Expression of the homeotic gene \textit{mab-5} during \textit{Caenorhabditis elegans} embryogenesis

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Summary

\textit{mab-5} is a member of a complex of homeobox-containing genes evolutionarily related to the \textit{Antennapedia} and \textit{bithorax} complexes of \textit{Drosophila melanogaster}. Like the homeotic genes in \textit{Drosophila}, \textit{mab-5} is required in a particular region along the anterior-posterior body axis, and acts during postembryonic development to give cells in this region their characteristic identities. We have used a \textit{mab-5}-lacZ fusion integrated into the \textit{C. elegans} genome to study the posterior-specific expression of \textit{mab-5} during embryogenesis. The \textit{mab-5}-lacZ fusion was expressed in the posterior of the embryo by 180 minutes after the first cleavage, indicating that the mechanisms responsible for the position-specific expression of \textit{mab-5}-lacZ act at a relatively early stage of embryogenesis. In embryos homozygous for mutations in the \textit{par} genes, which disrupt segregation of factors during early cleavages, expression of \textit{mab-5}-lacZ was no longer localized to the posterior. This suggests that posterior-specific expression of \textit{mab-5} depends on the appropriate segregation of developmental factors during early embryogenesis. After extrusion of any blastomere of the four-cell embryo, descendants of the remaining three cells could still express the \textit{mab-5}-lacZ fusion. In these partial embryos, however, the fusion was often expressed in cells scattered throughout the embryo, suggesting that cell-cell interactions and/or proper positioning of early blastomeres are required for \textit{mab-5} expression to be localized to the posterior.

Key words: \textit{C. elegans}, homeotic, homeobox, \textit{mab-5}, \textit{Antennapedia}.

Introduction

In organisms as diverse as insects, vertebrates and nematodes, evolutionarily conserved complexes of homeobox genes, the HOM-C genes, are important in generating pattern along the anterior-posterior body axis. The homeotic selector genes of the \textit{Antennapedia} and \textit{bithorax} complexes of \textit{Drosophila} each specify the identity of a particular region of the body along the anterior-posterior axis (Akam, 1987), and their vertebrate homologs appear to have similar functions (Balling et al., 1989; Wright et al., 1989; Chisaka and Capecchi, 1991; Cho et al., 1991). Four genes have been shown to be members of a \textit{C. elegans} HOM-C complex (Bürglin et al., 1991; Kenyon and Wang, 1991). One of these is the homeotic gene \textit{mab-5} (male abnormal). \textit{mab-5} mutations affect postembryonic development in the posterior region of the body (Kenyon, 1986), causing many posterior cells to adopt the fates of their anterior homologs. The cells affected by \textit{mab-5} mutations are not lineally related, and many different cell types are affected. In \textit{mab-5} mutants, sensory organs, muscles and epidermal structures that characterize this body region are not made. Using RNA in situ hybridization, it has been shown that \textit{mab-5} expression is localized to this region of the body (Costa et al., 1988).

In both insects and vertebrates, the HOM-C genes are expressed in restricted domains along the anterior-posterior axis, and the spatial order of these domains has been conserved (Graham et al., 1989; Gaunt and Singh, 1990; Kessel and Gruss, 1990). Although the expression patterns of the other HOM genes in \textit{C. elegans} are not yet known, the position-specific expression and function of \textit{mab-5} suggest two things: First, that a common ancestor of nematodes, insects and vertebrates may have used region-specific HOM gene expression to generate differences along the anterior-posterior body axis (Kenyon and Wang, 1991) and, second, that mechanisms of pattern formation along the anterior-posterior axis in different organisms may be more similar than expected. \textit{Drosophila}, \textit{C. elegans} and vertebrate embryos develop in very different ways, and yet all express HOM genes in similar region-specific patterns. Have regulatory mechanisms for the HOM-C genes been conserved throughout evolution, or do different organisms use diverse regulatory strategies to set up similar patterns of expression along the anterior-posterior axis? We wish to understand how the anterior-posterior pattern of expression of the HOM-C genes is established in \textit{C. elegans}, focusing on the posterior-specific expression of \textit{mab-5}.

In \textit{Drosophila}, the positional information directing the expression of the genes of the \textit{bithorax} and \textit{Antennapedia} complexes is fairly well understood. Anterior-posterior asymmetry is initiated by maternal factors localized within
the egg, which produce gradients of maternal morphogens within the syncytial blastoderm. Three independent maternal regulatory systems (anterior, posterior and terminal) act to define the anterior-posterior axis (Nüsslein-Volhard, 1991 for review). The zygotic gap, pair-rule and segment polarity genes elaborate this broad maternal positional information into the segmented pattern of the Drosophila embryo (Akam, 1987; Scott and Carroll, 1987; Ingham, 1988 for review). The action of these zygotic genes is required for correct expression of the HOM-C genes (Akam, 1987; Scott and Carroll, 1987; Ingham, 1988 for review).

At least superficially, the embryonic development of C. elegans seems quite different from that of Drosophila (Wood, 1988 for a review of C. elegans embryology). The early embryo is not syncytial. In contrast, early cleavages produce blastomeres with markedly different developmental potential. Segregation of factors to appropriate cells is thought to be important in establishing differences between early blastomeres. The first cleavage results in two blastomeres (AB and P1) which differ in size, cell cycle period, pattern of cleavage and the ability to give rise to specific differentiated cell types. This is accomplished through the segregation of factors to the P1 blastomere in a process requiring the action of the par (partitioning) genes (Kemphues et al., 1988). In addition, however, cell-cell interactions play a major role in establishing the identities of blastomeres (Priess et al., 1987; Priess and Thomson, 1987; Schnabel, 1991; Goldstein, 1992). For example, the two daughters of AB are initially equivalent, and thus must require interactions to establish their different fates (Priess and Thomson, 1987). The polarity of the anterior-posterior axis is established by the time of the first cleavage (Albertson, 1984), but it is not known either how polarity is established or how it is translated into later patterns of cell division and gene expression. The requirement for mab-5 activity in a specific region of the body, in cells not related by lineage, suggests the existence of cell-extrinsic positional information along the anterior-posterior axis. Does such information exist? If so, is it set up through the segregation mechanisms and cell interactions that set up the other asymmetries in the early embryo?

In the work described here, we used a fusion of the upstream regulatory sequences of mab-5 to the E. coli lacZ gene to study the expression of mab-5 during embryogenesis. In two transgenic lines with the mab-5-lacZ fusion integrated into different locations in the worm genome (Salser and Kenyon, 1991; see Materials and Methods), the fusion is expressed in the posterior body region during larval stages (Salser and Kenyon, 1991; data not shown), consistent with previous RNA in situ hybridization data. Thus expression of the mab-5-lacZ fusion can be used as an assay in asking what mechanisms direct the position-specific expression of mab-5.

Materials and methods

Strains
Characterization of β-galactosidase staining in wild-type embryos, and blastomere extrusion experiments were done using CF237 muls2 unc-31(e169) V and CF196 muls3 V. muls2 and muls3 are chromosomal integrations of a mab-5-lacZ fusion that has 7 kb of upstream sequences as well as the coding sequence for the first 17 amino acids of mab-5 fused to lacZ (Salser and Kenyon, 1991). These upstream sequences with the intact mab-5 gene are sufficient for rescue of a mab-5 mutation (Salser and Kenyon, 1991). muls2 was a spontaneous integration into chromosome IV of an extrachromosomal array of mab-5-lacZ and the cosmid C14G10 (carrying unc-31), into a strain with the unc-31(e169) mutation. muls3 maps to linkage group V. To generate this second integrated line, a strain carrying an extrachromosomal array of mab-5-lacZ and a different coinjection marker, rol-6(su1006) (Mello et al., 1991), was treated with 3800 rads using a Cs137 source (K. Cari, A. Fire, and R. Herman, personal communication). The expression of the mab-5-lacZ fusion during the stages of embryonic development described in this paper is identical for the two insertions. Later, at around the 2-fold stage and lasting through the larval stages, additional cells in the head are seen to stain in the muls2 strain. This staining is due to the unc-31 co-transformation DNA (data not shown.) Because of this non-mab-5 staining, experiments with strains carrying muls2 were done by injecting embryos for a defined period of time, long enough for control embryos to reach comma stage.

Strains used for par experiments were: (i) CF186 muls2 unc-31(e169)/nT1(IV); rol-4(e8) par-1(b274)/nT1(V), (ii) CF180 daf-7(e1372ts) par-2(it5ts) III; muls2 unc-31(e169) IV, (iii) CF190 lon-1(e185) par-3(it71)/qG1 III; muls2 unc-31(e169) IV, (iv) CF182 muls2 unc-31(e169) IV; unc-76(e424)/dpy-21(e1282) par-4(it33) V, (v) CF236 par-5(it55) dpy-20(e1282)/nT1 (IV); muls3/nT1 (V). The parental par strains from which these were constructed were obtained either from Ken Kemphues or from the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR). par-1(b274), par-3(it71), par-4(it33) are each strong reduction-of-function or null alleles (Kirby et al., 1990; Morton et al., 1992). par-2(it5) is a ts allele which at the non-permissive temperature (25°C) is indistinguishable from the strongest alleles of par-2 (Kirby et al., 1990). par-5(it55) is one of two alleles of par-5; the two have similar phenotypes (K. Kemphues, personal communication). Since par gene products are maternally contributed, Par embryos were obtained from a hermaphrodite homozygous for a par mutation.

For each par strain, we also tested the effect of the closely linked visible marker on mab-5-lacZ expression; the staining patterns of (i) muls2 unc-31(e169) IV; rol-4(e8) V, (ii) daf-7(e1372) III; muls3, (iii) lon-1(e185) III; muls2 unc-31(e169) IV, (iv) muls2 unc-31(e169) IV; dpy-21(e1282) V, and (v) dpy-20(e1282) IV; muls3 V were indistinguishable from those of muls2 and muls3.

Fixation and staining
Gravid hermaphrodites were cut open in water, and 1-cell to 4-cell embryos were picked using a drawn-out glass capillary. Embryos were placed in a drop of distilled water on a coverslip coated with a solution of polylysine (1 mg/ml), gelatin (0.2%) and chrome alum (0.01%), gently pressed into place using an eyelash attached to a toothpick, and incubated at 20°C or 25°C in sealed humidified boxes. After incubation, embryos were laser-permeabilized using the method of Priess and Hirsh (Priess and Hirsh, 1986) in egg salts based on Edgar and McGhee (1986), and fixed by floating the coverslip (with embryos stuck to it) on 2% glutaraldehyde (Grade I, Sigma G5882) in egg salts for 1 minute. After rinsing in egg salts, embryos were incubated for 2-24 hours in a variation of the β-galactosidase stain solution described by A. Fire (Fire et al., 1990), with 10 mM MgCl2. Embryos were observed and photographed using a Zeiss Axioshot.

Identification of stained cells
lacZ-expressing cells were identified by their positions relative to
other nuclei and to cell deaths, as visualized by DAPI. After the fixation procedure used here, embryo morphology was very well preserved. DAPI-stained nuclei were arranged in characteristic patterns at the different stages, recognizable by comparison to published drawings of lineage embryos (Sulston et al., 1983) and embryos lineaged by DC (data not shown). The identification of the first staining cells as ABplpppp(a/p) and ABprpppp(a/p) was based on lineaging, using a ‘4-D microscope’ (MRC 4-D Video Archiving System; BioRad) recording of several embryos. The position of the first cells to stain was consistent with the positions of these four cells. The fact that the descendants of these cells stained after another round of cell divisions lent support to this identification. At the 260-270 minute stage, cells were identified based on comparison of lacZ- and DAPI-stained embryos to Figures 2e, 7a, and 7b of Sulston et al. (1983). The identification of the hypodermal cells P7/8, P9/10, P11/12 and V6 at this stage was supported by the fact that at later stages, for example the comma stage and 11-fold stage, these cells are in very distinctive positions along the side of the animal, and can be identified with great certainty. At the 11-fold stage, Figure 8 of Sulston et al. (1983) was used for reference. In the case of the posterior ventral nerve cord precursors, we have not attempted to identify individual staining cells. These cells are tightly clustered together, and it would be difficult to distinguish specific staining cells. For this same reason it is possible that there are additional cells stained in this region that we have not identified.

Blastomere extrusion

Blastomeres were extruded as described by Priess and Thomson (1987). muIs2 embryos were incubated only for 5 hours at 25°C in order to avoid unc-31-dependent staining; muIs3 embryos were incubated overnight at 20°C. Before fixation the eggshells were re-permeabilized either using a needle or by laser. Embryos were then fixed and stained as described above.

Results

Background

For approximately the first 330 minutes of embryonic development (at 20°C) the C. elegans embryo undergoes a period of rapid cell proliferation (see Fig. 1 for an overview of embryogenesis). During this time almost all of the 558 cells found in the newly hatched L1 larva are generated. In the first 100 minutes, ‘founder cells’, blastomeres with different developmental potential, are generated by a series of unequal, stem-cell-like cleavages. From 100 minutes to 290 minutes, gastrulation occurs. By 350 minutes, the embryo is an oblong ball of cells that are, for the most part, in their final positions relative to one another. At this point morphogenesis begins, and the embryo elongates with little change in cell number or relative cell position (Sulston et al., 1983).

Although the anterior-posterior polarity of the embryo is established prior to the first cleavage (Strome and Hill, 1988, for review), the arrangement of the cells at early times does not correlate with the future anterior-posterior axis: descendants of all four cells of the four-cell embryo will later be found in the posterior of the embryo. During the cell proliferation phase of embryogenesis, the processes of gastrulation and cell division place cells in their appropriate positions relative to the anterior-posterior axis (Sulston et al., 1983). The sorting of anterior from posterior cells continues until the last round of cell division at 350 minutes (Sulston et al., 1983). For cells that express the mab-5-lacZ fusion in the embryo (see below), relative positions along the anterior-posterior axis are largely determined by the polarity of the cell divisions in the lineages that give rise to them, without large-scale cell movement.

The mab-5-lacZ fusion is expressed during early embryogenesis

In order to understand eventually how the position-specific expression of mab-5 is established, we decided to characterize the expression of the mab-5-lacZ fusion during embryogenesis. When and where is the mab-5-lacZ fusion first expressed? Northern analysis (Costa and Kenyon, unpublished) had shown that mab-5 mRNA is present in embryos. Low resolution RNA in situ hybridization experiments indicated that mab-5 expression is localized to one end of the embryo (Loer and Kenyon, unpublished). To analyze the mab-5-lacZ staining pattern during embryoge-

Fig. 1. Overview of C. elegans embryogenesis. For approximately the first 330 minutes of embryonic development at 20°C the C. elegans embryo undergoes a period of rapid cell proliferation, generating almost the entire complement of 550 cells that will be present at hatching. During this period, the processes of cell division and gastrulation place cells in their appropriate relative positions along the anterior-posterior axis. At the end of this time, morphogenesis begins, and the embryo elongates into a worm shape with little change in cell number or relative cell position. Times are shown in minutes at 20°C.

Approximate cell numbers are shown in boldface. The mab-5-lacZ fusion is first expressed at approximately 180 minutes, indicated by the arrow. The dorsal hypodermal cells fuse to form a syncytium (hyp?) at around 400 minutes, indicated by the * (Priess and Hirsh, 1986). For a more detailed description of embryonic development, see Sulston et al. (1983).
ness, we fixed and stained staged embryos carrying the fusion (see Materials and Methods). Consistent with the in situ results, the mab-5-lacZ fusion was expressed towards the posterior of the embryo. The fusion was first expressed during the period of cell proliferation, at 180 minutes after the first cleavage, in four cells located towards the posterior of the embryo on the ventral side, ABprpppp(a/p) and ABplpppp(a/p) (Fig. 2, A and B). At this point the embryo has approximately 200 cells. By 270 minutes, the embryo has undergone another round of cell division, and has around 350 cells. At this stage the mab-5-lacZ fusion was expressed in approximately 20 cells, all in the posterior third of the embryo (Fig. 2, C-E). These included lateral and ventral epidermal blast cells which are affected postembryonically by mab-5 mutations (Kenyon, 1986), dorsal hypodermal cells, intestinal cells and the eight descendants of ABprpppp(a/p) and ABplpppp(a/p). By the 1½-fold stage (around 430 minutes), after another set of cell divisions has occurred, additional cells were stained in the posterior body region. These included posterior ventral cord neuronal precursors, born in this round of cell division (Fig. 2K). In general, cells identified as expressing the fusion in the embryo were also stained in the newly hatched larva (Salser and Kenyon, unpublished), with an interesting exception. By the 1½-fold stage the 8 descendants of ABprpppp(a/p) and ABplpppp(a/p), located to the posterior of most of the expressing cells, were no longer stained (see Discussion).

In summary, the mab-5-lacZ fusion was first expressed during the cell proliferation stage of embryogenesis, after generation of the founder cells, in cells in the posterior of the embryo. These results indicate that the mechanisms responsible for initiating the position-specific expression of
Fig. 2. Expression of the mab-5-lacZ fusion during embryonic development. Staining is localized to the nuclei due to a nuclear localization signal in the fusion protein (Salser and Kenyon, 1991). Times indicated are normalized to development at 20°C. Anterior is to the left except in E, as noted. A, C, F and I are tracings of the embryos shown in B, D, G and J, with the stained cells outlined. (A and B) 180 minutes, ventral view, superficial plane. Earliest expression of the fusion. Two bilateral pairs of cells, ABplpppp(a/p) and ABprpppp(a/p), are the only cells stained at this stage. Gastrulation is in progress, and the ventral cleft can be seen (arrowhead). (C and D) 270 minutes, ventral view, superficial plane. The 8 granddaughters of ABplpppp and ABprpppp are visible in this focal plane. The polar body is indicated with an open arrowhead. (E) A diagram of the cells identified as staining at 270 minutes, ventral view, anterior to the top. The drawing is from Figure 7B of Sulston et al. (1983), with lacZ-expressing cells colored in. Stippled cells are cell deaths. At this stage, at least 22 cells are stained, including: 8 descendants of ABplpppp and ABprpppp [These cells will divide again and give rise to the following cells: PVP (L and R), rectal epithelial cell V (L and R), F, U, DVA, B, an anal depressor muscle, intestinal muscle, sphincter muscle, and a body muscle, 2 hyp10 nuclei, and 2 tail spike cells.]; 6 ventral hypodermal cells (P7/8, P9/10, and P11/12 on each side); two cells, one next to V6 on each side, which we believe to be ABplpapp and ABprapp (the sisters of P11/12 L and R, which give rise to ALN and PLM on each side); the two lateral hypodermal cells V6L and V6R; the dorsal hypodermal cells Caaapp and Cpaapp; and two posterior intestinal cells (not indicated). It is possible that additional cells within the region are stained and have not been identified. (F, G and H) 350 minutes, lateral view, superficial plane. The embryo has just begun elongation. Ventral hypodermal cells P7/8, P9/10, and P11/12, and the lateral hypodermal cell V6 (arrows) are visible staining in this plane of focus. H shows DAPI staining; the row of lateral hypodermal (V) nuclei (upper row of nuclei) and ventral hypodermal (P) nuclei are visible lined up along the side of the embryo. (I and J) 430 minutes (1½-fold), left lateral view, superficial plane. Ventral hypodermal cells P7/8, P9/10, P11/12, the lateral hypodermal cell V6, and the dorsal hypodermal nucleus Cpaapp (arrows) are visible. (K) 430 minutes, left lateral view, mid-focal plane (a different embryo than in Land J) Staining of ventral motor neuron precursors is visible. An unidentified stained cell behind the anus is also seen in this focal plane. Embryos in B, D, G and H were muIs2 unc-31(e169); embryos in J and K were muIs3. No differences were seen in the staining pattern of the two strains at these stages.

mab-5-lacZ are at work by this point during embryogenesis. Identification of many of the staining cells indicates that, consistent with the mab-5 phenotype, cells expressing the fusion were not closely related by lineage (Fig. 3).

The effect of par mutations on mab-5 localization

In C. elegans the segregation of factors to specific cells is critical in establishing the identities of early blastomeres and their subsequent developmental fates. The par gene products appear to be required for segregation of cytoplasmic factors to the appropriate cells during early cleavages (Kemphues et al., 1988; Kirby et al., 1990; Morton et al., 1992). In par mutants, many differences between blastomeres are abolished, and P granules are not properly localized. Intestinal tissue, the production of which is believed to require segregation of determinants into an intestinal founder cell (the E cell) is usually not made. par mutations do not block cell proliferation or differentiation, however, and embryos develop into disorganized masses of cells (Kemphues et al., 1988; Morton et al., 1992).

What would expression of the mab-5-lacZ fusion look like in a par mutant? To examine the effect of par mutations on expression of the mab-5-lacZ fusion, we introduced par mutations into strains carrying the fusion. Par embryos were isolated at the 1- or 2-cell stage, incubated until control embryos had reached comma to 1½-fold, fixed and stained. Mutations in each of the 5 par genes disrupted posterior-specific expression of mab-5-lacZ (Table 1 and Fig. 3).
4). The staining patterns were variable (Fig. 4), as are other aspects of development in Par embryos (Kemphues et al., 1988; Morton et al., 1992). However, a large fraction of Par embryos had staining cells scattered throughout the embryo (Table 1).

These results are consistent with the idea that the segre-

Table 1. Expression of mab-5-lacZ in Par embryos

<table>
<thead>
<tr>
<th>Position of stained cells</th>
<th>par-1</th>
<th>par-2</th>
<th>par-3</th>
<th>par-4</th>
<th>par-5</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>localized toward end</td>
<td>7 (32)</td>
<td>19 (79)</td>
<td>5 (26)</td>
<td>12 (48)</td>
<td>7 (41)</td>
<td>34 (100)</td>
</tr>
<tr>
<td>scattered, or central</td>
<td>13 (59)</td>
<td>5 (21)</td>
<td>14 (74)</td>
<td>10 (40)</td>
<td>9 (53)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>both ends</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>no staining</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>1 (6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>total</td>
<td>22</td>
<td>24</td>
<td>19</td>
<td>25</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>Average no. of cells stained</td>
<td>5</td>
<td>6</td>
<td>12</td>
<td>9</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Range</td>
<td>0-10</td>
<td>2-14</td>
<td>2-32</td>
<td>0-18</td>
<td>0-18</td>
<td>13-29</td>
</tr>
</tbody>
</table>

*aEmbryos homozygous for a par allele and the mab-5-lacZ fusion were incubated for 5 to 5.5 hours at 25°C, or 6.5 hours at 20°C, then fixed and stained as described in Materials and Methods. Numbers in each column represent numbers of embryos observed, with percentages in parentheses.

*bStaining was scored as localized toward one end of the embryo if ≥90% of the stained cells were in one half of the embryo. We did not determine the polarity of the Par embryos.

*cCF180 daf-7(e1372ts)/par-2(it5ts)III; muls2 unc-31(e169)IV hermaphrodites were incubated overnight at 25°C; embryos were then isolated and incubated at 25°C as described in Materials and Methods.

*dThe control column summarizes data from control embryos incubated in parallel with the Par embryos. These were, variously, muls2 or muls3 embryos, or heterozygous siblings of the par embryos containing the fusions. In each case, the staining patterns were the same.

*eThe variability in number of cells stained in control embryos reflects variability in degree of staining (sometimes fewer cells were stained) and also the difficulty of distinguishing stained cells that were closely packed (the ventral motor neuron nuclei were difficult to count for this reason, and may have been undercounted in many embryos).

Fig. 4. Expression of mab-5-lacZ fusion in par embryos. Par embryos were incubated for 5 to 5.5 hours at 25°C, then fixed and stained as described in Materials and Methods. The pattern of expression of the fusion was variable for every par strain (e.g. compare A and B; C and D; E and F; see also Table 1); the staining patterns shown are examples of the range of patterns seen for all the mutations and are not characteristic of the par allele shown per se. Staining could be scattered (e.g. A, C and E), clustered in the center (D), clustered at an end (F), or occasionally, clustered at both ends (B). (A and B) par-1(b274); (C and D) par-3(it71); (E and F) par-5(it55).
Table 2. Expression of mab-5-lacZ after blastomere removal

<table>
<thead>
<tr>
<th>Strain</th>
<th>Blastomere removeda</th>
<th>Gut granules</th>
<th>No. of embryos stainingc</th>
<th>No. of cells staining</th>
<th>No. of cells expectedd</th>
</tr>
</thead>
<tbody>
<tr>
<td>muIs3</td>
<td>P2</td>
<td>+</td>
<td>2/2</td>
<td>1; ≥14</td>
<td>10-12</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>–</td>
<td>2/2</td>
<td>1; ≥16</td>
<td>20-30</td>
</tr>
<tr>
<td></td>
<td>P2b</td>
<td>+</td>
<td>1/1</td>
<td>≥13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>+</td>
<td>1/1</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>–</td>
<td>3/3</td>
<td>2-3 cells each</td>
<td></td>
</tr>
<tr>
<td>muIs2</td>
<td>EMS</td>
<td>–</td>
<td>6/6</td>
<td>2; 5; ≥12; ≥18; ≥19; ≥21</td>
<td>20-30</td>
</tr>
<tr>
<td>muIs3</td>
<td>EMS</td>
<td>–</td>
<td>4/5</td>
<td>0; 4; 1-5; 14; 18</td>
<td></td>
</tr>
<tr>
<td>muIs3</td>
<td>ABa</td>
<td>+</td>
<td>4/4</td>
<td>7; 8; ≥8; ≥16</td>
<td>20-30</td>
</tr>
<tr>
<td>muIs3</td>
<td>ABp</td>
<td>+</td>
<td>2/3</td>
<td>0; 1; 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ABp</td>
<td>–</td>
<td>1/1</td>
<td>10-12</td>
<td></td>
</tr>
</tbody>
</table>

aBlastomeres were removed as described in Materials and Methods. muIs2 embryos were incubated 5 hours at 25°C; muIs3 embryos overnight at 20°C (see Materials and Methods).
bIn this case, P2 was enucleated. The cytoplasm of P2 eventually separated from the rest of the embryo, and by the end of the incubation period formed a detached blob at the posterior (see Figure 5F).
cAfter incubation, partial embryos were scored for degree of cell proliferation and for the presence of gut granules as a marker for overall development. Only partial embryos that had no visible damage to the remaining cells (abnormally large or non-staining nuclei visible by DAPI staining) and that had >150 cells (all but one listed here had >200; one had 150-200 cells) were counted.
dApproximate number of stained cells arising in the remaining three lineages in the undisturbed embryo, based on the data in legends to Figures 2 and 3.


gation of factors between cells is important for the posterior-specific expression of mab-5. It seems unlikely that the patterns of expression of the mab-5-lacZ fusion in Par embryos are due to dispersal or migration of cells initially localized at the posterior, for the following reasons: (1) the earliest detectable expression of the fusion in par-1 embryos was scattered (data not shown); and (2) staining cells in Par embryos were often widely separated. This would require extensive cell migration, which does not normally occur in C. elegans. In fact, a few Par embryos contained two clusters of staining cells, one at each pole of the embryo. It is difficult to imagine that such a pattern is generated by the long-range migrations of a subset of cells initially located in a single body region. Thus, the posterior-specific expression of the mab-5-lacZ fusion is dependent on the activity of the par genes, and is likely to be dependent on segregation of information between cells.

**Blastomere extrusions**

Cell interactions between the 2-cell and the 28-cell stages have been shown to be important for normal development of some blastomeres. Because the fates of ABa and ABp are known to be established by cell-cell interactions, it is clear that the detailed expression pattern of mab-5 requires intercellular signalling. But what about the overall localization of mab-5 expression to the posterior? Does posterior-specific expression of the mab-5-lacZ fusion require normal cell interactions in the early embryo? Priess and Thomson were able to show that cell signals were required to induce the development of the anterior pharynx in the ABa lineage by extruding individual blastomeres from the four-cell embryo (Priess and Thomson, 1987). We did a similar experiment to investigate the role of cell interactions in the posterior localization of mab-5 expression. Individual blastomeres were extruded at the four-cell stage according to the methods of Priess and Thomson (1987). The partial embryos were allowed to develop, and fixed and stained for β-galactosidase. After extrusion of any of the four blastomeres, cells in the remainder of the embryo could express the mab-5-lacZ fusion (Table 2 and Fig. 5).

Significantly, posterior localization of mab-5-lacZ expression was often completely disrupted by blastomere removal. Two classes of staining pattern were seen; in some partial embryos, staining was localized to the posterior, while in the others, staining cells were scattered throughout (Fig. 5). We asked whether staining was localized to the posterior or scattered after removal of a given blastomere. (Only embryos having ≥5 cells stained were considered, since with only a few cells stained it is not possible to evaluate realistically whether staining is localized or scattered.) After removal of EMS (7 embryos) or ABa (4 embryos), both classes of staining pattern were seen. After removal of ABp, only one embryo had enough stained cells to evaluate; this embryo had scattered staining. After removal of P2, staining was scattered (3 embryos); however, in an embryo in which P2 had been enucleated, staining was localized to the posterior (we do not know if the difference in technique is significant). These results suggest that cell-cell interactions and/or normal cell positioning during early embryogenesis are important for the spatial pattern of mab-5 expression. The variability of the staining patterns are consistent with the finding that, after irradiation of P2 or EMS, AB lineages and cell fates are variable (Schnabel, 1991).

The number of cells stained was variable in these experiments (Table 2). Due to several considerations, including: (i) the variability of AB lineages after EMS or P2 is killed (Schnabel, 1991), (ii) the fact that precise counts of staining cells were often difficult, and (iii) probable variability in the viability of the manipulated embryos, the precise numbers of staining cells are not necessarily significant.

**Discussion**

In this work we have studied the expression of a mab-5-lacZ fusion during C. elegans embryogenesis. The
expression pattern confirms earlier work indicating that \textit{mab-5} is expressed in a region-specific pattern, in cells not closely related by lineage. The data presented here provide a framework for asking how \textit{mab-5} expression is localized to the posterior of the embryo. They suggest that posterior-specific expression of \textit{mab-5} is dependent on both segregation of factors to appropriate cells, and on cell-cell interactions. They further suggest the possibility that multiple, independent decisions may be required for posterior-specific \textit{mab-5} expression.

\textit{Expression of the mab-5-lacZ fusion in the embryo}

The expression pattern of the \textit{mab-5-lacZ} fusion is consistent with earlier Northern hybridization experiments, which showed that \textit{mab-5} mRNA is present during embryogenesis, and RNA in situ hybridization experiments, which indicated that the mRNA is localized to one end of the embryo. The expression of the fusion was localized to the region affected by \textit{mab-5} mutations (Kenyon, 1986), and to the same region to which the \textit{mab-5} transcript was localized by RNA in situ hybridization (Costa et al., 1988), so is likely to reflect the actual expression of the \textit{mab-5} transcript in many respects. Thus, although we cannot be certain that in detail the expression pattern of the \textit{mab-5-lacZ} fusion corresponds to that of the native \textit{mab-5} gene, overall the expression of the fusion provides an assay for investigating the regulation of the posterior localization of \textit{mab-5} expression.

The fusion is expressed in cells from many different lineages in a localized region along the anterior-posterior axis. This expression pattern supports the prediction of genetic studies of \textit{mab-5} (Kenyon, 1986) that suggested that \textit{mab-5} is expressed and is acting in a particular spatial domain. The similarities between the HOM-C complex in \textit{C. elegans} and those in other organisms, along with these results, suggest that the expression of other members of the complex may also be spatially localized.

The timing of the initial expression of the \textit{mab-5-lacZ} fusion indicates that the information directing the posterior expression of the fusion is present by at least 180 minutes after the first cleavage (approximately 23\% of embryogenesis). This is after the generation of the founder cells, during the period of cell proliferation and gastrulation. The fusion was expressed before formation of the dorsal hypodermal syncytium (see Fig. 1), ruling out models for \textit{mab-5} localization involving this syncytium. Thus, for example, graded positional information within the hypodermal syncytium

\textbf{Fig. 5.} Expression of the \textit{mab-5-lacZ} fusion after removal of individual blastomeres from the 4-cell embryo. (A) AB\textit{a} extruded; (B) AB\textit{p} extruded; (C and D) EMS extruded; (E) P\textsubscript{2} extruded. Anterior is to the left. As discussed in the text, staining patterns were variable. A, B, C, and E show examples of partial embryos in which staining was not localized to the posterior; (D) a partial embryo in which staining was localized to the posterior. The arrowhead indicates the position of the polar body in E.
cannot be responsible for initiating the position-specific expression of mab-5-lacZ.

The mab-5-lacZ expression pattern did not arise all at once, but sequentially in different sets of cells. Anterior-posterior cell divisions resulting in the final alignment of cells along the anterior-posterior axis continue until approximately 350 minutes after the first cleavage. Expression of the fusion arose in a pattern that corresponded to the birth of posteriorly-localized cells. Many of the cells that expressed the mab-5-lacZ fusion were sisters of anterior cells that did not express the fusion (data not shown.) For example the lateral epidermal cells V4 and V6 are sisters. The mab-5-lacZ fusion was expressed only in the posterior sister, V6, and not until after V4 and V6 were born.

Even after the expression pattern was complete, the mab-5-lacZ fusion was not expressed in all cells in the region (see Fig. 2). This is consistent with the idea that mab-5 is regulated in part by cell-specific, as opposed to region-specific, controls. This is not unexpected, since several genes that appear to affect mab-5 expression in subsets of its normal domain have been identified (Waring and Kenyon, 1990, 1991; Harris and Kenyon, unpublished).

The first cells to express the mab-5-lacZ fusion were two bilateral pairs of cells, which appear to be the daughters of ABplpppp and of ABprpppp. These divided to give eight staining cells by around 220 minutes. These cells are located in the portion of the embryo that, after elongation, becomes the posterior-most region of the animal. By the time of hatching, the descendants of ABplpppp and of ABprpppp no longer expressed the mab-5-lacZ fusion (for the identities of these cells, see legend to Fig. 2). Interestingly, these early staining cells are not within the region of the animal affected by mab-5 mutations. Instead, they lie just posterior, within the region affected by mutations in egl-5. egl-5 is a good candidate for the gene adjacent to mab-5 in the C. elegans HOM cluster (Chisholm, 1991). These observations suggest that there might be interactions between HOM-C genes in C. elegans analogous to those in Drosophila. In Drosophila, each homeotic selector gene is down-regulated in the regions posterior to its region of influence by the more posteriorly-acting genes in the complex (e.g. Struhl and White, 1985; Carroll et al., 1986). It is possible that, likewise, mab-5 is first expressed within the egl-5 region, but later down-regulated by egl-5 activity. However, preliminary experiments have found no difference in the expression of the mab-5-lacZ fusion in egl-5 mutants (D.C. and C.K. data not shown; Hunter and Kenyon, unpublished).

Factors affecting posterior-specific expression of mab-5-lacZ

In Par embryos, expression of the fusion was no longer localized to the posterior. With strong alleles of several par genes, the majority of embryos expressed the fusion in cells scattered throughout the embryo. Thus, since par mutations are known to disrupt segregation of factors to appropriate cells during early cell divisions, these results suggest that normal expression of mab-5 requires the appropriate segregation of factors during these early cell divisions. This requirement could be quite indirect; par mutations might disrupt the posterior localization of mab-5 expression simply as a consequence of the disorganization of the entire embryo. While this result was not surprising, since the par genes act very early during embryogenesis (Morton et al., 1992), it was not necessarily obvious that par mutations would affect all early patterning events in the embryo. For example, some aspects of patterning could be established by pre-localized, or independently localized, factors. (One such example would be a molecule deposited on a posterior region of the vitelline membrane during oogenesis that acted to set up a gradient of positional information after cell proliferation had placed cells in the appropriate positions along the a-p axis.) However, our results indicate that the same mechanisms responsible for other aspects of anterior-posterior polarity in the embryo are also required for position-specific expression of mab-5.

We tested the involvement of cell interactions in the localization of mab-5-lacZ expression by extruding individual blastomeres at the four-cell stage. By extruding the blastomere EMS (see Fig. 3) from the four-cell embryo, Priess and Thomson had previously shown that a specific inductive interaction between a descendant of that cell and descendants of ABa was required for descendants of ABa to produce the anterior pharynx (Priess and Thomson, 1987). In contrast, we did not find evidence for any simple induction of posterior-specific mab-5 expression. After extrusion of any blastomere of the four-cell embryo, descendants of the remaining three blastomeres were still capable of expressing the mab-5-lacZ fusion. This suggests that no one of these four cells is absolutely required for descendants of other blastomeres to express the fusion. However, we cannot rule out the induction model completely, since we cannot exclude the possibility that after extrusion of one blastomere that normally gave rise to an inducing cell, the fate of another blastomere was altered to now produce an inducing cell.

What was really surprising about these results was that the mab-5-lacZ fusion was often expressed in individual cells scattered throughout the embryo, both in par mutants and after blastomere extrusion. The normal posterior-specific expression of the mab-5-lacZ fusion suggests that expression of mab-5 might be regulated regionally, by some sort of cell-extrinsic positional information. After disruption of a source of positional information, one might expect expression to be eliminated, expanded, or in an altered position, but nevertheless localized to specific regions. These sorts of pattern disruptions, for instance, are seen in Drosophila patterning mutants (Nüsslein-Volhard, 1991), or after ligation of insect embryos (Sander, 1975). Instead, the mab-5-lacZ fusion was sometimes expressed in many individual cells scattered throughout the embryo (for example, see Fig. 4E, and Fig. 5A, B, and E.) These results are consistent with the idea that multiple, independent decisions in different lineages are directing the final pattern of expression, rather than one, or even a few, posterior-specific sources of positional information.

In Drosophila the expression of the ANT-C and BX-C genes is clearly under regulation of localized position-specific morphogens. It could be that mab-5 expression is also under the control of a position-specific morphogen, but if so, then the proper positioning of many blastomeres, presumably allowing the correct cell-cell interactions, must be
achieved in order to set up or maintain position-specific cell identities. Alternatively, it could be that \textit{mab-5} expression is not regulated by region-specific controls, but rather by multiple, independent decisions in different lineages. Whatever the case, the fact that different genetic and physical manipulations can completely abolish the localization of \textit{mab-5} expression in \textit{C. elegans} embryos, which is not seen for \textit{Drosophila} HOM gene expression, suggests that there are significant differences in the way localized HOM gene expression is achieved in different organisms.

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\section*{References}


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