Developmental Determinants in Embryos of Caenorhabditis elegans[1]

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Abstract: *C. elegans* is proving useful for the study of cell determination in early embryos. Breeding experiments with embryonic lethal mutants show that abnormal embryogenesis often results from defective gene function in the maternal parent, suggesting that much of the information for normal embryonic development is laid down during oogenesis. Analysis of a gut-specific differentiation marker in cleavage-arrested embryos has provided evidence that the potential for this differentiation behaves as a cell-autonomous internally segregating developmental determinant, which is present from the 2-cell stage onward and is partitioned into the gut precursor cell during early cleavage divisions. Visible prelocalized cytoplasmic granules that segregate with a particular cell lineage have been observed in the embryonic germline precursor cells by fluorescent antibody staining. Whether these granules play a role in germline determina-

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More than 100 years ago, early European embryologists defined the enigma of embryonic development in terms of two kinds of questions. First, how is the sameness of cells and organisms maintained during development and reproduction, and what factors transmit this hereditary information? Second, how do the cells of an embryo become different; what factors dictate that a particular cell at a particular time and position becomes committed to a particular developmental pathway?

In the intervening century, the extensive information we have acquired about the genetic machinery and how it works has largely answered the first question. In contrast, we have gained little new understanding of the epigenetic mechanisms responsible for temporal and positional control of cell determination in embryos. How this process operates remains a central problem of contemporary developmental biology.

EMBRYONIC DEVELOPMENT

In several respects *C. elegans* provides an excellent experimental system with which to approach questions of early embryogenesis (1). The embryos are transparent and develop rapidly, giving rise to first-stage larvae with only 549 cells. Although inconvenient for physical manipulation, they are well suited for analysis by light and electron microscopy. Moreover, the organism is convenient for genetic analysis, so that mutations that perturb embryogenesis can be easily isolated and studied for clues to normal developmental mechanisms.

From microscopic observations of embryogenesis in living specimens, the lineages and developmental fates of all the cells in the embryo are now known (4; J. Sulston and E. Schierenberg, personal communication). These relationships are shown for the first few cleavages in Figure 1. The zygote (P_0) undergoes a series of asymmetric divisions, each giving rise to a somatic precursor and a germline precursor (P) cell. Most of these divisions take place in the direction of the anterior-posterior axis, but several have dorsal-ventral or left-right components. The resulting positions of the cells at each stage, as well as the fates of their progeny, are invariant from embryo to embryo (4,9,10).

How does the same cell in every embryo become committed to the same developmental fate? We can propose two general kinds of mechanisms, either or both of which could explain these observations. 1) The fate of a cell may be determined by cues from outside that depend upon its position and/or upon interaction with neighboring cells in the embryo. 2) The fate of a cell may be determined in response to lineally

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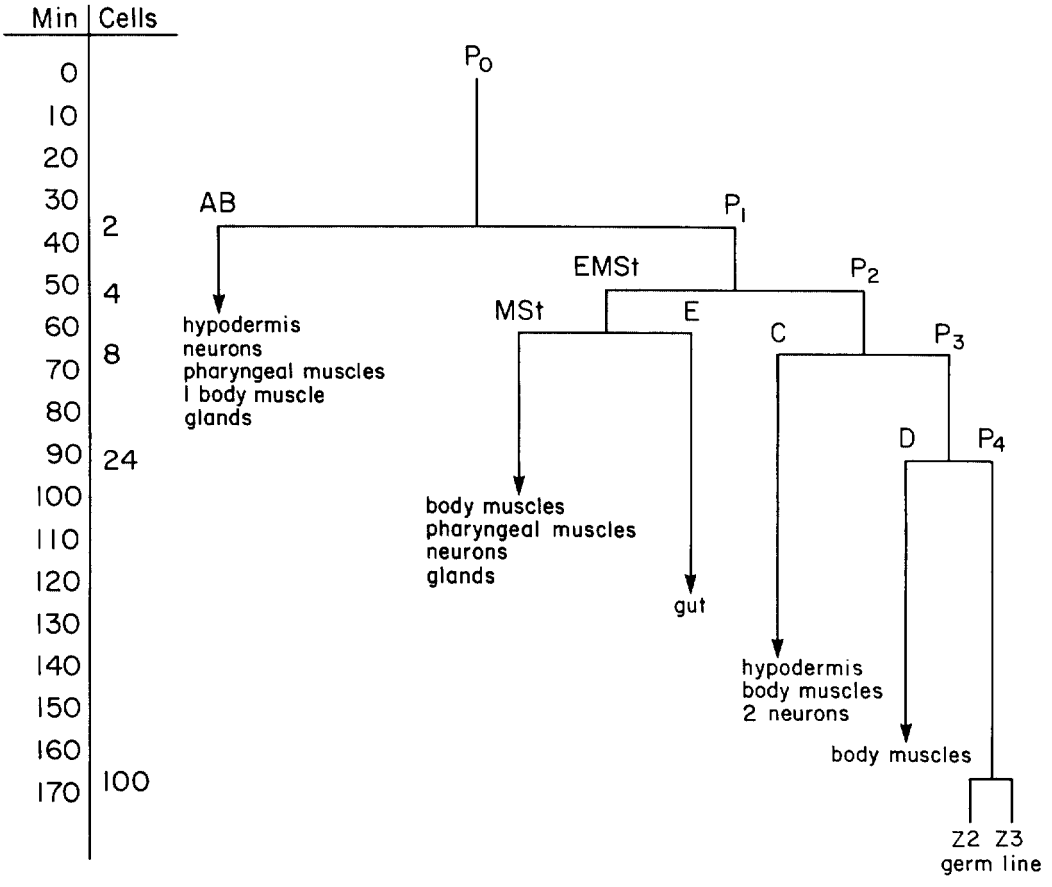


Fig. 1. Lineage relationships, timing, and progeny cell fates in the early cleavage divisions of *Caenorhabditis elegans* embryos at 25 C. Progeny cell fates are based on published (4,9) and unpublished data (J. Sulston and E. Schierenberg, personal communication).

transmitted, internally segregating determinants. Because the lineages, positions, and fates of cells in the embryo are invariant, causal relationships between these parameters cannot be deduced from observations of normal development, but must be investigated by perturbing the system.

MUTATIONAL PERTURBATION

Temperature-sensitive mutants of *C. elegans* that show lethal defects in embryogenesis at 25 C but not at 16 C have been isolated and characterized by D. Hirsh and collaborators (6,14). Genetic analysis showed that 21 out of 24 of these mutants define genes that show maternal effects; 11 are strict maternal mutants for which viability of the embryo depends only on the maternal genotype and is independent of the embryonic genotype (16). Similar results have been obtained with mutants isolated independently by G. von Ehrenstein and coworkers (2,12). Therefore, in *C. elegans,* as in most organisms, normal embryogenesis depends on many gene products made during maternal gametogenesis before embryonic development begins.

Several of the strict maternal mutants show striking morphological perturbations in the first two cleavages (Fig. 2), but then continue division to 100 cells or more before development arrests and the embryos die (16; N. Wolf and D. Hirsh, personal communication). Analysis of the defects in these mutants eventually could provide information about the normal developmental process. However, because the mutant embryos do not develop far enough to allow identification of differentiated tissues by morphology, such analysis requires more sophisticated means of assaying at early stages

for developmental potential and for the presence of specific macromolecules as markers for determinative events. In developing and exploiting such assays, we also have perturbed embryos chemically and physically, with results that provide information about the nature of at least one determinant for differentiation in a particular somatic cell lineage.

SEGREGATION

In experiments with the sea squirt *Ciona intestinalis,* Whittaker (15) showed that cytochalasin-treated or colchicine-treated early embryos, although arrested in cell division, nevertheless would produce enzymes characteristic of specific differentiated tissues approximately on schedule. Furthermore, production of these marker enzymes was restricted to the correct tissue progenitor cells. Such experiments provide a means of assaying the differentiation potential of cells at early cleavage stages.

In similar experiments with *C. elegans,* we determined the potential of embryonic cells to express a gut-specific differentiation marker after blocking cleavage of early embryos with cytochalasin B and colchicine. As a marker we used the so-called rhabditin granules (3), fluorescent refractile bodies containing tryptophan catabolites and normally appearing in gut cells at the 100-cell stage of embryogenesis. All the gut cells in *C. elegans* are derived from the E cell, which arises at the third embryonic cleavage as a descendant of the P1 and EMSt cells (Fig. 1). When embryos were blocked at the 2-cell stage, gut granules developed only in the P1 cell. When embryos were blocked at the 4-cell and 8-cell stages, granules developed only in the EMSt cell and the E cell, respectively. The same results were observed in cleavage-blocked partial embryos, in which one or more cells were ablated at the 2-cell, 4-cell, or 8-cell stages (Fig. 3). Therefore, the potential for this gut-specific differentiation depends neither on cell division after the 2-cell stage nor on the positional

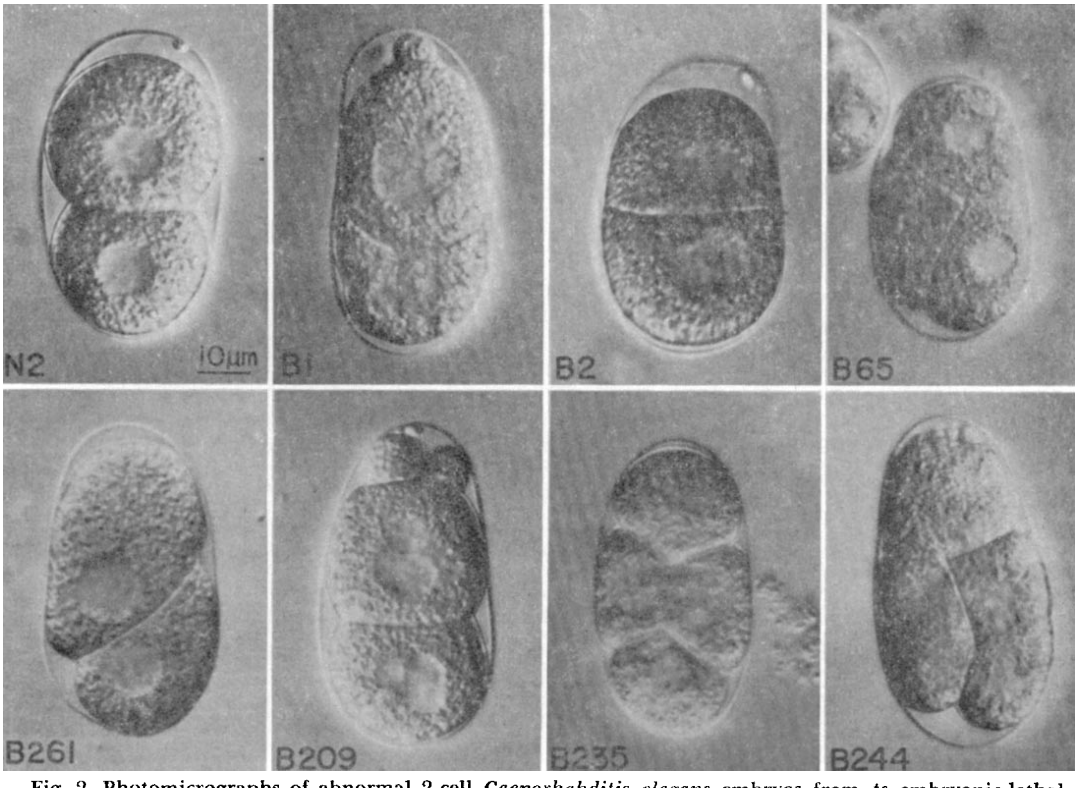
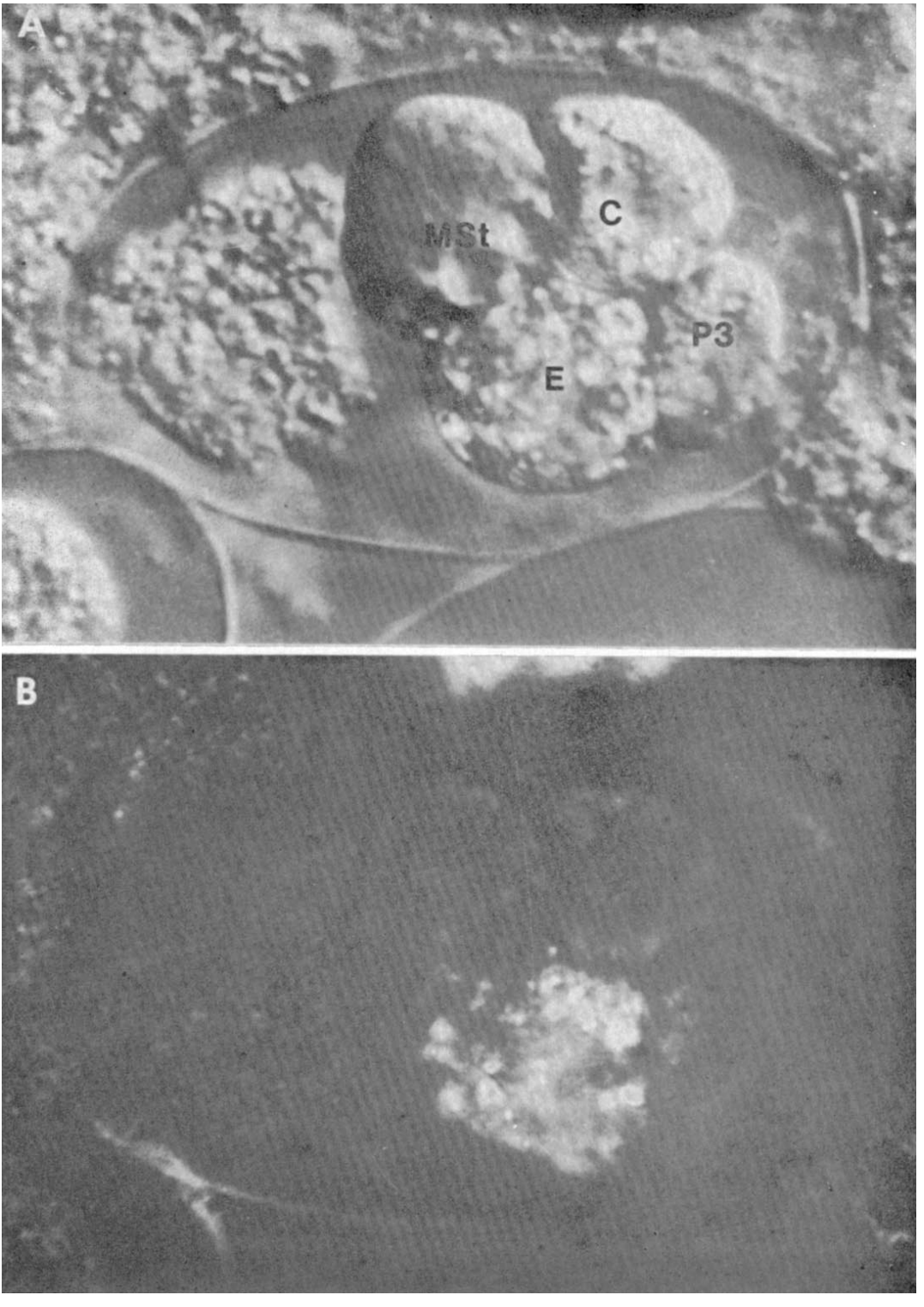


Fig. 2. Photomicrographs of abnormal 2-cell *Caenorhabditis elegans* embryos from *ts* embryonic-lethal mutants. Embryos oriented with the anterior pole at the top were photographed shortly after first cleavage. The upper left panel shows the normal first cleavage of a wild type (N2) embryo to form the larger AB and the smaller P1 cell. From Wood et al. (16).


A
MSt
C
P3
E
B

relationships or cell contacts characteristic of untreated embryos. Rather, it depends on cell-autonomous, internal determinants, which are partitioned from the P1 cell into the EMSt cell and thence into the E cell and its descendants (10).

These determinants could be either nuclear or cytoplasmic. However, Laufer and von Ehrenstein (11) have shown that removal of substantial portions of cytoplasm from early embryos does not prevent normal development. These investigators observed that puncture of the eggshell with a laser microbeam often leads to extrusion of a membrane-bounded bleb of cytoplasm, followed by resealing of the puncture and continued development of the embryo. They obtained larvae that grew into fertile adult worms from embryos that had lost up to 20% of the cytoplasm from either end of the uncleaved egg or up to 60% of the cytoplasm from any one of several different cells during early cleavage stages. These results suggest that if there are prelocalized cytoplasmic determinants essential for development, they may be anchored in some manner to cell components that are not free to flow with the bulk cytoplasm through a small hole. Further experiments are in progress in our laboratory to test more directly whether determinants that control somatic differentiation are nuclear or cytoplasmic.

PRELOCALIZATION

Immunofluorescence microscopy provides a powerful method for determining the location of specific macromolecules in cell or tissue preparations. Using the hybridoma technique of Kohler and Milstein (8), we have generated a collection of monoclonal antibodies from mice immunized with nematode embryo and adult tissue homogenates. These mouse antibodies have been employed, in combination with fluorochrome-conjugated rabbit anti-mouse antibody, to determine the distribution of several specific antigens in fixed *C. elegans* embryos and larvae (S. Strome, M. Hobbs, and W. B. Wood, unpublished). The most interesting results from these studies so far, however, were derived from the chance finding that a preparation of fluorochrome-conjugated rabbit anti-mouse antibody (F-RAM) reacts directly, in the absence of mouse antibodies, with cytoplasmic components specific to germline cells (Fig. 4). The F-RAM antibody has allowed us to follow the segregation and distribution during development of these components, which we have called P-granules (13).

P-granules are detectable in the uncleaved zygote as prelocalized particles at the posterior pole of the embryo near the male pronucleus. After the first cleavage, they are detected only in the P1 cell. In the subsequent divisions, they are progressively segregated to the P2, P3, and P4 cells. At the 100-cell stage, P4 divides into the germline precursor cells Z2 and Z3 (Figure 1),

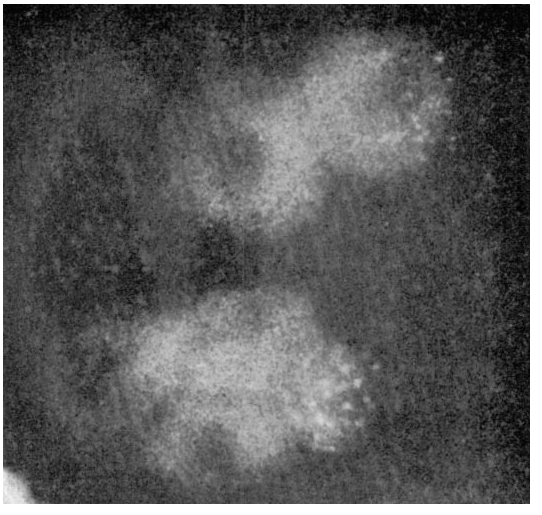


Fig. 4. Staining by F-RAM of P-granules in a 2-cell and a 4-cell embryo of *Caenorhabditis elegans*. See text for further description. From Strome and Wood (13).

Fig. 3. Formation of gut granules in the E cell of a third-cleavage partial *Caenorhabditis elegans* embryo incubated in the presence of cytochalasin B and colchicine to prevent further cell division. At the 2-cell stage this embryo was subjected to local pressure causing breakage of the shell and ablation of the AB cell. The P1 cell was allowed to undergo two more divisions before addition of the drugs. The resulting four cells were identified from the cleavage pattern. After 15 hours of incubation at room temperature the embryo was photographed with Nomarski differential interference-contrast optics (A) and with polarization optics (B), which show rhabditin granules as bright refractile bodies. For experimental details see Laufer et al. (10).

which remain the only stained cells until the embryo hatches as a first-stage larva.

In each of the first three cleavages, the P-granules become prelocalized, during prophase, to the region of the cytoplasm that will be included in the next P-cell daughter. After the second cleavage, they change in size and distribution. In 1-cell to 4-cell embryos, they are numerous and small, located apparently randomly in the cytoplasm during interphase and near the cortex during cell division. However, by about the 16-cell stage, the small granules seem to have coalesced, forming 3–5 large granules, which are located around the nucleus.

In larvae, the antibody also stains nongonadal tissues, as if new antigens recognized by F-RAM appear after hatching. Nevertheless, in germline cells of the developing larval gonad and later in the distal arm of the adult hermaphrodite gonad, stained perinuclear granules are still visible. As oocytes mature, the granules appear to disperse from the nucleus, leading to diffuse cytoplasmic staining. More detailed characterization of developing gametes is in progress.

The origin of anti-P-granule antibodies in the F-RAM serum remains unclear. However, the staining of other larval tissues in addition to gonad lends support to the possibility that a nematode infection in one or more of the rabbits used to make the serum elicited production of antibodies that cross-react with *C. elegans* antigens (13).

Germline-specific cytoplasmic elements referred to as nuage have been observed by electron microscopy as electron-dense bodies in a variety of both vertebrate and invertebrate organisms (5), including *C. elegans* (9). Like P-granules, nuage is restricted to P cells during early cleavage stages in *C. elegans*. Moreover, the number, size, and distribution of nuage change during early cleavages in the same manner and at the same time as observed for P-granules, from numerous small cytoplasmic bodies to fewer larger perinuclear bodies (N. Wolf and D. Hirsh, personal communication), suggesting that the F-RAM antibody could be reacting with a component of nuage.

The functional significance of nuage is unclear, although the widespread occurrence of these germline-specific elements suggests that they may play a role in the determination of germline cells during early embryogenesis and/or in the functions of germline cells during gametogenesis. Consistent with a determinative role is the demonstration by Illmensee and Mahowald (7) that in *Drosophila* embryos, transplanted oocyte cytoplasm containing the nuage-like elements known as polar granules can cause normally somatic embryonic cells to become functional germline precursors. The function of nuage in *C. elegans* is unknown.

CONCLUSION

We are still a long way from understanding epigenesis in the early *C. elegans* embryo. As promising leads to pursue, however, we have evidence and a functional assay for a cell-autonomous somatic determinant, whose location and nature we can explore further. We also have evidence for a germline-specific component, whose unknown nature, mechanism of segregation, and function we can now investigate using the F-RAM antibody as a specific reagent. The results of these studies with *C. elegans* should contribute toward answering the question of how, in general, cell fates are determined during embryogenesis.

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