results from these studies are consistent with the cell-autonomous view of nematode development; cells do not appear to change their fate after neighboring cells are ablated (Sulston et al., 1983). In a series of experiments on more than 37 different embryonic cells, only two exceptions to this pattern were observed. Both of these cases involved regulation between two similar cells and occurred late in embryogenesis.

The large blastomeres in embryos before the 28-cell stage are difficult to kill with the laser microbeam, and ablated blastomeres maintain contact with neighboring cells. Hence experiments on the very early embryos have involved physically removing blastomeres by rupturing the egg (Laufer et al., 1980). The most detailed experimental studies on cell determination in the very early C. elegans embryo have concerned the intestinal cells and their precursors (Laufer et al., 1980). All intestinal cells are derived from a single early cell, the E blastomere, which generates only intestinal cells. Cellular interactions do not appear to be required for the E blastomere to generate intestinal cells; only the precursors to the E blastomere appear capable of generating intestinal cells when isolated from surrounding blastomeres. Moreover, the "potential" for forming intestine-specific granules appears to be segregated at each of the early divisions into the intestinal precursors (Laufer et al., 1980; Cowan and McIntosh, 1985), much as cytoplasmic particles are known to be segregated into the germ-line precursors (Strome and Wood, 1982).

The lineal origins of both the intestinal and germ-line cells can be traced back to single early embryonic blastomeres that generate clones of similar cells. However, none of the other tissues formed in C. elegans embryogenesis are generated as homogeneous clones from single ancestors. Instead, tissues generally are polyclonal in origin (Sulston et al., 1983). The complexity of the lineage can be illustrated by considering the origins of body-wall muscles. Eight blastomeres in the 28-cell stage embryo normally generate body-wall muscles. While three of these blastomeres produce homogeneous clones of body-wall muscles, only between 3%–50% of the progeny of the other blastomeres become muscles. Thus a question emerges of whether the developmental information enabling several blastomeres to generate the same cell type is a property segregated to each of these blastomeres, as appears to be the case for the intestinal lineage.

In this paper we examine how embryonic blastomeres become committed to making two types of cells that are formed from multiple, complex lineages: the pharyngeal muscles and body-wall muscles. We test whether different precursors are committed to making pharyngeal muscles by removing surrounding cells. We further examine whether regulation can occur between muscle and nonmuscle precursors by interchanging a blastomere that normally does not make pharyngeal muscles with another that does. Our results suggest that cell–cell interactions are important in the early embryo for generating at least some of the pharyngeal and body-wall muscles, and that the fates of certain blastomeres that normally do not make pharyngeal muscles can be altered such that they do.

Results

Background

The pharynx is a muscular organ used in feeding. It connects the mouth to the intestine and is separated from other body-tissues by a basement membrane. The phar-

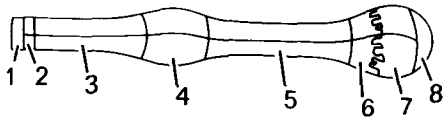

Figure 1. Diagram of Pharyngeal Muscles Viewed from the Lateral Surface of the Pharynx

The pharyngeal muscles are organized in eight sets, each set containing between one and six morphologically similar cells. One cell from each set is labeled (redrawn from Albertson and Thomson, 1976).

ynx consists of several cell types, including muscles, neurons, and glands. Pharyngeal muscles express pharyngeal-specific myosins, designated myosins C and D (Epstein et al., 1974; Waterston et al., 1982); these can be stained by antibodies specific for myosin C (see Figure 5c and Epstein et al., 1982). The pharyngeal muscles are arranged in an anterior–posterior series of eight sets of cells (Figure 1 and Albertson and Thomson, 1976). The pharyngeal muscles within each set are morphologically identical, and are arranged with a 3-fold rotational symmetry around a central lumen.

Both blastomeres in the 2-cell embryo produce pharyngeal muscles (Figure 2 and Sulston et al., 1983). The anterior blastomere, AB, produces most of the muscles in the anterior of the pharynx, and the posterior blastomere, P1, produces most of the posterior cells. Each of the middle sets of pharyngeal muscles, 3-5, include descendants of AB as well as P1 descendants. Thus the apparently identical muscles within some sets have very different lineal origins.

Although pharyngeal muscles are not generated clonally, the origins of all pharyngeal cells can be traced back to three AB and two P1 descendants in a 28-cell embryo (Figure 2 and Sulston et al., 1983). These AB and P1

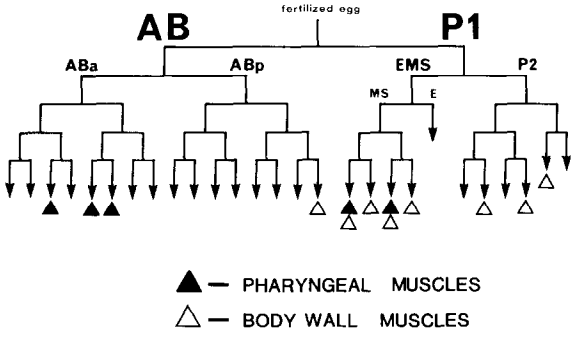

Figure 2. The Embryonic Cell Lineage

The diagram is an abbreviated version of the embryonic lineage to about the 28-cell stage, showing the origins of the precursors to the pharyngeal muscles (closed triangles) and body-wall muscles (open triangles). Each of the pharyngeal precursors divides several additional times to generate largely, but not solely, pharyngeal cells. Note that the AB daughter, ABa, makes all the AB-derived pharyngeal cells, and that the P1 daughter, EMS, makes the P1-derived pharyngeal cells. Also note that both daughters of P1 make body-wall muscles. The E blastomere is descended from P1, and continues division to yield a clone of intestinal cells (adapted from Sulston et al., 1983). Embryogenesis takes about 15 hr at 20°C, and most cell divisions are competed by about 6 hr. The lineage diagram shown represents approximately the first 2 hr of embryogenesis.

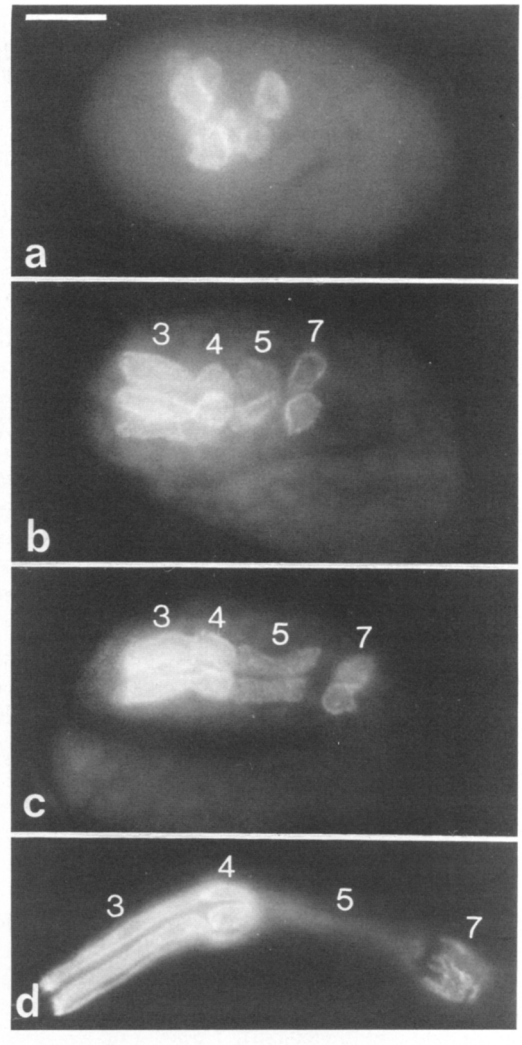

Figure 3. Immunofluorescence Staining of Pharyngeal Muscle Cells in Embryos and a Larva by the 3NB12 Antibody

The series shows 3NB12 staining of the pharynx in embryos about 6.5 hr (a), 7.5 hr (b), and 8 hr (c) in development, and in a larva hatching at about 13 hr (d). Each picture is taken through a top focal plane of the embryo, such that only a few of the muscle cells within each set are visible. Note that the cells within the m3 and m5 sets lengthen greatly during embryogenesis. Bar = 10 μm.

descendants produce predominantly, but not solely, pharyngeal cells. Tracing back further in the lineage, the AB-derived pharyngeal precursors are descended only from the AB daughter ABa, and the P1-derived pharyngeal precursors from the P1 daughter EMS. Thus when the AB and P1 blastomeres divide, one daughter of each will generate pharyngeal muscles while the other daughters, ABp and P2, will not.

The body-wall muscles differ from the pharyngeal muscles in several anatomical aspects (Albertson and Thomson, 1976; Zengel and Epstein, 1980) and express unique myosin genes (Epstein et al., 1974; Waterston et al., 1982; Miller et al., 1983). There are 81 body-wall muscles; 80 are descended from the P1 blastomere and one from AB (Figure 2). Both of the daughters of the P1 blastomere gen-

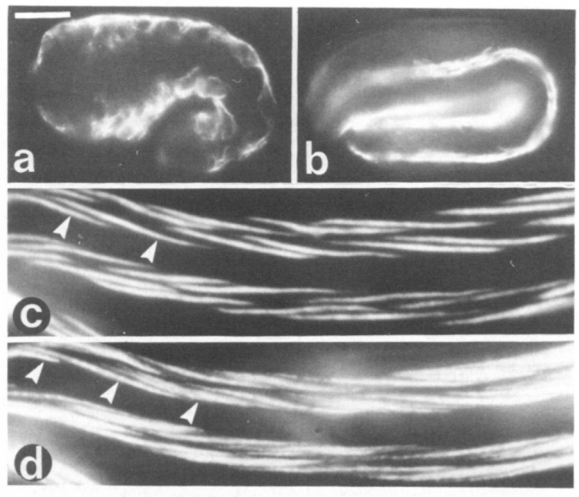

Figure 4. Immunofluorescence Staining of Body-Wall Muscles in Embryos and a Larva by the Anti-Myosin Antibody 5-6

(a, b) Embryos about 5 hr (a) and 9 hr (b) in development stained with 5-6. Staining initially appears throughout the cells, but later becomes localized in filaments (see Gossett et al., 1982). (c, d) A posterior body fragment from a newly hatched larva stained simultaneously with RITC-labeled 5-6 (c) and FITC-phalloidin (d) to visualize bands of thick and thin filaments, respectively. Each body-wall muscle contains two sarcomeres at hatching, and thus is expected to show two bands of thick filaments (arrowheads, c) and three bands of thin filaments (arrowheads, d; see Zengel and Epstein (1980) for further description of nematode muscle structure). The pattern of thick filament staining correctly maps onto the pattern of thin filament staining, indicating that 5-6 is staining all body-wall muscles present. (a, b) bar = 10 μm. (c, d) bar = 10 μm.

erate body-wall muscles, while only one AB daughter produces a body-wall muscle.

3NB12 Stains a Subset of AB- and P1-Derived Pharyngeal Muscle Cells

3NB12 is a monoclonal antibody that was isolated in a search for tissue-specific antibodies to C. elegans (Okamoto and Thomson, 1985). The antigen recognized by 3NB12 first appears in embryos about 6 hr in development, which is shortly after the divisions generating the pharyngeal cells are completed (Figure 3). By comparison with the ultrastructural anatomy of the fully formed pharynx in adults (Albertson and Thomson, 1976) and late embryos (J. Priess, unpublished results), and the positions of cells in the embryonic pharynx (Sulston et al., 1983), we conclude that 3NB12 recognizes an antigen present near the surfaces, and, within, all cells in the pharyngeal muscle sets 3, 4, 5, and 7. The embryonic lineage indicates that seven of the cells stained by 3NB12 are derived from the AB blastomere, and 14 are from P1. Staining was not observed for cells in sets 1, 2, 6, or 8 during any stage of embryogenesis. The pharyngeal muscle cells are recognized by the 3NB12 antibody several hours before these cells express myosin, as determined by staining with an antibody specific for pharyngeal myosin (see Figure 5). In addition to recognizing the pharyngeal muscle cells, late in embryogenesis 3BN12 also stains four cells in the tail; two of these are AB-derived intestinal muscles and the other two are small cells of undetermined identity.

An Anti-Myosin Antibody, 5-6, Stains Both AB- and P1-Derived Body-Wall Muscles

The monoclonal antibody, 5-6, specifically stains myosin-thick filaments located in body-wall, but not pharyngeal, muscles (Miller et al., 1983). Staining in embryos is first visible about 4.5 hr in development (Figure 4), as reported previously by others using a different anti-myosin antibody (Gossett et al., 1982). As described above, only one of the 81 embryonic body-wall muscles is derived from the AB blastomere. It is difficult to identify specifically the sole AB-derived muscle in whole mounts of embryos prepared for immunocytochemistry and thus prove that the 5-6 antibody stains this cell. However, we have not observed any gaps in the pattern of body-wall muscles stained with 5-6 that would indicate certain muscles were not recognized. In addition, we have examined body fragments from about 30 animals that were fixed immediately after hatching and stained with both RITC-labeled 5-6 and FITC-labeled phalloidin (Wulf et al., 1979; Faulstich et al., 1983) to visualize actin filaments (Figures 4c and 4d). Because all body-wall muscles identified with FITC-phalloidin also stained positively with 5-6, we conclude that 5-6 can be used as a marker for both AB- and P1-derived body-wall muscles.

The Blastomeres That Generate Pharyngeal Muscles Appear to Be Determined by the 28-Cell Stage

At the 28-cell stage the three AB- and two P1-derived blastomeres that generate pharyngeal cells are clustered on the ventral side of the embryo (Figure 5a). It is possible to destroy any or all of these five pharyngeal precursors by irradiation with a laser microbeam (Sulston and White, 1980; Sulston et al., 1983). The ablated cells usually detach completely from the surface of the embryo, and thus are removed from their normal positions. When all five of the AB- and P1-derived pharyngeal precursors were ablated, the resulting embryos did not contain any cells in the head that were stained by 3NB12 or by a second antibody specific for pharyngeal myosin (Figures 5d and 5e and Table 1). A few cells were stained by 3NB12 in the tails of some experimental embryos, as observed in normal embryos late in development. When only the two P1-derived precursors were ablated, the embryos developed an anterior half-pharynx with variable morphology that stained with both pharyngeal antibodies (Figures 5f and 5g and Table 1). When only the three AB-derived precursors were ablated, a posterior half-pharynx with variable morphology developed which stained with both antibodies (Figures 5h and 5i and Table 1).

The P1 Blastomere Does Not Require AB-Derived Cells in Order to Generate Pharyngeal and Body-Wall Muscle Cells

The AB blastomere was removed as completely as possible with a microneedle (see Experimental Procedures) from 2-cell embryos to test whether AB, or AB descendants, were necessary for P1 decendants to differentiate into muscles (Figure 6 and Table 2). Almost all of the partial embryos derived from P1 developed several pharyngeal muscle cells and body-wall muscles, as detected by

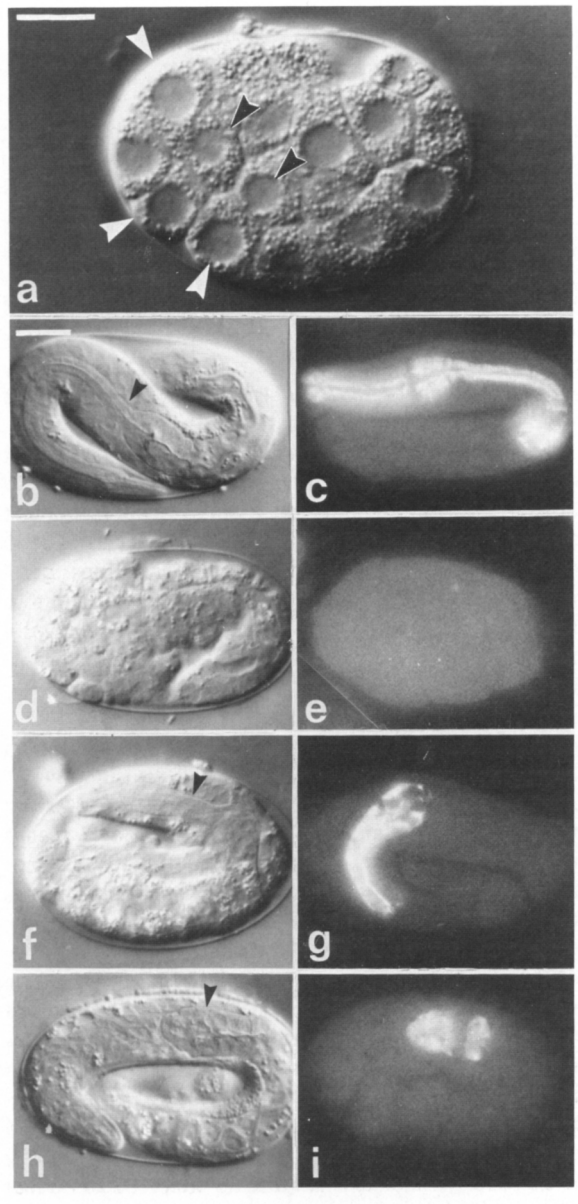

Figure 5. Development in Embryos Following Ablation of Pharyngeal Precursors at the 28-Cell Stage

The top panel (a) is a light micrograph of the ventral surface of a 28-cell embryo showing the three AB-derived pharyngeal precursors (white arrowheads) and two P1-derived precursors (black arrowheads). These five cells are indicated in the lineage diagram in Figure 2 by the closed triangles. Note that each AB-derived precursor contacts a P1-derived precursor. The lower left column shows light micrographs of normal (b) and experimental (d, f, h) embryos, and the right column shows comparable embryos stained with 9.2.1., a monoclonal antibody that recognizes pharyngeal myosin C (Epstein et al., 1982). (b, c) Normal embryos. The outline of the pharynx is visible in the light micrograph (arrowhead, b). (d, e) Embryos following ablation of all five pharyngeal precursors. No pharyngeal structures are visible in the light micrograph (d) or after staining with anti-pharyngeal myosin (e). (f, g) Embryos following ablation of only the P1-derived pharyngeal precursors. A small, anterior pharynx with cells expressing pharyngeal myosin has developed (arrowhead, f). (h, i) Embryos following ablation of only the AB-derived pharyngeal precursors. A small, posterior pharynx has developed (arrowhead, h). (a) bar = 10 μm; (b–i) bar = 10 μm.

Table 1. Fraction of Embryos Showing Staining after Ablation of Pharyngeal Precursors at the 28-Cell Stage

	Pharyngeal Precursors Ablated		
Antibody	3[AB][a]	2[P1][b]	(3[AB] + 2[P1])[c]
3NB12	11/11	7/7	0/4[d]
Anti-Pharyngeal Myosin[e]	7/7	8/8	0/6

[a] The three AB-Derived pharyngeal precursors: ABalpa, ABaraa, ABarap (see Sulston et al., 1983).
[b] The two P1-derived pharyngeal precursors: MSaa, MSpa (see Sulston et al., 1983).
[c] All five pharyngeal precursors: ABalpa, ABaraa, ABarap, MSaa, MSpa (see Sulston et al., 1983).
[d] No cells were stained in the head, where the pharynx forms. A few cells were stained in the tails of two embryos, as observed in normal development with this antibody.
[e] Anti-Myosin C (9.2.1) Described in Epstein et al. (1982).

staining with 3NB12 and 5-6, respectively. Intestinal cells also were produced by P1, as in normal development. Four P1-derived partial embryos were fixed and prepared for electron microscopic analysis. Although the morphologies of the embryos were severely abnormal, pharyngeal cells, body-wall muscles, and intestinal cells were recognizable in each. The pharyngeal cells were organized around a central cavity and surrounded by a thick basement membrane as in normal development. In addition, a specialized pharyngeal structure called the grinder, which is formed by the sixth set of pharyngeal muscle cells (Albertson and Thomson, 1975), was present in two of the embryos examined (data not shown). Abundant but disorganized thick filaments were visible within the body-wall muscles of the partial embryos viewed in the electron microscope, consistent with the 5-6 staining patterns. However no thick filaments were present in the presumptive pharyngeal muscle cells. This correlates with our observation that partial embryos derived from P1 did not stain positively with antibodies against pharyngeal myosin (data not shown), though pharyngeal muscle cells were present. The failure to express pharyngeal myosin in these cells could mean interactions with AB descendants are necessary for full pharyngeal muscle differentiation, or simply reflect the fact that pharyngeal myosin normally is expressed much later than the antigen recognized by 3NB12.

The AB Blastomere Requires P1-Derived Cells in Order to Generate Pharyngeal and Body-Wall Muscle Cells

The P1 blastomere was removed from a total of 59 2-cell embryos in two sets of experiments. In the first set, the P1 blastomere was removed as completely as possible from the embryo. For the second set of experiments, essentially only the nucleus and centrosomes were removed from P1 in an effort to minimize any possible damage to the AB blastomere. Laufer and von Ehrenstein (1981) have shown that C. elegans embryos develop and hatch after extrusion of up to 20% of the P1 blastomere (not including the nucleus), and we have found similar results using our different blastomere removal techniques and culture con-

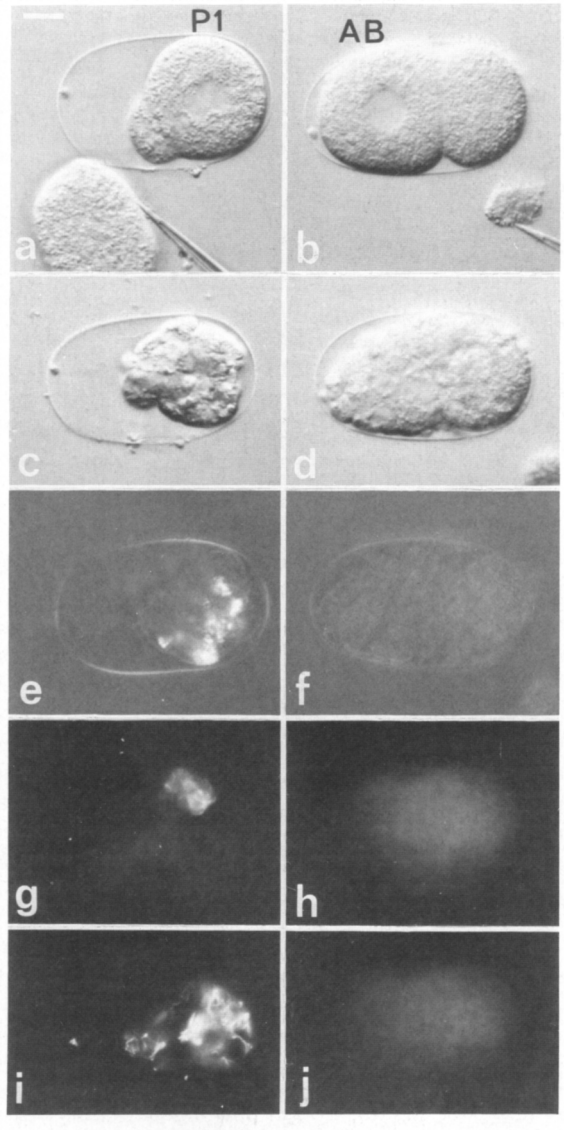

Figure 6. Differentiation in a P1-Derived Partial Embryo and a AB-Derived Partial Embryos

The left column shows development of the P1 blastomere after removal of most of the AB blastomere, and the right column shows AB development after enucleation of P1. (A normal 2-cell embryo is shown in Figure 8a.) (a, b) Light micrographs of embryos shortly after the experiment. (c, d) Light micrographs of the partial embryos after overnight incubation. The intact blastomere has generated a large number of descendants in both experiments. The enucleated P1 blastomere in (d) has not divided and does not appear to have degenerated. (e, f) The embryos viewed with polarization optics. Intestine-specific granules are highly birefringent, and appear as bright spots within differentiated intestinal cells (Laufer et al., 1980). As in normal development, the P1 blastomere has generated intestinal cells (e; see Figure 2 for normal lineage). (g, h) The partial embryos stained with 3NB12 to visualize pharyngeal muscle cells. The P1-derived embryo contains several pharyngeal muscle cells (g), while the AB-derived embryo does not contain any cells that stain with 3NB12 (h). (i, j) The embryos stained with 5-6 to visualize body-wall muscles. Only the P1-derived embryo shows expression of body-wall muscle myosin (i). Bar = 10 μm.

ditions (data not shown). The volume of cytoplasm removed in such experiments can be much greater than the total volume removed in enucleating P1. Cytoplasmic movements continue in enucleated P1 blastomeres, and the cytoplasts do not appear to degenerate during the experimental incubation period (Figure 6).

No pharyngeal or body-wall muscle cells were visible in either set of AB-derived partial embryos stained with 3NB12 or 5-6, respectively (Figure 6, Table 2). Several AB-derived embryos were examined in the electron microscope, and all of these showed evidence of cellular differentiation (data not shown). Hypodermal cells could be recognized by their pattern of desmosomal attachments (Sulston et al., 1983; Priess and Hirsh, 1986) and the secretion of an extracellular layer called the embryonic sheath (Priess and Hirsh, 1986). Small cells resembling neurons in nuclear morphology were present, as were several cells apparently undergoing programmed cell death (Robertson and Thomson, 1982; Sulston et al., 1983).

The P2 or EMS blastomere was removed from embryos at the 4-cell stage to test which P1 daughter was necessary in order for AB descendants to become pharyngeal muscles (see lineage chart, Figure 2). Almost all of the partial embryos formed from AB plus EMS (P2 removed) developed both pharyngeal and body-wall muscle cells as indicated by staining with 3NB12 and 5-6, respectively (Figure 7, Table 2). Two properties of these partial embryos suggest that some of the pharyngeal muscle cells they contained were descendants of the AB blastomere. Each of the embryos formed from AB plus EMS contained more pharyngeal muscle cells than were observed in embryos derived from P1 alone or from embryos following ablation of the three AB-derived pharyngeal precursors (compare Figures 7g and 6g). Moreover, many of the embryos formed from AB plus EMS developed a pharynx that extended to the tip of the head (Figure 7b). Only the AB-derived pharyngeal cells form the anterior tip of the pharynx in normal development, and an anterior pharynx was not observed in any embryo after ablating the three AB-derived pharyngeal precursors (Figure 5h). Although all of the AB plus EMS embryos examined produced body-wall muscles in addition to pharyngeal muscle cells, it was not determined whether any of the body-wall muscles were descended from AB.

The EMS blastomere was removed from 4-cell stage embryos in three sets of experiments. In the first set, the EMS nucleus and variable amounts of EMS cytoplasm were removed. The majority of these partial embryos formed by AB plus P2 (22/24) did not develop any pharyngeal muscle cells, as indicated by the lack of staining with 3NB12 (Table 2). However two of these embryos contained a few cells that stained with 3NB12 (see below). In the second set, essentially all of the EMS blastomere was removed (Figure 7; Table 2). None of the resulting embryos (0/14) contained any cells that stained with 3NB12 (Figure 7h). Although pharyngeal muscle cells were not observed, other types of differentiated cells were apparent in these embryos. Hypodermal cells could be recognized by their morphology in the light microscope (Sulston et al., 1983) and several of these partial embryos underwent fairly normal tail morphogenesis (Figure 7d) which requires the proper functioning of hypodermal cells (Sulston et al.,

Table 2. Fraction of Embryos Developing Intestinal Granules or Showing Staining with 3NB12 or 5-6 after Removal of Early Blastomeres

	Blastomere Removed								
	AB	P1[b]	P1[c]	ABa	ABp	EMS[d]	EMS[e]	EMS[f]	P2[g]
Intestinal Granules[a]	30/32	0/45	0/14	7/7	10/10	0/33	0/19	0/6	26/28
3NB12	14/14	0/25	0/7	7/7	10/10	2/24	0/14	6/6	22/23
5-6	17/18	0/20	0/7	NA	NA	9/9	NA	NA	5/5

[a] Intestinal granules were scored with polarization optics (Laufer et al., 1980).
[b] The P1 blastomere was removed as completely as possible.
[c] The P1 blastomere was enucleated.
[d] Variable amounts of EMS were removed.
[e] The EMS blastomere was removed as completely as possible.
[f] The EMS blastomere was enucleated late in the cell cycle.
[g] These numbers do not include several embryos (13) that did not make intestinal granules when P2 was removed immediately after the division of P1 into P2 and EMS. These embryos contained abnormally large cells and did not make intestinal granules, pharyngeal cells, or body-wall muscles. EMS and P2 remain connected by a cytoplasmic bridge after division, and we feel the most likely explanation for the absence of intestinal and muscle differentiation in these embryos is that EMS was damaged by the early removal of P2.
NA = not analyzed.

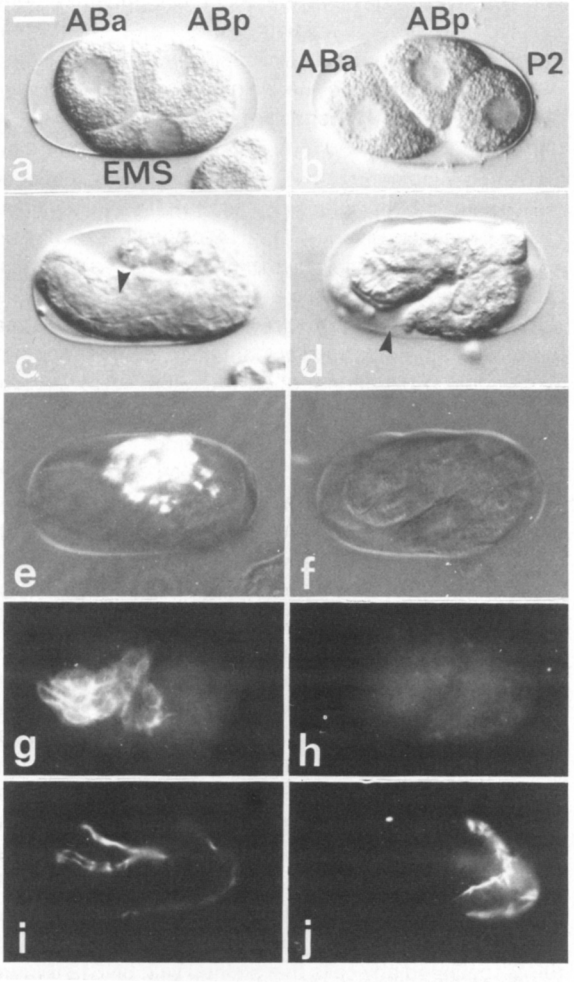

Figure 7. Differentiation in an AB plus EMS-Derived Embryo, and an AB plus P2-Derived Embryo

The left column shows development of an embryo following removal of the P2 blastomere at the 4-cell stage (a), and the right column shows an embryo following removal of EMS (b). (A normal 4-cell embryo is shown in Figure 8i.) The series of micrographs shown here are the same kind and in the same sequence as in Figure 6, and are described there in detail. (c, d) Both types of partial embryos have undergone some degree of morphogenesis after overnight incubation. A well-formed head containing a pharynx (arrow) is visible in the AB plus EMS-derived embryo (c), and a fairly organized tail with a tail-spike (arrowhead) has developed in the AB plus P2-derived embryo (d). (e, f) The AB plus EMS embryo, but not the AB plus P2 embryo, develops intestine-specific granules, consistent with normal development (see Figure 2). (g, h) The AB plus EMS embryo has developed pharyngeal muscle cells, as in normal development, but the AB plus P2 embryo has not, in contrast to normal development. Note that more cells are stained in the AB plus EMS embryo than in the P1-derived embryo in Figure 6g, suggesting that AB descendents also have differentiated into pharyngeal muscle cells. (i, j) Both embryos have developed body-wall muscles. It was not determined whether any of the body-wall muscles were derived from the AB blastomere in either experiment.

1983; Priess and Hirsh, 1986). Hypodermal cells and cells resembling neurons were also identified in partial embryos that were fixed and examined with the electron microscope (data not shown). In the third set of experiments, the EMS blastomere was enucleated late in the EMS cell cycle, leaving the cytoplasm of the EMS blastomere largely intact. All of these embryos (6/6) contained one or two cells that were stained by 3NB12 (Table 2). While the positively staining cells are candidates for pharyngeal muscle cells, it is possible that these cells are instead some of the tail cells that 3NB12 also stains late in embryogenesis.

The Daughters of AB Are Probably Equivalent Initially

In normal development only the ABa daughter of AB generates pharyngeal muscles; the ABp daughter does not (see lineage chart, Figure 2). Because the results described above suggest that an interaction between AB- and P1-derived cells is necessary for any AB descendants to become muscles, we were interested in testing whether ABp descendants could become pharyngeal muscles if they occupied the positions normally assumed by ABa descendants. We found that it is possible to interchange the ABa and ABp blastomeres with a blunt-ended micropipette needle and micromanipulator as shown in Figure 8 and described in Experimental Procedures. The ABa and ABp blastomeres are interchanged as the cleavage

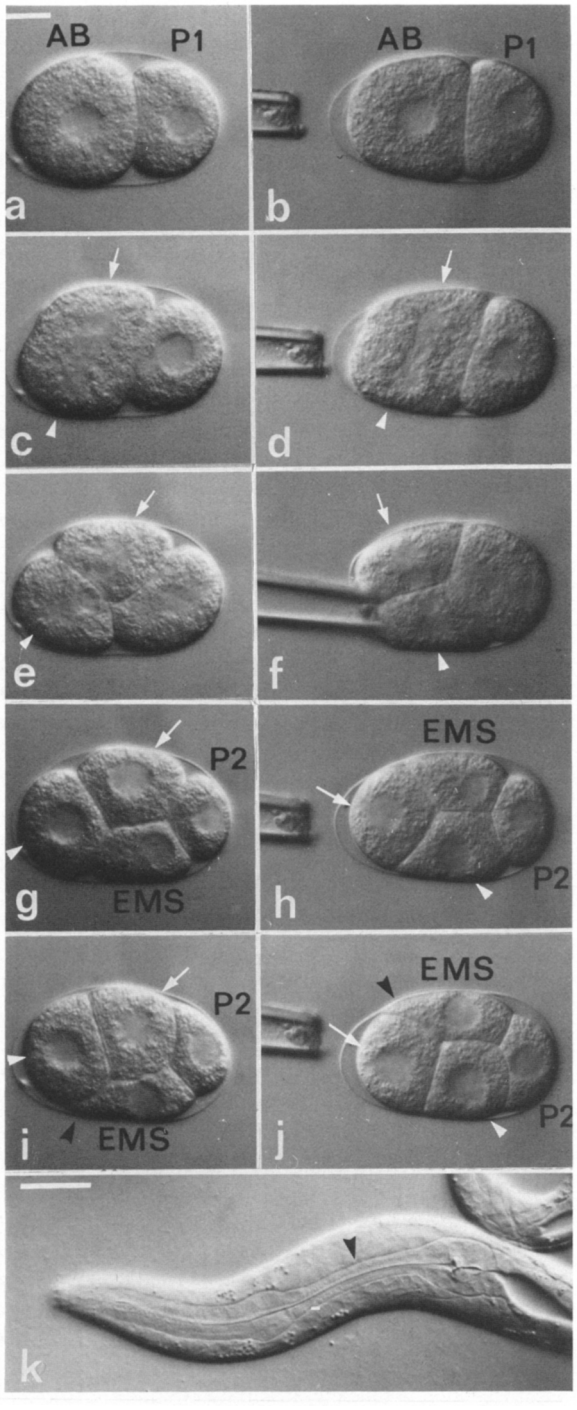

Figure 8. Interchange of the ABa and ABp Blastomeres

The left column shows the division of a normal 2-cell embryo into four cells. The right column shows the experimental inversion of the ABa and ABp blastomeres in a second embryo. (a, b) 2-cell embryos. (c, d) The AB spindle forms at right angles to the long axis of the egg, and now is shown skewing toward that axis. As the AB cleavage furrow progresses inward (visible on the left of the embryos) the anterior daughters normally become ABa and the posterior daughters ABp. (e) The AB division is completed and P1 divides parallel to the line connecting the two AB daughters. (f) A blunt-ended micropipette needle capped with cellular debris is used to gently push the anterior daughter (arrowhead) posteriorly. The P1 spindle becomes reoriented, and divides parallel to the new axis. (g, h) Early 4-cell stages. Note that in (h) the cell that normally would have been ABa (arrowhead) is now

furrow separates the two cells, but before separation is complete. By the time separation is complete, the P1 blastomere also has divided; it proved impossible to then interchange ABa and ABp without simultaneously displacing both P2 and EMS, and such embryos are severely abnormal (data not shown).

ABa and ABp were interchanged in a total of 11 embryos. To our surprise, all 11 embryos developed into apparently normal larvae and hatched (Figure 8). Specifically, the pharynx appeared normal in morphology, and the ventral nervous system and hypodermis were examined in three larvae and found to have the correct number and patterns of cells. Eight of the larvae were placed on culture plates for continued observation; all developed into fertile adults with no visible abnormalities in morphology or movement. Two adults were fixed and processed for electron microscopy, and semi-serial sections through the tip of the head were examined. The anterior sensory sensillae, which are complex assemblages of neurons and supporting cells (Ward et al., 1975), appeared completely normal in both animals. For example, all six inner labial neurons with striated rootlets, which normally are ABa descendants, and the amphid sheath cells, which normally are ABp descendants, were present and in the correct positions.

The development of two embryos with interchanged ABa and ABp blastomeres was followed by Nomarski microscopy continuously to the 28-cell stage and then checked intermittently until hatching to address the possibility that the descendants of the interchanged ABa and ABp blastomeres were migrating back into their normal positions. No rearrangement of cells was observed; instead, the overall pattern of cells and timing of divisions appeared normal, with no abnormalities apparent in later embryonic stages.

Because ABp normally forms the dorsal surface of the embryo, interchanging ABa and ABp results in an inversion of the dorsal-ventral axis (compare Figures 8i and 8j). Therefore it was of interest to determine whether the polarity of the left-right axis also was inverted in these embryos. The left-right axis can be determined by examining left-right asymmetries in the cleavage patterns of early embryos, and particularly the positions of blastomeres at the 28-cell stage (Sulston et al., 1983). The left-right axis in two embryos with interchanged ABa and ABp blastomeres was found to be oriented normally relative to the new dorsal-ventral axis. The orientation of the left-right axis was also examined and found to be normal for the intestine and Q-derived cells (Sulston and Horvitz, 1977) during post-embryonic development in six larvae from embryos with

posterior. (i, j) Late 4-cell stages. The EMS blastomere develops a process (black arrowhead) that extends across the most anterior cell, normally ABa. Note that the embryo with interchanged ABa and ABp blastomeres (j) has its dorsal-ventral axis inverted relative to the normal embryo (i). Thus the surfaces of the P1 blastomere at the 2-cell stage (b) that normally would become dorsal and ventral now are ventral and dorsal, respectively. (k) High magnification of newly hatched larva from embryo with inverted ABa and ABp blastomeres. The pharynx (arrowhead) appears completely normal. (a–j) bar = 10 μm (k) bar = 10 μm.

interchanged ABa and ABp blastomeres (C. Kenyon, personal communication).

Discussion

Cellular Interactions

A cell is considered to be "determined" or "committed" if it differentiates normally outside of its normal environment. Classical studies on many types of invertebrate embryos showed that certain early embryonic blastomeres were capable of developing normally when separated from other blastomeres, and thus the fate of these blastomeres appeared to be determined very early. These results led to the hypothesis that "determinants" might exist in the fertilized egg that could specify the fates of early cells (reviewed in Davidson, 1976). A cell that inherited a certain type of determinant would differentiate according to that determinant.

Because the complete cell lineage of C. elegans is known (Sulston et al., 1983), it now is possible to compare in detail the normal development of an embryonic cell with its behavior in isolation. Although the development of isolated intestinal precursors in C. elegans is consistent with the hypothesis that determinants are segregated to these cells (Laufer et al., 1980), the results presented here suggest strongly that at least some other blastomeres are not determined in this fashion. In normal development the AB blastomere generates several pharyngeal muscles. However, none of the descendants of the AB blastomere appear to differentiate into muscles after the P1 blastomere is removed from the embryo. In normal development, the only AB daughter that produces pharyngeal cells is ABa. However, embryos in which ABp and ABa have been interchanged during division appear to develop a normal pharynx containing ABp descendants. These two lines of evidence argue against the possibility that AB descendants become pharyngeal muscles as a result of inheriting preexisting muscle determinants, and that instead, the information that allows AB descendants to become muscles is acquired through interactions with other cells in the embryo.

At present we do not know which P1-derived cells the AB descendants must interact with in order to become muscles. The absence of pharyngeal muscles in embryos after removal of EMS suggests that EMS is required and that the AB-derived pharyngeal precursors become committed after the 4-cell stage. In addition, the laser ablation studies reported here suggest that the fates of pharyngeal muscle cells may already be determined by the 28-cell stage. Because the AB pharyngeal precursors are directly adjacent to the P1 pharyngeal precursors between these stages, an intriguing possibility is that the P1 pharyngeal precursors themselves influence the differentiation of the AB descendants.

Cellular interactions in the early embryo are probably required for more than the specification of pharyngeal muscle cell fates. The AB blastomere does not appear to produce any body-wall muscles when isolated from other cells. Furthermore, the ABa-ABp interchange experiments reported here specifically rule out the possibility that the nuclei, asters, or peripheral cytoplasm of these blastomeres are fundamentally different. Although ABa and ABp are connected at the time they are interchanged, there was no indication that cytoplasm was being pushed from one cell into the other. Therefore we consider it very likely that ABa and ABp are equivalent initially, and adopt different fates as a consequence of their positions in the embryo.

The observation that embryos develop normally when ABa and ABp are interchanged argues against there being specific determinants for any of the types of cells normally made by only one of these blastomeres. For example, all of the GABA-containing motoneurons in the nematode's ventral nervous system are descended from ABp in normal development (Sulston et al., 1983; S. McIntyre, J. White, and R. Horvitz, personal communication), and ablation studies indicate that animals lacking these motoneurons should be severely uncoordinated (Chalfie et al., 1985). However, larvae from embryos with interchanged ABa and ABp blastomeres move normally, and appear to have a morphologically normal ventral nervous system. Since it is very probable that the GABA-containing motoneurons of these embryos were derived from what normally would have been the ABa blastomere, ABp does not appear to uniquely inherit special determinants for producing these cells.

Cell Autonomy

Although the ABa–ABp interchange experiments here suggest that special determinants do not exist for any of the cell types that only ABa or ABp produce, our results do not rule out the possibility that determinants specify cell types that both ABa and ABp produce. In normal development, ABa and ABp both produce descendants that differentiate into hypodermal cells or neurons, or that undergo programmed cell death. After removal of the P1 blastomere, AB descendants appear to differentiate into hypodermal cells, undergo programmed cell death, and become cells that are candidates for neurons. We do not know at present whether these cell fates are determined through cellular interactions between AB descendants, or cell-autonomously according to a fixed lineage pattern.

Our results also do not rule out the possibility that determinants specify the fates of the pharyngeal precursors descended from P1. P1 descendants differentiate into pharyngeal muscle cells after removal of AB, as do descendants of the AB and EMS blstomeres after removal of P2. The latter result suggests that EMS does not require its sister, P2, in order to produce pharyngeal muscle cells, although this has not been tested directly. Therefore it is possible that cellular interactions are not required for P1 descendants to become pharyngeal muscle cells.

Establishment of the Embryonic Axes

Because interchanging ABa and ABp also switches the polarity of the dorsal-ventral axis of the embryo, this result suggests that this axis is not already determined at the 2-cell stage. Moreover, if the polarity of the left-right axis were already determined at the 2-cell stage, inverting the dorsal-ventral axis should create embryos that were mirror

images of normal embryos. Instead, we find that left-right asymmetries are normal relative to the new dorsal-ventral axis in both embryos and larvae, indicating that the left-right axis has reversed. These results suggest there are no fundamental asymmetries between the dorsal and ventral, or left and right, regions of the 2-cell embryo.

The AB spindle normally forms at right angles to the anterior–posterior axis of the egg; as the spindle elongates it skews toward this axis, thereby defining the future dorsal-ventral axis of the embryo. Because the present study demonstrates that the direction of skewing can be reversed easily, and no cytoplasmic asymmetries have been reported that can successfully predict the direction of skewing, it is possible that the direction of skewing is random. Position-dependent interactions between the AB and P1 descendants at or beyond the 4-cell stage might then result in some of the dorsal-ventral differences in cell fate observed in C. elegans embryogenesis.

Experimental Procedures

Strain and Culture

Caenorhabditis elegans strain Bristol was cultured as described by Brenner (1974). Cultures were maintained at 20°C. All ages of embryos stated in this paper refer to time elapsed beyond the 2-cell stage at 20°C.

Microscopy

Differential interference contrast (DIC) and polarization microscopy were done using a Zeiss inverted microscope. Fluorescence microscopy was done using a Zeiss RA microscope equipped for epifluorescence. All photographs were taken on Kodak Technical Pan film at ASA 100 and developed in HC110 developer.

Blastomere Removal and Interchange

Embryos were cut from gravid adults in dH_2O, and pronuclear stages were collected with a micropipette and placed on a cover slip coated with 0.1 mg/ml polylysine (Sigma, MW >350,000). Embryos were pressed gently onto the cover slip with an eyelash affixed to a toothpick, then the excess dH20 was removed and the embryos covered with a small drop of embryonic culture medium. The culture medium consisted of 4 mg/ml hyaluronic acid (Sigma, Type 111S), 10% fetal calf serum (Gibco), 90 mM NaCl, 50 mM KCl, and 0.5 mg/ml gentamicin sulfate (Cowan and McIntosh, 1985). The culture medium was covered immediately with a drop of 10S voltalef oil. The embryos were manipulated at 15°C on a Zeiss inverted microscope. Blastomeres were removed or interchanged with Clark GC12015 needles drawn using a two-stroke electrode puller, and a Zeiss/Jena micromanipulator. Blastomeres were removed by puncturing the eggshell adjacent to a blastomere, then pulling the eggshell gently toward one side of the lesion; the pressure on the egg squeezes the blastomere out. To remove the large AB blastomere, it also was necessary to press on the top of the egg gently with the needle. The egg was monitored constantly in the microscope during extrusion of a blastomere; slightly more pressure was required to squeeze nuclei through the lesion than cytoplasm, and it was necessary to reduce the pressure immediately once the nucleus was extruded.

For interchanging blastomeres, the tips of the needles were broken off by gently pushing the needle against the edge of a cover slip, monitoring the procedure with the microscope. The broken needle was capped with cellular debris to cover any sharp edges by pushing the needle repeatedly into an embryo. After blastomere removal or interchange, the embryos were incubated overnight at 20°C.

Laser Ablation

Eggs were cut from gravid adults in dH_2O, and 2-cell stages were collected and allowed to develop for 30 min at room temperature. The embryos were placed on an agar pad, covered with a cover slip, examined with DIC optics, and cells in appropriately oriented 28-cell embryos were irradiated with a laser microbeam (Sulston and White, 1980). Following irradiation, the embryos were incubated overnight at 20°C. The cover slips then were removed and the embryos collected and processed for immunofluorescence.

Immunofluorescence

After incubation the culture medium and oil covering the embryos was replaced by an isotonic solution consisting of 0.1 M NaCl and 3.7% sucrose (Priess and Hirsh, 1986). To ensure permeability, the eggshells were penetrated with a needle according to the general procedure for extruding blastomeres. Almost all the isotonic medium then was removed, and the cover slip laid on dry ice for about 10 min. The cover slip was placed in methanol at −20°C for 4 min followed by acetone at −20°C for 4 min, then air dried.

The fixed embryos were preincubated with Tris-buffered saline (TBS, 150 mM NaCl, 50 mM Tris-HCl [pH 7.4]) containing 0.5% Tween 20 for 20 min at room temperature, then incubated for 1 hr at 37°C in primary antibody. Primary antibody was removed by rinsing in TBS-Tween 20. The embryos then were incubated with a 1/100 dilution of fluorescein-labeled rabbit anti-mouse IgG (Miles) for 1 hr at 37°C. The embryos were rinsed in TBS-Tween 20, and mounted in 1 mg/ml p-phenylenediamine (Sigma) in 10% phosphate-buffered saline (PBS) and 90% glycerol (pH 8.0).

Fragments of newly hatched larvae were prepared for immunofluorescence according to Priess and Hirsh (1986). Eggs were collected in dH_2O from gravid adults and allowed to hatch. Larvae were collected and placed between two cover slips coated with 0.1 mg/ml polylysine. The excess fluid was removed until the top cover slip slightly compressed the larvae. The larvae then were frozen by placing the cover slips on dry ice for at least 10 min. The cover slips were separated and immersed in methanol at −20°C for 4 min, then acetone at −20°C for 4 min. The larval fragments were rehydrated quickly through a graded acetone series (95%, 75%, 50%, 30%, 15%) and three changes of PBS. The larvae were stained with 1 μg/ml fluorescein-isothiocyanate-conjugated phalloidin (FITC-phalloidin, kindly provided by T. Wieland) in PBS for 30 min at room temperature, then rinsed in TBS-Tween 20 and stained with tetramethylrhodaminyl-conjugated 5-6 (RITC-5-6, kindly provided by D. Miller) for 1 hr at 37°C. The slides were rinsed and mounted for immunofluorescence microscopy as above.

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