

Establishment of gut fate in the E lineage of *C. elegans*: the roles of lineage-dependent mechanisms and cell interactions

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SUMMARY

The gut of *C. elegans* derives from all the progeny of the E blastomere, a cell of the eight cell stage. Previous work has shown that gut specification requires an induction during the four cell stage (Goldstein, B. (1992) *Nature* 357, 255-257). Blastomere isolation and recombination experiments were done to determine which parts of the embryo can respond to gut induction. Normally only the posterior side of the EMS blastomere contacts the inducing cell, P₂. When P₂ was instead placed in a random position on an isolated EMS, gut consistently differentiated from the daughter of EMS contacting P₂, indicating that any side of EMS can respond to gut induction. Additionally, moving P₂ around to the opposite side of EMS in an otherwise intact embryo caused EMS's two daughter cells to switch lineage timings, and gut to differentiate from the descendants of

what normally would be the MS blastomere. The other cells of the four cell stage, ABa, ABp, and P₂, did not form gut when placed in contact with the inducer. To determine whether any other inductions are involved in gut specification, timed blastomere isolations were done at the two and eight cell stages. In the absence of cell contact at the two cell stage, segregation of gut fate proceeded normally at both the two and four cell stages. Gut fate also segregated properly in the absence of cell contact at the eight cell stage. A model is presented for the roles of lineage-dependent mechanisms and cell interactions in establishing gut fate in the E lineage.

Key words: *C. elegans*, gut, induction, segregation of fate, blastomere isolation

INTRODUCTION

Two types of cues generate diversity among lineally related cells: some cells inherit particular cellular contents (see Davidson, 1986 and Horvitz and Herskowitz, 1992, for reviews), and some cells of an equivalent group of cells use cues from their neighbors (see Jessell and Melton, 1992 and Greenwald and Rubin, 1992 for reviews). Often embryos use both types of cues to establish a tissue. For example, mesoderm in amphibians forms from the marginal zone cells of the blastula, via an induction from the neighboring vegetal cells. Before the induction occurs, only the animal cap and marginal zone cells inherit the ability to respond to induction (Nieuwkoop, 1969; Nieuwkoop and Ubbels, 1972). Thus the specification of mesoderm in the marginal zone cells depends on both the inheritance of cellular contents and on cell-cell interactions.

The nematode *C. elegans* is useful for investigating the mechanisms that generate cellular diversity during animal development, as mutants can be analysed (Brenner, 1974) and the complete cell lineage is known (Sulston and Horvitz, 1977; Deppe et al., 1978; Kimble and Hirsh, 1979; Sulston et al., 1983). In *C. elegans*, the gut (or 'intestine') derives

from all the progeny of one cell (the E cell) of the eight cell stage (see Fig. 1 for names of early blastomeres). The gut is the only somatic tissue which derives from a single founder cell (Deppe et al., 1978). Previous studies have suggested that gut is specified in the E lineage solely by virtue of the contents inherited by E (Laufer et al., 1980; Edgar and McGhee, 1986). These experiments involved isolating cells from their neighbors and determining whether the E cell or its precursors could form gut in the absence of potential signals from other cells. Cells were isolated by applying physical pressure on embryos inside their eggshells and then picking out individuals where all cells except the cell of interest had lysed. The result, that gut often differentiates from an isolated E cell or its precursors, suggested that the potential to form gut is restricted to E independently of cell interactions, and hence solely by virtue of the segregation of cellular contents into E. However, cells are not completely isolated by this method, as the intact cells generally remain in contact with the lysed remnants of other cells inside the eggshell. Other work, in which cell fragments or complete cells were extruded through a hole in the eggshell, has suggested that cell interactions may be required for gut differentiation. Extruding a P₂ cell from a four cell stage

embryo prevented gut differentiation (Schierenberg, 1987). It was not clear, however, whether extruding P₂ prevented a cell interaction with EMS or simply damaged EMS. When this experiment was done by Priess and Thomson (1987), gut differentiation occurred in most cases.

In none of the above experiments was the time in the cell cycle of each isolation noted; therefore interactions occurring during only the beginning of a cell cycle could not be detected. The development of a culture medium which supports embryonic development after removal of the eggshell and vitelline membrane has since allowed complete cell separations and recombinations (L. Edgar, personal communication). Using this medium, blastomere isolation and recombination experiments demonstrated that P₂ induces EMS to form gut in its E lineage, early in the four cell stage (Goldstein, 1992).

As part of an effort to identify the cues involved in blastomere diversification, two questions have been asked in order to determine further how the E lineage acquires the potential to form gut. (1) Does the induction that occurs during the four cell stage play a role in restricting gut potential to the E lineage, or is E the only part of the embryo capable of responding to gut induction? This has been answered by determining if gut can form from other parts of EMS or from the other cells of the four cell stage when in contact with P₂. (2) Are other cell interactions required for gut to differentiate in only the E lineage? A potential role for cell interactions has been tested during each of the cleavage stages until the formation of E, the gut founder cell, at the eight cell stage. Previous experiments have found no cell interactions required for gut specification at the two or eight cell stage (Laufer et al., 1980; Edgar and McGhee, 1986), however the finding that an interaction occurs early in the four cell stage (Goldstein, 1992) raises the possibility that cell interactions may also be occurring at other stages. Cell interactions have been identified by determining whether, after cells are isolated, gut differentiates from the normal cells. For each cleavage stage where cell contacts are found to be dispensable for segregation of fate to the proper cell, segregation of fate must occur by a lineage-dependent mechanism, i.e., as a consequence of the contents cells inherit.

MATERIALS AND METHODS

Removal of eggshell and vitelline membrane, and embryo culture

Embryos were obtained by cutting gravid hermaphrodites in egg salts (Edgar and McGhee, 1986) on a depression slide. Methods for removing the eggshell and vitelline membrane were developed by Dr Lois Edgar (personal communication). Eggshells were removed by treatment with 15% hypochlorite solution (Aldrich) in egg salts for 3-4 minutes followed by two washes in egg salts, and treatment with chitinase and chymotrypsin (0.3 units/ml chitinase (Sigma), 780 units/ml α -chymotrypsin (Sigma, type II), 1% pen-strep solution; Gibco) for 6-9 minutes. De-eggshelled embryos were then washed through two changes of embryonic growth medium (medium includes 40% fetal bovine serum, amino acids, salts, and other components; developed by Dr. Edgar, methods available upon request). Vitelline membranes were removed by drawing embryos (by mouth pipetting) into a glass needle whose

tip had been cut to an open diameter of approximately 25 μ m, just narrower than the short axis of the egg. Needles were made by pulling capillary tubes (A-M Systems, 1 mm outer diameter, 0.58 mm inner diameter) on a double puller (Narishige, PP-83), and were cut under a dissecting microscope at a magnification of 50 $\times$. Capillary tubes were pre-siliconized (Prosil-28, PCR, Inc.) to prevent embryos from sticking in them. De-vitellinized embryos were cultured in wells on depression slides, in 5-10 ml of embryonic growth medium under a layer of halocarbon 100 oil (Halocarbon Products, Inc.). Gut differentiation was scored by checking for birefringent rhabditin granules, an early marker for gut differentiation, under polarized light 8-24 hours after first cleavage. Rhabditin granules, which are products of tryptophan catabolism (Babu, 1974; Babu and Siddiqui, 1980; Siddiqui and Babu, 1980), develop only in the gut, 4-6 hours after first cleavage, and are the only birefringent structure in the embryo (Chitwood and Chitwood, 1974; Babu, 1974; Laufer et al., 1980). Rhabditin granules require zygotic transcription to appear (Edgar and McGhee, 1986). Rhabditin granules form in 92% of all de-vitellinized and further unmanipulated embryos (Goldstein, 1992).

Cell isolations and recombinations

Cells were isolated from two cell and four cell embryos by drawing de-vitellinized embryos in and out of a glass needle whose tip had been cut to an open diameter of approximately 25-40 μ m, prepared as described above. Moving embryos in and out of the needles without delay reduces the number of embryos stuck in capillary tubes. Upon isolation, cells were moved to another well. Cells were placed back in contact with each other by blowing medium (through the needle, by mouth pipetting) behind a cell, moving it up against another. The position of contact between two cells was unknown as isolated cells look approximately spherically symmetrical. The orientation of contact was additionally randomized by putting isolated cells down in the second well without regard to their positions in the first well. In this way cells could then be placed in contact with each other without regard to their original positions. Cells stick to each other immediately upon contact. The plane of contact between two cells then increases in area during the first 30 seconds to 1 minute of contact. All cell manipulations were done under a dissecting microscope at a magnification of 50 or 100 $\times$. After manipulations, cells were observed with a compound microscope (Leitz Orthoplan) with Nomarski DIC optics.

In cases where only some cells were removed without isolating all cells, and also in cases where separations were done after the four cell stage, cells were separated from each other by a different method: the fine end of a glass needle, prepared as described above but without cutting the tip, was laid down between cells to separate cells from each other. In the case where P₂ was removed and placed back on an otherwise intact four-cell stage embryo, the three remaining cells were left undisturbed as P₂ was moved, so that it could be known on which side P₂ was originally.

Photographs of embryos and isolated cells illustrate the experimental design, but do not show the same embryo through each step of manipulation, except for Fig. 3, which shows three embryos at successive stages. Limits in the optics set-up used to make all the figures except for Fig. 3 necessitated using a new embryo for each photograph. In all figures, except Fig. 3, embryos were flattened somewhat for the photographs of rhabditin granules, to increase the number of granules visible in the plane of focus. Photos of embryos with rhabditin granules do not show all of the granules and hence are intended to show only that granules were present.

Determinations of timing

Cells removed from the eggshell divide in the same order as they normally would (except when the P₂-EMS interaction is blocked, in which case the E lineage takes on the timing of an MS lineage; see Goldstein, 1992). Thus after identifying isolated cells by size,

division order was used to check that no mistakes were made. A cell lineage, showing cell division order, is shown in Fig. 1B. Cells were isolated from one embryo at a time and were kept separate from each other to avoid 'confusing' cells. The time in the cell cycle when isolations were done during the two and four cell stages was determined by recording the time at which each separation was done and then the time at which the next cytokinesis began in the gut precursor cell. As division order was consistent with cell size, it is unlikely that the isolations altered the cell cycle length of manipulated cells. Recording the time of the preceding cleavage, in addition to the following cleavage, would help to determine if cell cycle times are affected in manipulated cells; however, it is not feasible to record exactly the time of the preceding cleavage for every embryo, as only some embryos in each batch can be used (many lyse during operation) and manipulations must be done very quickly. In cases where it has not been recorded, this time has been estimated by arranging embryos in order of age while preparing to remove eggshells. This allows one to record when the preceding division occurs in a few embryos, and then to recognize if any embryos develop much more slowly or quickly than the few recorded. The time in the cell cycle when eight cell stage separations were done was determined by simply recording the time when cytokinesis began in EMS and then the time when EMS's daughter cells were separated.

In some experiments, cells of the four cell stage were isolated before gut was specified by induction. The time when gut is specified has been determined previously by isolating EMS at various times throughout the four cell stage; isolating EMS 10 or more minutes before EMS cleaves consistently prevents gut differentiation (Goldstein, 1992). Subsequent observations confirm this finding in embryos whose EMS cell cycle length has been recorded: of 11 EMS cells isolated just 10 minutes before EMS cleaved, each of which had an EMS cell cycle length of 14-15 minutes, gut differentiated in only one case (B.G., unpublished observation).

Time lapse videomicroscopy

In cases where one must know whether cells move around each other or rotate, embryos were viewed at 600 $\times$ to 1000 $\times$ to see cytoplasmic granules, and were time-lapse videorecorded (Gyrr Timelapse Videorecorder) at 12-18 times real time. Embryos were mounted for viewing in culture medium on flat slides. A cover slip with a piece of clay at each corner (for support) was placed on top, and halocarbon oil (Halocarbon Products) was added around the culture medium to prevent dessication during observations longer than 10-12 minutes. Cover slips were pushed down only to the level where the objective lenses could swing into place and focus, to prevent immobilizing cells. In some cases the cell mass could be seen rotating (though as discussed in the results, in no case did one cell move relative to another) and in no case were the cells seen to flatten. Both of these observations indicate that cells were not stuck between the slide and cover slip and were free to move. Cells were recorded on videotape starting within 20 to 90 seconds after placing them in contact with each other. It is unlikely that cells rotate around each other before this time, as cell asymmetries visible under low magnification (eccentric nuclei, oblong cells) never changed position relative to the site of cell-cell contact during this time. Videotapes were played back at real time, and also at 12-18 times real time in order to detect slow movements.

Lineaging was done using a multiple focal plane time-lapse recording system (described in Hird and White, 1993). In cases where EMS was lineaged, the first three to six divisions of EMS were followed, recording the time of cell division for each descendent of EMS. After this time it became difficult to determine when some of the descendants divide, and therefore the location of the lineages were followed, rather than recording the time of each successive cell division. After recording in Nomarski optics for 8-12

hours, a multiple focal plane image under polarized light was recorded. The lineage that formed the rhabditi granules was determined by comparing the location of each lineage and the location of the rhabditi granules.

RESULTS

The role of contact between P₂ and EMS during the four cell stage

Previous work has shown that gut is induced during the four cell stage; in order for gut to differentiate from EMS's E

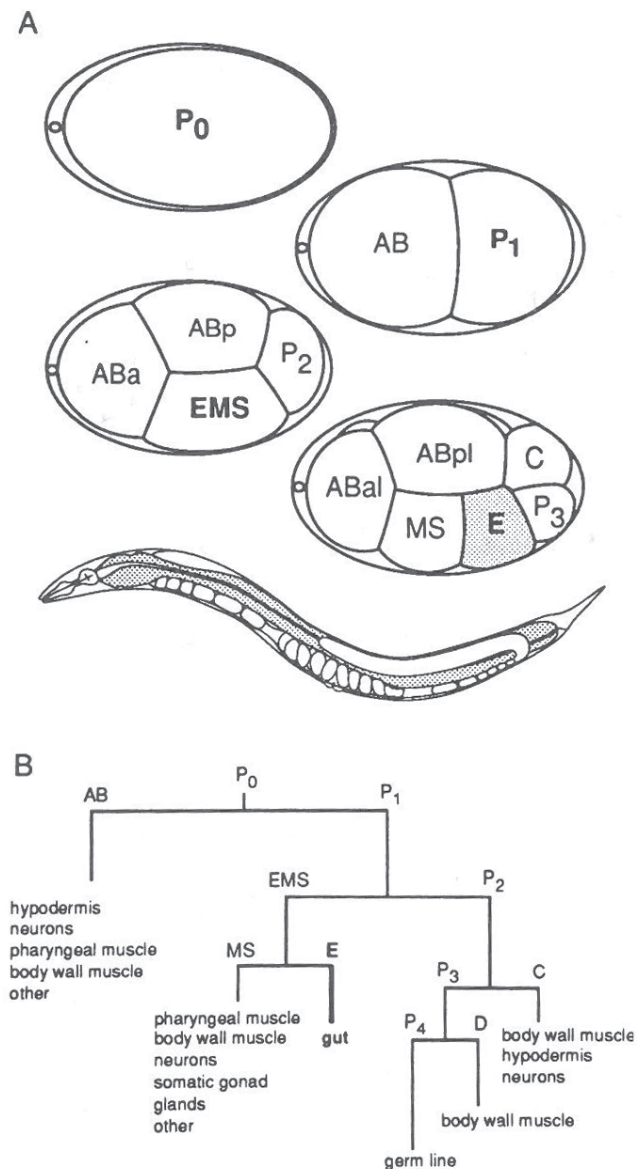


Fig. 1. (A) One, two, four and eight cell stage embryos and adult hermaphrodite worm (not to scale). The gut (shaded in worm) derives from all the progeny of the E cell (shaded in 8 cell stage). Gut precursor cells are labelled in bold type. (B) Cell lineage, showing the six founder cells and the tissues they form (after Sulston et al., 1983).

lineage, EMS must contact P₂ (Goldstein, 1992). Under normal circumstances, only the future E side of EMS contacts P₂ (Fig. 1). It is not yet known if the induction plays a role in establishing the site of gut specification; i.e., the future MS side of EMS might have the potential to form gut if placed in contact with P₂, or alternatively, the future E side of EMS might be the only part of the embryo that can respond to the induction. In order to determine whether only the E side can respond to gut induction, cells were separated from each other before gut specification had occurred (10 or more minutes before EMS cleavage) and then P₂ was placed back on the isolated EMS in a random position (see Materials and Methods). EMS then divided, in a manner that left only one of its daughter cells contacting P₂. Which daughter of EMS formed gut was determined by one of two methods. (1) In five cases, cell division times were recorded to see which daughter took on the timing of an E lineage. E lineage cell divisions normally occur more slowly than those of the MS lineage after E and MS divide once (Deppe et al., 1978). In the absence of the induction, both cells take on MS-like cell cycle timing (Goldstein, 1992). In all five cases, the daughter of EMS contacting P₂ took on the E-like lineage timing. (2) In five other cases, EMS's two daughter cells were separated from each other and were cultured separately for 12-24 hours, to see which produced rhabditin granules, a marker for gut differentiation (Fig. 2A,B). In each case rhabditin granules formed from the side of EMS which P₂ had contacted. These findings suggested that any side of EMS can form gut in response to P₂.

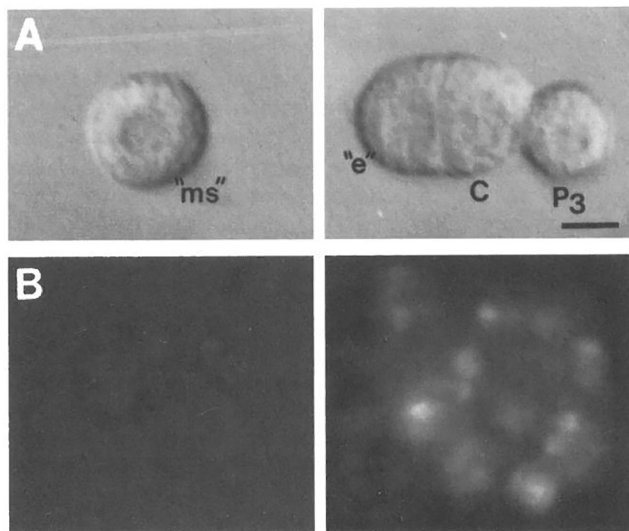


Fig. 2. (A) EMS's two daughter cells separated from each other by laying a glass needle between them. P₂ was left in contact, as it can no longer induce gut or reposition where gut will form by this time (B.G., unpublished results). Note that EMS's daughter cells, E and MS, cannot be distinguished from one another; the one contacting P₂'s daughters (P₃ and C), which was found in this experiment to form gut, is labelled with a lower case 'e' and its sister is labelled 'ms'. (B) The progeny of the isolates 12-24 hours later under polarized light. Rhabditin granules have developed only from the side of EMS on which P₂ had been placed. Scale bar, 10 µm.

To be sure that the induction establishes the site of gut differentiation in EMS, one must know that when P₂ is placed on EMS, it does not then move back to its original site. This was checked by again isolating the cells of the four cell stage before specification had occurred, recombining P₂ and EMS, and then watching the cells to see if P₂ moves or EMS rotates in some cases. At a magnification of 600-1000×, cytoplasmic granules can be visualized, allowing one to see if either cell rotates or moves around the other cell. Each case was also time-lapse videotaped to allow detection of slow movements. P₂ consistently stayed in the position where it had been placed on EMS (6 out of 6 cases). This result, along with the finding that gut consistently differentiates from the side of EMS where P₂ was placed, demonstrates that any side of EMS can respond to the induction. Hence gut normally differentiates only in the E lineage by virtue of P₂'s position on EMS.

If any side of EMS can respond to gut induction, one would expect that moving P₂ to the future MS side of EMS would cause gut to differentiate from what would normally be the MS lineage. To determine if this occurs, P₂ was taken off of a four cell stage embryo by removing the eggshell and

Fig. 3. Three embryos in which P₂ was either removed (A-E), removed and placed back in the same position (F-J), or removed and then placed on the MS side of EMS (K-O). Arrowheads mark the dividing cells, and point toward the forming cleavage furrows. The embryos have been tipped up onto the four AB daughters just after they form, to facilitate viewing and lineaging descendants of EMS; hence all views except (K) are ventral. (K) shows an embryo before it was tipped. The view is from the left side of the embryo. Only part of an embryo can be seen in each view, as Nomarski optics show thin planes of optical section. The complete results are summarized in Fig. 4. (A) EMS cleaving in an embryo in which P₂ was removed. The four AB daughter cells are in a lower plane of focus (B). (C) Two EMS daughters dividing to form four cells. The daughter of EMS shown on the bottom of the photo is dividing through the plane of observation; hence only one of its daughter cells can be seen. (D) The four daughters of EMS are dividing simultaneously to form eight cells, 37 minutes after forming. Two of the four dividing cells can be seen in this view. (E) Same embryo, next day. Rhabditin granules did not form (not shown). (F) EMS cleaving in an embryo in which P₂ has been removed and placed back in the same place. Posterior is to the right. (G) E (right) and MS (left) cleaving. (H) The two daughters of E (marked by a double arrow) cleaved twenty-five minutes after this time. (I) Same embryo, next day. As seen under polarized light in J, rhabditin granules are present. Partial lineaging (see Materials and Methods) showed that they formed in the area of the E descendants but not in the area of the MS descendants. (K) Six cell stage embryo in which P₂ was removed and placed onto the future MS side of EMS. The asterisk marks the original position of P₂. (L) Embryo tipped up onto the four daughters of AB (and turned, hence the view is with posterior to the left in L-O). EMS has divided to form two daughters. Note that the daughter of EMS which is next to P₃, which normally would have been MS, has its nucleus eccentrically placed, apposed to P₃. This occurs in E in normal embryos (Schierenberg, 1987). (M) The two daughters of what normally would have been E, dividing 30 minutes after they formed. The two daughters of what normally would have been MS (marked by a double arrow) divided 22 minutes after this time. (N) Same embryo, next day. As seen under polarized light (O) rhabditin granules are present. Partial lineaging showed that they formed from the descendants of what normally would have been MS. Scale bar in A, for A-E and in F for F-O, 10 µm.

then laying a glass needle down between P₂ and its neighbors and embryos were lineage (see Materials and Methods). When such embryos were left with P₂ off, gut did not differentiate and both of EMS's daughters took on the lineage timings of an MS blastomere (4/4 cases), confirming earlier work (Goldstein, 1992). In four additional cases, P₂ was removed in this way and placed onto the future MS side of EMS. In all four cases, the E and MS blastomeres switched their normal cell cycle timings and gut differenti-

ated from the descendants of what normally would have been MS. This result is not an artifact of manipulation, as removing P₂ and placing it back in its original position caused E and MS to have their normal lineage timings and gut differentiated from descendants of E (3/4 cases; in the fourth, both daughters of EMS took on MS-like lineage timings and gut did not differentiate). The results are shown in Fig. 3, and the experiments and results are diagrammed in Fig. 4.

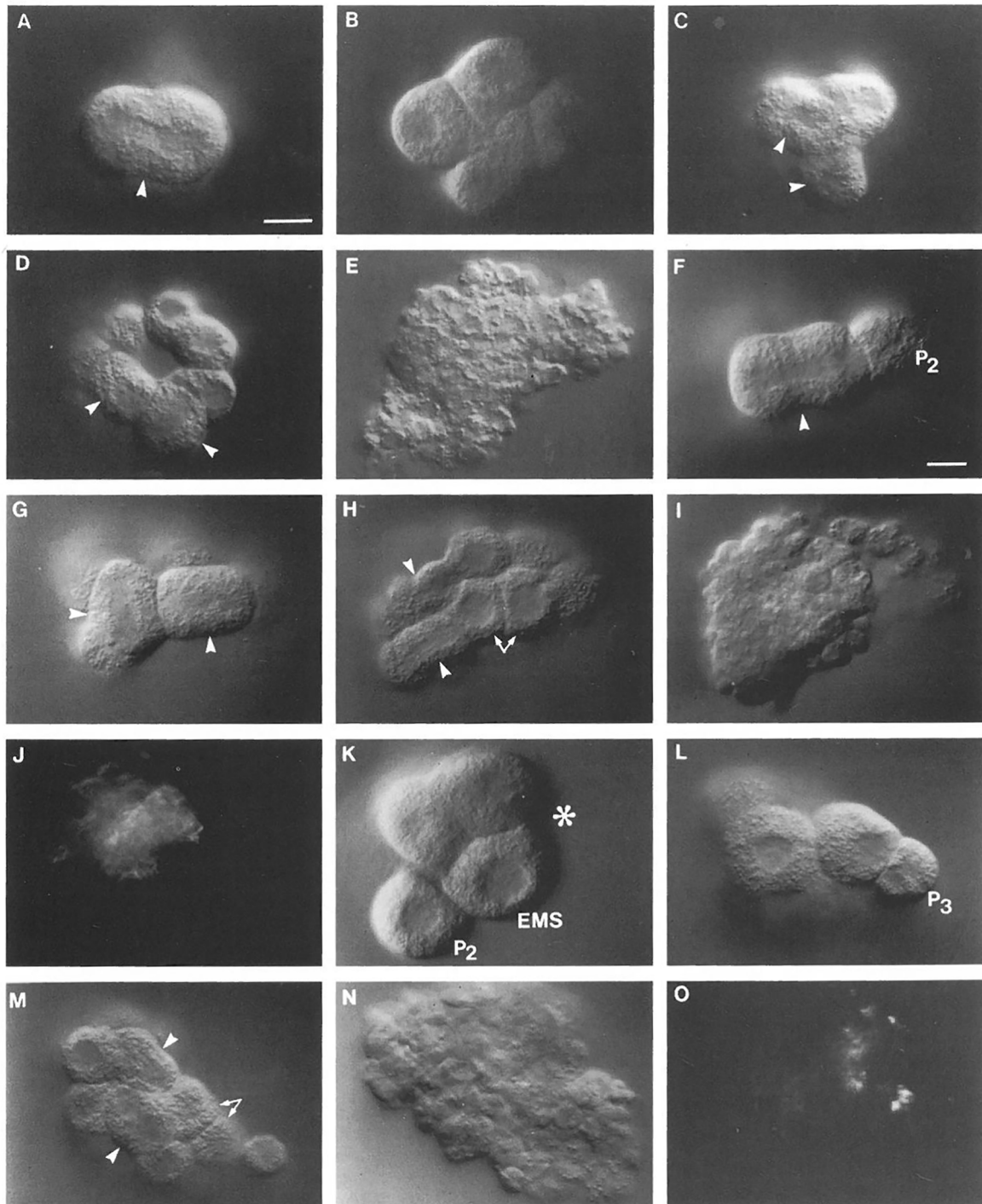


Fig. 3

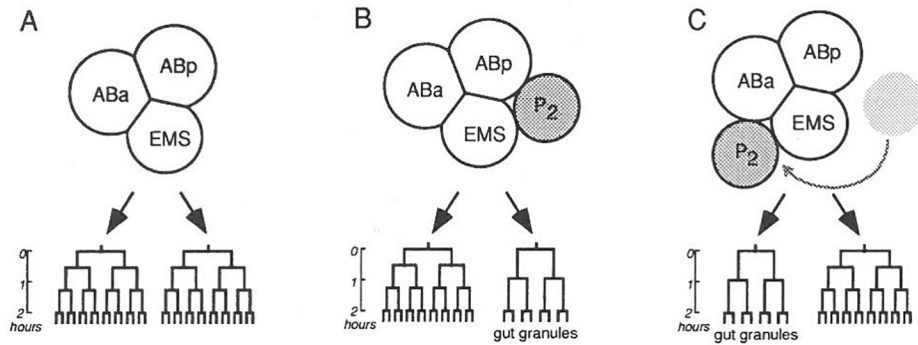


Fig. 4. P₂ removed from a four cell stage embryo (A), and P₂ removed and then placed back on the embryo either in the same position (B) or on the other side of EMS (C). Lineages and resulting gut differentiation, as assayed by the presence of birefringent rhabditi granules, diagram the experiments shown in Fig. 3. Lineages were followed for three to five divisions of EMS. Presence of gut granules was scored 12-24 hours into development, by which time more cell divisions had taken place than is shown in the lineages (see Fig. 3 for photos of embryos at this time). Note that one case of the manipulation diagrammed in B did not result as shown (see text for details). After P₂ was moved (C), the EMS cleavage plane formed approximately in the axis it normally does in three cases, and shifted obliquely 20-30 degrees in the fourth case. In all four cases, the more anterior daughter of EMS touched P₂. This daughter, which normally would have been MS, took on an E-like lineage timing and differentiated rhabditi granules.

Can the other cells of the four cell stage respond to gut induction?

Although the P₂ cell, which induces gut in EMS, also contacts ABp (Fig. 1), gut does not differentiate in any AB progeny. AB progeny may be prevented from forming gut in response to P₂ either because of the contents they inherit (for example, they may lack a necessary receptor) or because of inhibitory signals from the other cell of the four cell stage, EMS. In order to determine whether EMS inhibits AB progeny from responding to P₂, four cell stage partial embryos lacking EMS were generated. This was done by separating the cells of the four cell stage and then placing P₂ in contact with ABa and/or ABp. Gut did not differentiate from this combination (0 out of 6 cases, see Fig. 5). Although the lack of gut differentiation is a negative result, it is unlikely that it is caused by damage; ABa and ABp continued to cleave with normal timing after the manipulation, and in a similar experiment in which P₂ and EMS were combined, gut differentiated in every case (Goldstein, 1992). Hence the result indicates that AB progeny intrinsically lack the competence to respond to P₂.

If direct cell-cell contact is required for a cell to respond to gut induction, then in a normal embryo P₂ may not experience an induction signal. For this reason the ability of P₂ to respond to gut induction was also tested. P₂ cells were isolated from embryos at various times during the four cell stage and were placed in contact with each other in pairs. Gut did not differentiate in any descendants of the eight P₂ cells tested, indicating that P₂ cannot form gut in response to the induction.

The role of cell contact at the two cell stage

The possibility that segregation of gut fate may also require a cell interaction at the two cell stage was tested by separating AB and P₁. Gut differentiation was assayed in each isolate 12-24 hours later (see Materials and Methods). Note that the E lineage derives from the P₁ cell of the two cell

stage (Fig. 1B). Cells continued to divide in the normal order after the separation. Gut usually differentiated (13 out of 16 cases) in some of the progeny of each isolated P₁ cell (Fig. 6A-C), suggesting that P₁ does not require contact with AB for gut to differentiate. Because previous work has shown that a cell interaction can occur between sister cells, with specification occurring within minutes after the cells form

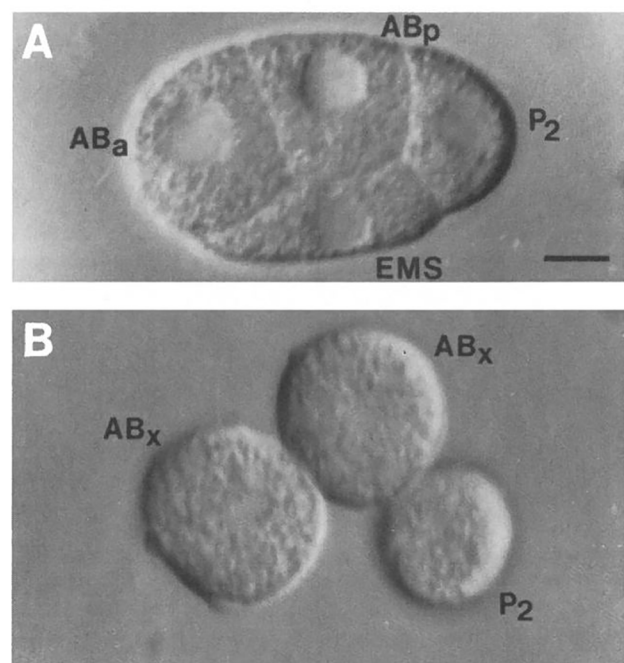


Fig. 5. Four cell separation followed by recombination of AB's daughter cells and P₂. (A) four cell stage. (B) ABa, ABp, and P₂. Note that ABa and ABp are labelled ABx as these cannot be distinguished from each other after isolation. Rhabditi granules did not develop from this combination (data not shown). Scale bar, 10 μm.

(during the four cell stage; Goldstein, 1992), the time when each separation was done was examined to see if isolating P₁ early in the two cell stage prevents gut from differentiating. This time was determined by recording the time when

each separation was done and also the time when P₁ cleaved. The time when first cleavage had occurred was also recorded when possible and estimated in other cases (see Materials and Methods) to be sure that the operation did not alter the

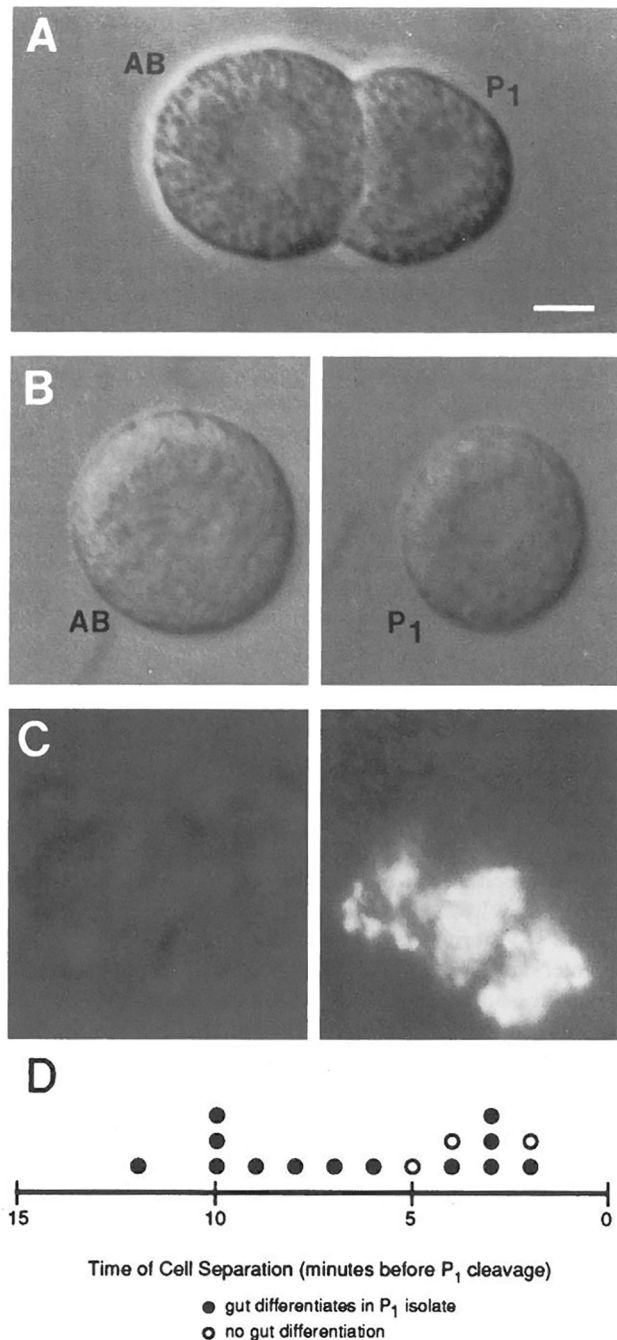


Fig. 6. Cell isolation during the two cell stage. (A) The two cell stage. (B) AB and P₁ separated. (C) The progeny of the AB and P₁ isolates 12-24 hours later, under polarized light to visualize gut-specific rhabditin granules, which have developed in some progeny of P₁ but not in AB progeny. (D) Diagram showing when each separation was done and whether rhabditin granules developed in the P₁ isolate. Each circle represents one case. Note that separations cannot be done in the first 3 minutes after cytokinesis begins in P₀, as the cleavage furrow will retract. Scale bar, 10 μ m.

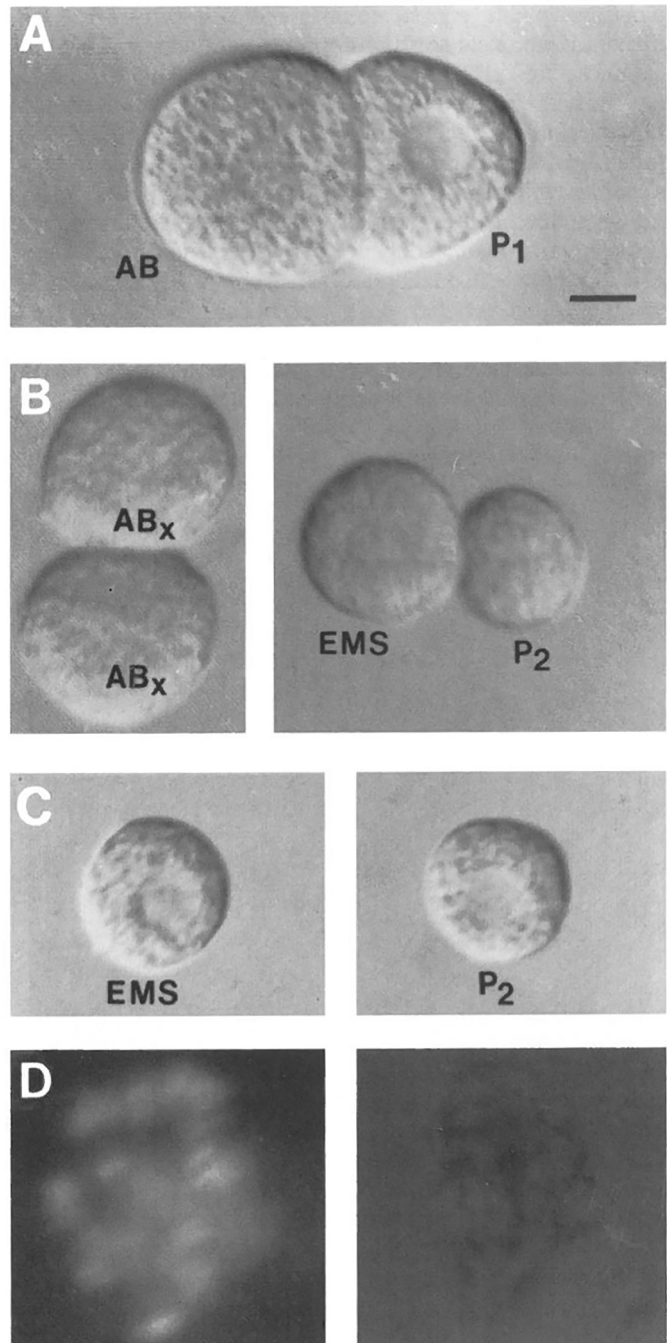


Fig. 7. Cell isolations during the two and four cell stage. (A) The two cell stage. (B) AB and P₁ isolated; each has divided once. ABa and ABp are both labelled ABx as these cannot be distinguished from each other. (C) P₂ and EMS separated from each other. (D) The progeny of P₂ and EMS 12-24 hours later, under polarized light. Rhabditin granules developed in some progeny of EMS but not in P₂. This occurred in all six cases; AB-P₁ separations were done at 8, 9, 9, 10, 10, and 12 minutes before P₁ cleavage. Scale bar, 10 μ m.

length of the cell cycle. As shown in Fig. 6D, gut differentiated in the P₁ isolate even when the separation was done at the beginning of the two cell stage, indicating that gut-forming potential arises in the P₁ cell independently of contact with AB. Gut never differentiated in the progeny of isolated AB cells (0 out of 16 cases) although cell divisions continued with normal timing, indicating that the AB lineage is prevented from forming gut independently of contact with P₁.

While the above results suggest that contact with AB is dispensable for gut to be specified in P₁'s progeny, AB might play a role in restricting gut fate to EMS, one of P₁'s daughter cells. For example, AB, which is anterior to P₁, might induce a localization of gut-forming potential into P₁'s anterior side and hence into P₁'s anterior daughter (EMS), preventing the posterior daughter (P₂) from also forming gut. To determine if this occurs, P₁ was isolated from AB and then, 10–15 minutes after P₁ had divided, EMS was separated from P₂ to see if gut differentiates in one or both isolates. Both cells continued to divide with normal

timing. Gut differentiated only in the EMS isolate (6 out of 6 cases), regardless of when the two cell separation was done (Fig. 7), demonstrating that the localization of gut potential into EMS occurs independently of cell contact at the two cell stage.

The role of contact between E and other cells of the eight cell stage

In order to determine whether contact between E and its sister cell, MS, is required for only the E lineage to form gut, cells were separated from each other during the last 10 minutes of the four cell stage. After EMS divided, its two daughter cells were separated from each other and were cultured individually. Gut usually differentiated from only one of EMS's daughters (10 out of 13 cases; in the other three, gut did not differentiate from either daughter). This result suggests that contact between E and MS is not necessary for specification of gut in the E lineage. The time of each separation was also examined to check the possibility that contact between E and MS might be necessary briefly at the beginning of the eight cell stage. Fig. 8 shows that gut differentiation occurred in one of EMS's daughters even when cells were separated just after EMS had divided. This result indicates that contact between E and MS is not necessary for specification of gut potential in E, nor for the prevention of gut differentiation in MS.

DISCUSSION

Previous work has shown that an induction is involved in gut specification: in order for gut to differentiate from EMS's E lineage, EMS must contact P₂ during the four cell stage (Goldstein, 1992). In this paper, the role of this induction in positioning gut fate into the E lineage was determined, and a search was done for other cell interactions involved in gut specification. It was found that, (1) while P₂ normally resides at EMS's posterior end and induces gut in EMS's posterior daughter, any side of EMS can form gut when apposed to P₂. Hence the future E and MS sides of EMS are equivalent until the induction makes E different from MS. (2) EMS does not inhibit ABp from responding to P₂; ABp (as well as ABa) intrinsically lacks the competence to form gut in response to P₂. (3) P₂ cannot form gut in response to induction from another P₂. (4) Contact between AB and P₁ during the two cell stage is dispensable for segregation of gut fate into P₁ and then into EMS. (5) Contact between E and MS is dispensable for segregation of gut fate to E. Based on these results a model is presented for the roles of induction and segregation of cell contents in establishing gut fate in the E lineage (Fig. 9). During first and second cleavages, P₁ and then EMS inherit some factor(s) (indicated by shading in Fig. 9) which give EMS and only this blastomere the competence to respond to induction and form gut. P₂ then induces gut fate in the side of EMS that it contacts (indicated as the black part of the shaded area) establishing E as the site from which gut differentiates.

Generation of asymmetry in the zygote

First cleavage generates two cells with distinct cell fates, presumably as a result of determinants segregated to partic-

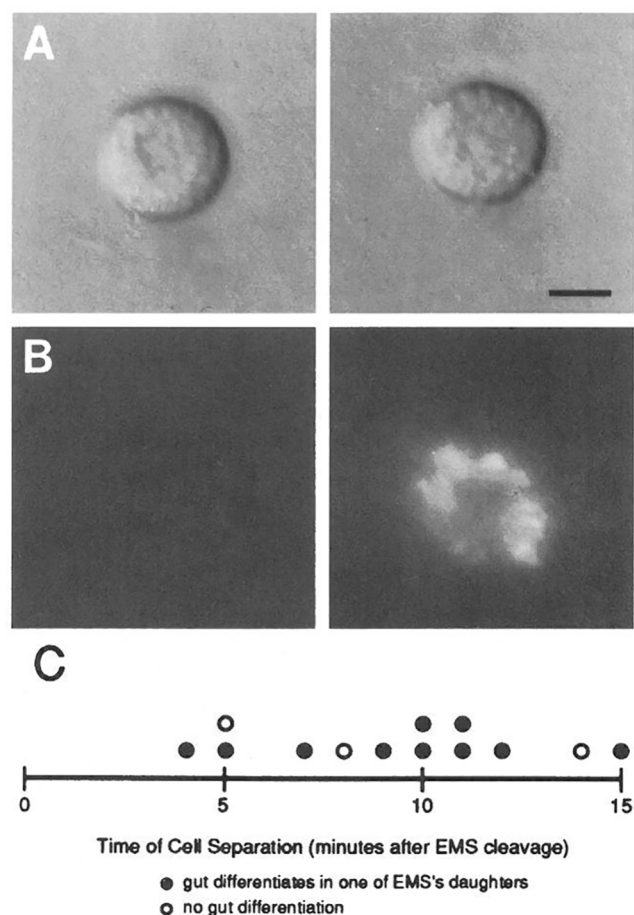


Fig. 8. Gut differentiation after separating EMS's daughter cells from one another. (A) EMS's two daughter cells. (B) The progeny of the two isolates the next day under polarized light. Gut has differentiated in only one. (C) Diagram showing when each separation was done and whether rhabditiin granules developed in one daughter. Each circle represents one case. In no cases did both daughters form gut. Note that separations cannot be done in the first three minutes after cytokinesis begins in EMS, as the cleavage furrow will retract. Scale bar, 10 μ m.

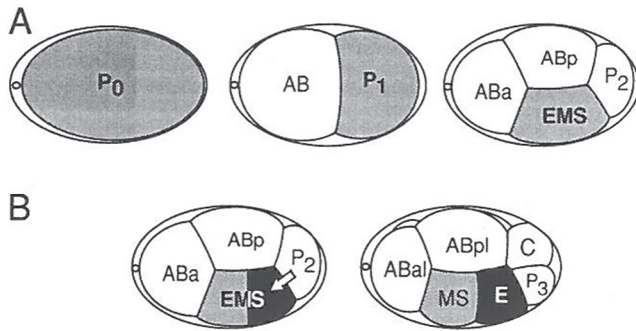


Fig. 9. Model for specification of gut in the E lineage. (A) 1, 2, and 4 cell stages. Shading indicates proposed factor(s) that confer on EMS the competence to respond to gut induction. Such factors might be localized as shown, or they might be localized to the future EMS-forming region of the embryo as early as the zygote or two cell stage. (B) P_2 induces gut fate (black shading) in the side of EMS it contacts, and therefore in the E lineage.

ular parts of the cell before the cleavage. A segregation event is known to occur before first cleavage, as the pseudo-cleavage furrow forms and the anterior membrane contracts (Nigon, 1960), the pronuclei migrate toward each other (Albertson, 1984), P granules are redistributed posteriorly (Strome and Wood, 1982, 1983), and cortical and cytoplasmic flows occur (Hird and White, 1993). However no segregated determinants have yet been found.

Work on gut specification suggests that AB and P_1 inherit distinct cell fates independent of later cell interactions, as isolating the cells from each other does not prevent them from differentiating normally. The gut derives from only P_1 progeny. When P_1 was isolated by lysing AB, P_1 progeny still formed gut (Laufer et al., 1980; Edgar and McGhee, 1986). Experiments presented here contribute to this argument, by showing that progeny of P_1 can form gut when physically separated from AB early in the two cell stage.

Previous evidence for gut determinants

Previous work has demonstrated that exposing a non-E nucleus to gut precursor cytoplasm can cause, in other lineages, nuclear activity necessary for gut differentiation: while gut will not differentiate from an enucleated P_1 , fusing an enucleated P_1 cell to one of AB's daughters often allows gut to differentiate, despite the lack of a gut precursor nucleus (Wood et al., 1984). The result suggests that at least one necessary component of the gut differentiation pathway is localized in gut precursor cells. The *par* genes are required for partitioning of cytoplasmic components in early embryos. The finding that mutations in two of these, *par-1* and *par-4*, lack gut granules also suggests that localization of a determinant is required for gut specification (Kemphues et al., 1988; Morton et al., 1992). These results are also consistent with induction being involved in gut specification, as the important cellular components, which are localized, may be either determinants or factors that confer on a cell the ability to respond to an induction. As induction has been found to be required for gut to differentiate in EMS's E lineage (Goldstein, 1992), what is localized in P_1 and then EMS could be a gut determinant that requires the induction to cue its concentration past a threshold in one side of EMS,

or it could be a factor that confers upon EMS the competence to respond to the induction.

Specification of EMS fate

It is not known when the ability to form gut in response to the induction is localized to the future EMS-forming region of the zygote. This could occur during the cytoplasmic rearrangement that occurs during pronuclear migration in the uncleaved zygote (Nigon et al., 1960; Hirsh et al., 1976; Strome and Wood, 1983; Strome, 1986; Hird and White, 1993), in a similar way to what probably occurs in ascidian embryos for factors that allow cells to respond to neural induction (Conklin, 1905; Rose, 1939). Alternatively, localization into EMS could occur sequentially at each cleavage, as is the case for P granule localization into the P_4 cell (Strome and Wood, 1983). Experiments in which parts of the zygote were extruded from the eggshell after the zygote's cytoplasmic reorganization (Laufer and von Ehrenstein, 1981; Schierenberg, 1987, 1988) address this issue. Although the region of the zygote that will form EMS has never been removed in a case where gut differentiation can be assayed, the part of the egg posterior to this region (the posterior 15-20% of the egg) has been removed (Laufer and von Ehrenstein, 1981). In this experiment it is unlikely that EMS received the cytoplasm it normally would have received from the zygote. Nevertheless, a normal worm developed, suggesting that the proposed factor(s) that confer on EMS the ability to respond to gut induction are not yet restricted to the future EMS-forming region in the zygote. Alternatively, such factor(s) could be bound to a cytoskeletal network that cannot be extruded by this method.

The *skn-1* gene product may encode an EMS determinant (Bowerman et al., 1992a). In *skn-1* mutants, EMS cell fates are altered in a way that mirrors P_2 cell fates. Additionally, ABa progeny do not make pharyngeal muscle, however this may be due to the respecification of EMS fate, as EMS is known to be required for ABa progeny to make pharyngeal muscle (Priess and Thomson, 1987). The *skn-1* gene encodes a protein with sequence similarity to the basic regions of bZIP transcription factors (Bowerman et al., 1992a).

In *pie-1* mutant embryos, P_2 takes on an EMS-like fate (Mello et al., 1992). In these embryos gut is specified in both EMS-like cells in the absence of a P_2 -like cell. There are several possible explanations for this finding. One is that the mutation may cause P_2 to differentiate as an EMS-like cell, but still retain the ability to induce gut in EMS. Another is that the mutation causes both EMS and P_2 to bypass the requirement for gut induction. Alternatively, EMS may normally possess the ability to induce gut in another EMS, such that two EMS-like cells would induce gut in each other. Consistent with this last hypothesis, in four cell stage *pie-1* embryos the sides of the EMS-like cells that will form the gut face each other.

Evidence for the P_2 -EMS interaction

An alternative explanation for the finding that gut does not form when EMS is isolated (Goldstein, 1992) is that EMS is simply damaged, such that gut will not form. Previous results made this an unlikely explanation as only the P_2 cell has the ability to rescue gut fate in an early-isolated EMS,

and in the absence of P₂, E does not stop dividing and it does not divide erratically; it continues to divide with the timing of its sister cell, MS (Goldstein, 1992). This alteration in cell cycle timing also occurs in the mutants that do not form gut (Morton et al., 1992; T. Stiernagle, D. C. Sigurdson, and J. Shaw, personal communication). Results presented here show additionally that contact with P₂ is sufficient for gut specification: the presumptive MS side of EMS can also form gut if placed in contact with P₂.

Inducing a cell fate change in part of a single responding cell

Results suggest that only one of EMS's two daughter cells has its fate altered by the induction. In the absence of the inducing cell both daughters take on MS-like lineage timings, and in the presence of the inducing cell one daughter keeps MS-like lineage timings, while the other takes on an E-like lineage and forms the gut. Hence only part of EMS, a single responding cell, has its fate altered by the induction. This and the finding that the induction occurs between sister cells within a single cell cycle (Goldstein, 1992) raises the possibility that gut induction may occur by a qualitatively different mechanism than vertebrate embryonic inductions, which affect the fates of large numbers of cells and take hours or days (see Jacobson, 1966, and Jones and Woodland, 1987 for examples.) As inductions in invertebrates tend to occur between small numbers of cells, it is possible that some others might occur with a timing similar to *C. elegans* gut induction, however little is known about the timing of invertebrate inductions. Bowerman et al. (1992b) have recently reported that in *C. elegans* P₂ also induces development of the ABp-derived intestinal valve cells, at about the same time as gut induction.

It is striking to note that many fate alterations produced in early *C. elegans* development, by manipulations or mutations, involve changes in the identities of early blastomeres, rather than between particular tissue types. In *skn-1* mutants, EMS takes on a P₂-like fate (Bowerman et al., 1992a), in *pie-1* mutants P₂ takes on an EMS-like fate (Mello et al., 1992), and in *mex-1* mutants the four daughters of AB take on MS-like fates (Mello et al., 1992). Cell manipulations described here suggest that the presumptive E cell can take on an MS-like fate and that the presumptive MS cell can take on an E-like fate. The alterations in identities of early blastomeres may indicate that rather than making early cell fate decisions about tissue types, the embryo makes early cell fate decisions about the identity of early blastomeres, and the diversity of tissue types among the descendants of each blastomere is generated later.

Prospects for determining the mechanisms involved in induction

Some gene products that may act in the gut specification pathway have already been identified by mutation analysis (Bowerman et al., 1992; Mello et al., 1992; Morton et al., 1992; T. Stiernagle, D. C. Sigurdson, and J. Shaw, personal communication) and by deletion analysis of the upstream promoter of a gut-specific gene (Aamodt et al., 1991). The ability to isolate and recombine cells may aid in dissecting the mechanics that underlie gut induction. The methods used

here may also be useful in identifying the bases for the specification of other cell types in early *C. elegans* development.

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