Induction of gut in *Caenorhabditis elegans* embryos

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TWO types of developmental events can cause an embryonic cell to adopt a fate different from that of its neighbours: during a cell division particular contents may be segregated to only one daughter cell and cells may experience different external cues, commonly in the form of inductive cell interactions. Work on development in the nematode *Caenorhabditis elegans* suggests that most cell fates are specified without a need for cell interactions. In particular, the gut cell lineage of *C. elegans* has been used as a primary example of specification by differential segregation of determinants[1]. Here I re-examine the role of induction in gut specification by isolating early blastomeres. In *C. elegans*, the gut derives from all the progeny of a single blastomere (E) of the eight-cell stage[2]. When a gut precursor cell (EMS) is isolated during the first half of the four-cell stage, gut does not differentiate. Gut differentiation is rescued by recombining EMS with its posterior neighbour (P_2), but not by recombining EMS with one or both of the other two cells of the four-cell stage. These results demonstrate that P_2 induces EMS to form gut in *C. elegans*.

Cells were isolated from the influence of their neighbours by removing egg shells from cleavage-stage embryos and physically separating cells from each other (Fig. 2 legend). After isolating cells during the four-cell stage, gut often differentiated from the EMS isolate (21 out of 40 cases) but never from the P_2, ABa or ABp isolates (Figs 1 and 2). An investigation of the timing during the four-cell stage of each isolation suggests that EMS may need to contact one or more of its neighbours early in the four-cell stage for gut to differentiate: EMS cells isolated during the first half of the four-cell stage never differentiated gut, whereas those isolated during the second half of the four-cell stage often did (Fig. 3*a*). In EMS isolates that did not differentiate gut, the normal slowing of E-cell cycles after one division did not occur; the E lineage divided synchronously with the MS lineage (Fig. 4). This suggests that in the absence of the induction E may take on the fate of MS; however, differentiation of other tissues has not yet been assayed in uninduced EMS cells. This alteration in the timing of the E-lineage cell cycles also occurs in mutants that do not form gut (J. Shaw and D. Morton, personal communication.)

The finding that gut will not differentiate if EMS is isolated early in the four-cell stage suggests that during the four-cell stage a cell interaction occurs that specifies gut in EMS. Alternatively, separating cells from each other early in the cell cycle may damage cells so that although EMS continues to cleave, it will not form gut. To distinguish between these two possibilities, cells were separated from each other during the first half of the four-cell stage, and then within one to two minutes all four cells were placed back in contact with each other. If separating cells simply damages EMS, then gut should not differentiate. If, however, separating cells prevents them from interacting to specify gut, then recombining cells should allow the interaction to continue, and gut should differentiate. In such an experiment, gut differentiates (5 out of 5 cases). Thus EMS requires contact with one or more of the other cells during the four-cell stage in order for its E lineage to differentiate gut.

To determine which of the other three cells induce gut in EMS, cells were separated during the four-cell stage and EMS was then recombined with either P_2, or one or both of ABa and ABp. When cells were separated from each other during the four-cell stage and EMS was immediately placed back in contact with one or both of ABa and ABp, gut differentiation was not rescued (Fig. 3*b*). When cells were separated from each other during the four-cell stage and EMS immediately placed back in contact with P_2, gut differentiated in every case (Fig. 3*c*). These experiments demonstrate that P_2, which normally sits at the posterior end (the 'E' end) of EMS, induces EMS to form gut in its E lineage.

These results should not be misconstrued as proof that segregation of determinants plays no part in gut specification. Although it remains to be tested, it is possible that segregation may localize gut potential to EMS, and the induction may then act as a positional cue to make the two daughter cells of EMS differentiate along different pathways. These experiments do, however, demonstrate that there is an induction during the four-cell stage that is necessary for specification of gut.

It has previously been suggested[3] that an interaction between P_2 and EMS specifies gut: out of six cases in which a P_2 cell was removed from a four-cell embryo, in none did gut differentiate. It was not clear, however, whether removing P_2 prevented an interaction with EMS or simply damaged EMS, and in a similar experiment[4] gut in fact usually differentiated.

The original suggestion that gut specification occurs independently of induction derives from experiments in which cells were isolated by applying pressure on embryos and then individuals were picked out in which all cells except the cell of interest were lysed[5,6]. When the E cell or its precursors were isolated by this method at the two-, four-, or eight-cell stage, gut often differentiated[5,6]. This result suggested that gut is specified independently of cell interactions and thus solely by virtue of determinants segregated into E during the divisions leading to its formation. But because the time in the cell cycle when cells were isolated was not noted, this method could not detect the type of interaction described here, which occurs between sister cells soon after their formation.

Several cells are specified by cell interactions during late embryonic and post-embryonic development in *C. elegans* (reviewed in ref. 7). Interactions are also necessary during early embryonic development to specify the fates of several cells in

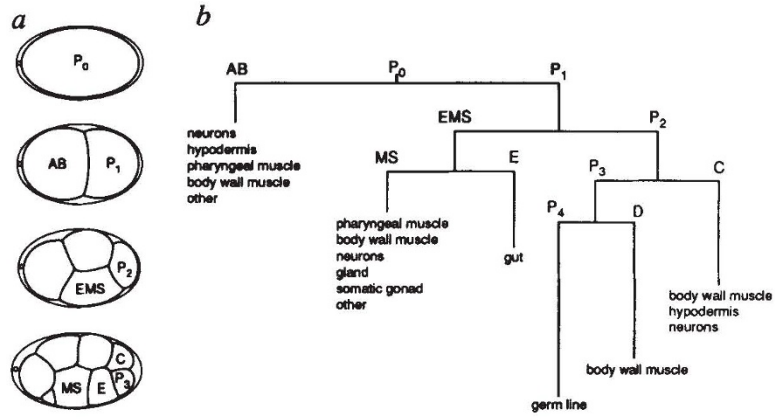

FIG. 1 Early development and segregation of fate in *C. elegans*. *a*, Early development; 1, 2, 4 and 8 cell stages. Names of blastomeres are shown for all cells except progeny of AB. *b*, Segregation of fate, showing the six founder cells and the tissues to which each contributes (after ref. 2).

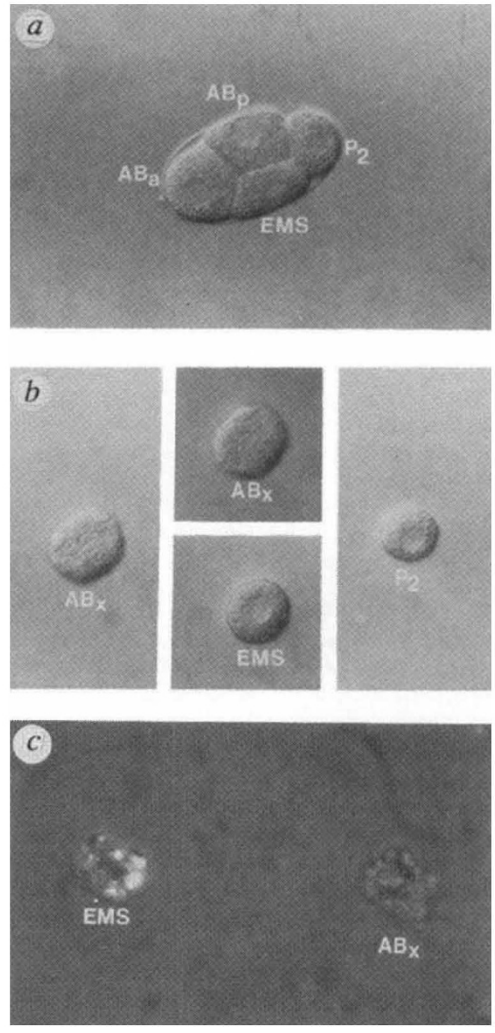

FIG. 2 Cell isolation at the four-cell stage. *a*, Intact four-cell stage embryo. *b*, Four cells after isolation. ABa and ABp are labelled ABx after the separation as these cannot be distinguished from each other. Nuclei have broken down in ABx cells in preparation for cell division. *c*, EMS and ABx isolates 12 h later under polarized light. Birefringent rhabditin granules have developed in the EMS isolate but not in the ABx isolate.

METHODS. Animals were maintained by standard techniques[11] at 21.5 °C. Gravid hermaphrodites were cut in egg salts[6] on a depression slide to release embryos. Early cleavage stages were collected and transferred to a 15% solution of hypochlorite (Aldrich) in egg salts. After 3 min, embryos were transferred through two changes of embryonic growth medium (EGM, developed by L. Edgar, personal communication; methods available on request) and then to a solution of 20 units per ml chitinase (Sigma), 20 mg ml^{-1} *a*-chymotrypsin (Sigma, type II), 1% penicillin–streptomycin solution (Gibco). After 6–9 min, as the eggshells softened and cells began to round up, an equal volume of trypsin inhibitor (10 mg ml^{-1}) (Worthington) in EGM was added. After 30 s, embryos were transferred through two more changes of EGM. The vitelline membrane was removed in EGM by drawing each embryo in and out of a pulled glass needle (glass capillary tubes, A-M Systems) whose tip had been cut to an open diameter slightly shorter than the short axis of the embryo. Capillary tubes were siliconized in Prosil-28 (PCR, Inc.) before pulling. Embryos allowed to develop unperturbed from this point differentiate gut in 92% of all cases ($n = 57/62$.) Cells were separated from each other as a result of the turbulence created by drawing each embryo several times in and out of this needle or one of a slightly larger tip diameter. In about half of these cases, two or more cells lysed and the embryo was discarded; the remainder were used. Cells removed from the egg shell invariably divided in the normal order. In more than 95% of all cases, ABa and ABp divided synchronously, EMS divided 3–4 min later, and P_2 divided 3–5 min after EMS. Thus after separating cells and identifying them on the basis of size, division order was a useful check to see that no mistakes had been made. Embryos were disaggregated one at a time and were kept separately to avoid confusing cells. The time in the cell cycle when EMS was isolated was established by recording the time of separation and then the time at which cytokinesis began in EMS. As only some embryos in each batch can be used because many are damaged, and manipulations must be rapid, it is not feasible to record the time of P_1 cleavage as well for every embryo. This time has been at least estimated for every embryo: while preparing to remove egg shells, embryos were arranged in order of age as AB and P_1 divided in each embryo. This method allows one to know when P_1 divides in a few embryos, and then to recognize embryos that develop much more slowly or quickly than the few recorded. In this way the EMS cell cycle in disaggregated embryos can be estimated at about 15 to 20 min, compared with about 15 min for embryos inside their eggshells. In the cleavages that follow, cells continue to divide in the proper order, only up to twice as slowly as embryos inside their eggshells. Differentiation of gut was scored by checking for the presence of rhabditin granules, a gut-specific differentiation marker[5,12], under polarized light at 8–24 h after first cleavage.

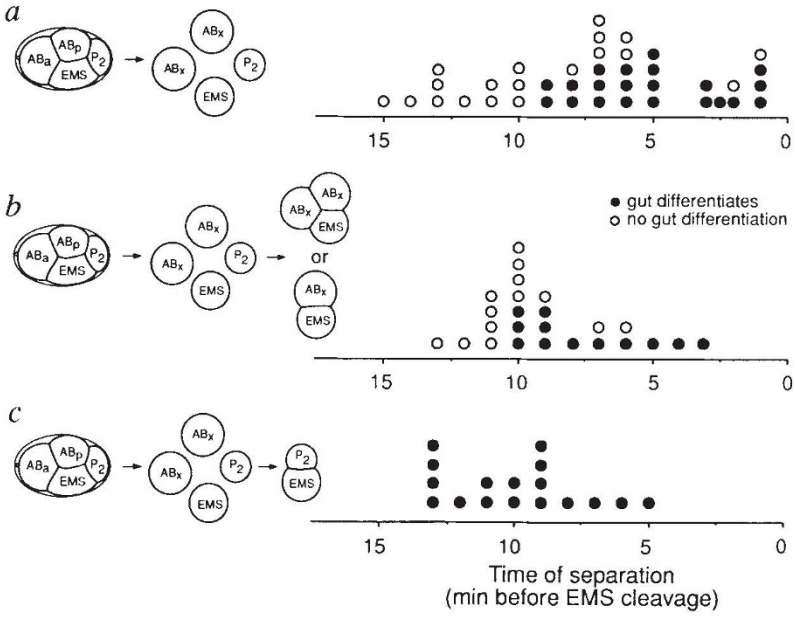

FIG. 3 Results of isolation and recombination experiments. Figures on the left show the experiments and histograms on the right show the results. Each circle in the histograms represents one case; cases are stacked vertically over the time at which each separation was done. Time lines each represent the entire four-cell stage. Time of separation was determined as described in Fig. 2 legend. *a*, Gut differentiation after isolating EMS at various times throughout the four-cell stage. *b* and *c*, Gut differentiation after isolating EMS at various times throughout the four-cell stage and then (within 1–2 min) recombining EMS with either one or both of ABa and ABp (*b*) or with P_2 (*c*). ABa and ABp are both labelled ABx after a separation, as these cannot be distinguished from each other. Cases where EMS was recombined with only one ABx cell and gut did not differentiate are at 12 min (one case) and 11 min (two cases) before EMS cleaved, and at 10 (three cases), 9 (one case), 8 (one case), 5 (one case) and 3 (one case) min before EMS cleaved in which gut did differentiate. In all other cases in *b*, EMS was recombined with both ABx cells. As expected, in both recombination experiments (*b*, *c*) gut differentiated in most cases if the separation was done late in the four-cell stage, as results in *a* indicate that gut is induced by this time. Thus the results from separation and recombination experiments during the first half of the four-cell stage indicate whether or not, after isolating EMS, gut differentiation was rescued by the recombination.

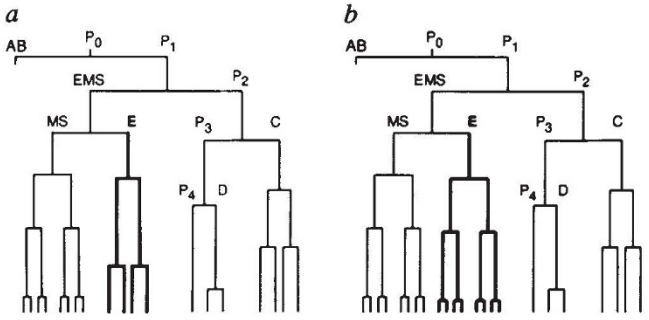

FIG. 4 *a*, Partial lineage of intact embryo. *b*, Partial lineage of embryos in which P_2 and EMS were separated and gut did not differentiate, showing the relative order of division of cells. The E lineage takes on MS-like cleavage times when the induction is blocked. (9/9 cases observed, from the 19 that did not differentiate gut, shown in Fig. 3*a*.)

the AB lineage[4,8,9]. No other lineages were previously known to require cell interactions during early development.

Two pieces of information raise the possibility that many other inductions may be occurring during early *C. elegans* development. First, this work shows that previous methods could not detect all cell interactions. Second, most issues in *C. elegans* arise from many distantly related cells, not from the progeny of a single founder cell[2] (Fig. 1*b*); segregation of determinants would have to be extraordinarily complex to generate this sort of lineage independently of inductions, considering that determinants for at least several cell types are not prelocalized in the egg[10].

Given the organism's amenability to genetic analysis, the finding that gut is induced and the possibility that many other inductions may be occurring in *C. elegans* may afford us a chance to understand the molecular mechanisms that underlie embryonic inductions. □

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The rate of actin-based motility of intracellular *Listeria monocytogenes* equals the rate of actin polymerization

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THE Gram-positive bacterium *Listeria monocytogenes* is a facultative intracellular pathogen capable of rapid movement through the host cell cytoplasm[1]. The biophysical basis of the motility of *L. monocytogenes* is an interesting question in its own right, the answer to which may shed light on the general processes of actin-based motility in cells. Moving intracellular bacteria display phase-dense 'comet tails' made of actin filaments, the formation of which is required for bacterial motility[2,3]. We have investigated the dynamics of the actin filaments in the comet tails using the technique of photoactivation of fluorescence, which allows monitoring of the movement and turnover of labelled actin filaments after activation by illumination with ultraviolet light. We find that the actin filaments remain stationary in the cytoplasm as the bacterium moves forward, and that length of the comet tails is linearly proportional to the rate of movement. Our results imply that the motile mechanism involves continuous polymerization and release of actin filaments at the bacterial surface and that the rate of filament generation is related to the rate of movement. We suggest that actin polymerization provides the driving force for bacterial propulsion.

L. monocytogenes can move in many cell types, and we chose to infect the flat and easily injectible potoroo kidney epithelial (PtK2) cell line. As reported[1], *L. monocytogenes* induced the formation of actin-filament-rich phase-dense comet tails and moved at rates of up to 0.4 μm per second through the cytoplasm of these cells. The tails appear substantially shorter by phase contrast than by rhodamine–phalloidin labelling, which marks filamentous actin (Fig. 1*a*, *b*). Fluorescence intensity profiles of phalloidin-labelled tails reveal that there is a pronounced gradient of actin filament density through the tail, such that the filament density is highest closest to the bacterium and decreases exponentially towards the distal top (Fig. 1*c*). The tail is visible by phase microscopy as long as 30–50% of the peak density of actin filaments in the tail persist.

To probe *L. monocytogenes* tail actin filament dynamics, infected cells were microinjected with purified rabbit skeletal muscle G-actin covalently coupled to caged resorufin (CR)[4]. CR-actin is nonfluorescent and readily incorporates into endogenous actin structures in microinjected cells, including stress fibres and lamellipodia. Upon illumination with ultraviolet light at 360 nm, CR is rapidly and efficiently converted to the bright red fluorescent parent compound, resorufin. The movement and turnover of activated labelled actin filaments can then be followed by fluorescence videomicroscopy[4]. Short segments of the actin tails of moving *L. monocytogenes* were photoactivated[5]. The marked filaments were imaged by fluorescence and the moving bacteria by phase-contrast; the two images were superimposed electronically. We marked the tails of 22 bacteria moving at rates ranging from 0.02 to 0.20 μm per second. In each case, the photoactivated mark on the tail remained stationary in the cytoplasm as the bacterium moved away from the mark (Fig. 2). Movement or splitting of the activated region was never observed; given the limits of sensitivity of this technique, at least 95% of the actin filaments in the tail are stationary in the cytoplasm as the bacterium moves. We conclude that filaments must appear continuously at the bacterium surface, and be released from the bacterium as it moves on, so the rate of bacterium movement is simply equal to the rate of actin filament appearance.

To investigate the stability of tail actin filaments, we measured the decay in intensity of the fluorescent mark under conditions of negligible photobleaching. Fluorescence decay was exponential, and the average turnover rate was 33 s (s.d. = 16, $n = 22$). By comparison, the average half-life of actin filaments in stress fibres in PtK2 cells is 230 s (ref. 4). There was no correlation between filament half-life and rate of bacterium movement