Deficiency Screen Based on the Monoclonal Antibody MH27 to Identify Genetic Loci Required for Morphogenesis of the Caenorhabditis elegans Embryo

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ABSTRACT The monoclonal antibody MH27 recognizes an adherens junction protein present around hypodermal cells in the pharynx and the intestine. By using this antibody and an antisera against the LIN-26 protein, which is present in hypodermal and glial-like cells, I have examined the morphogenesis of the embryo in embryos homozygous for 91 chromosomal deficiencies that cover approximately 74% of the Caenorhabditis elegans genome. Most deficiencies were found to affect both the morphogenesis of the embryo and the organogenesis of the pharynx. By contrast, the intestine was generally normal. I have classified deficiencies according to their hypodermal staining abnormalities. I identified a few deficiencies that appeared to affect more specifically anterior-directed migration of hypodermal cells or extension of the margins of ventral hypodermal cells, integrity of hypodermal membranes, elongation of the embryo, and hypodermal cell fusions. This work opens the way for a genetic analysis of morphogenesis in C. elegans. Dev. Dyn. 1997;210:19–32. © 1997 Wiley-Liss, Inc.

Key words: Caenorhabditis elegans; embryo; hypodermal cells; morphogenesis

INTRODUCTION

Epithelial cells play a critical role in many morphogenetic events during embryogenesis through epiboly, invagination, and ingestion accompanied by changes in cell shapes. This is particularly visible during gastrulation, when a single-layered epithelium is transformed into three germ layers (see Hardin, 1994; Shih and Keller, 1994; Smith et al., 1996). Closure of the neural tube also involves important changes in the neuroepithelium and the surrounding ectodermal layer (Schoenwolf and Smith, 1990). Concerted movements of epithelial cells are important for organogenesis: For instance, in vertebrates, the epicardium migrates from the splanchnopleuric mesoderm to cover the myocardium (Hiruma and Hirakow, 1989). Although proteins that are required for spreading and histogenesis of epithelia and that modify the shape of cells by regulating the cytoskeleton or by modifying cell adhesion and/or migration mechanisms are beginning to be known (see Ashkenas et al., 1996; Gumbiner, 1996), so far, morphogenesis has been investigated very little at the genetic level.

The simplicity and rapid reproduction rate of Caenorhabditis elegans make this animal an ideal system to analyze morphogenesis using genetic tools. In C. elegans, hypodermal cells (the equivalent of epidermal cells) that constitute the external epithelium are essential for morphogenesis. Hypodermal cells are born and initially located in a dorsal and posterior position (Sulston et al., 1983). From there, the anteriormost cells migrate toward the anterior part of the embryo. Simultaneously, four leading ventral hypodermal cells located on both sides of the embryo at the head/body junction send filopodia toward the ventral midline; once they have attached to each other, ventralmost hypodermal cells in the body extend their margins ventrally to finally attach to their contralateral homologs (Williams-Masson et al., 1997). These concerted movements result in full enclosure of the embryo. After enclosure, the shape of hypodermal cells changes under the influence of cytoskeletal elements, which causes the embryo to elongate (Sulston et al., 1983; Priess and Hirsh, 1986).

Mutations that cause hypodermal cells to degenerate (Labouesse et al., 1994) or elimination with a laser beam of certain hypodermal precursors (Sulston et al., 1983) or of certain hypodermal cells (Priess and Hirsh, 1986; Williams-Masson et al., 1997) block ventral enclosure or elongation and cause the embryo to rupture. Drugs that inhibit polymerization of actin block ventral enclosure (Williams-Masson et al., 1997); similarly, drugs that inhibit actin or microtubules block elongation (Priess and Hirsh, 1986). Very little is known about proteins that control enclosure and elongation except, as mentioned above, that actin filaments and microtubules within hypodermal cells play an essential role.

Recently, we have examined the expression of the nuclear protein LIN-26 in embryos homozygous for 90 chromosomal deficiencies representative of a large fraction of the C. elegans genome (Chanal and Labouesse, *Correspondence to: IGBMC, CNRS/INSERM/ULP, BP 163, 67404 Illkirch Cedex, CU de Strasbourg, France.
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aspects of the morphogenesis of the embryo. The LIN-26 protein is expressed in all hypodermal cells and in all glial-like cells of the animal and is required for development of these cells (Labouesse et al., 1994, 1996). The main goal of that deficiency screen was to identify genes involved in specifying the fates of ectodermal cells. To assess hypodermal differentiation, we also stained embryos with the monoclonal antibody MH27, which recognizes an adherens junction protein present around all hypodermal cells and in all desmosomes of the intestine and the pharynx (the mAb MH27; Francis and Waterston, 1991). The gene encoding the MH27-reactive epitope and its function are not known. By using both markers to monitor the numbers and the positions of hypodermal and glial-like cells, we identified six loci that are necessary for normal lin-26 expression (Chanal and Labouesse, 1997). Here, I describe the MH27-staining phenotypes observed in embryos homozygous for the same chromosomal deficiencies that were examined by Chanal and Labouesse (1997), with particular emphasis on hypodermal defects. These deficiencies define a number of loci that could encode proteins involved in controlling different aspects of the morphogenesis of the embryo.

RESULTS

Brief Overview of the MH27 Pattern in Wild Type Embryos

Priess and Hirsh (1986) and Podbilewicz and White (1994) have previously described the hypodermal MH27 staining pattern in wild type embryos. I will briefly review this pattern, focusing on aspects that are directly relevant to the present study. Hereafter, I will refer to the antigen recognized by MH27 as the MH27-reactive antigen. This antigen can first be detected around each hypodermal cell as a punctate ring (which will be called the “MH27 ring”) about 240 min after fertilization (at 20°C), soon after the generation of hypodermal cells (Podbilewicz and White, 1994; Fig. 1E), when they still occupy the posterior two-thirds of the dorsal surface of the embryo. The ventral and anterior areas of the embryo are not stained by MH27 at that stage (Fig. 1A,E). As development proceeds, hypodermal cells modify their positions to enclose the embryo, which can be visualized with MH27 and anti-LIN-26 antibodies (Fig. 1). In particular, cells from the outer row on each side of the embryo (there are three rows of hypodermal cells on each side; see Fig. 1C,D) extend their margins ventrally (Fig. 1E–G) and finally attach to each other through adherens junctions (Sulston et al., 1983; Podbilewicz and White, 1994). A few additional hypodermal cells are generated anteriorly and posteriorly (Sulston et al., 1983) at this time. Several cells fuse together between 360 min and 420 min, which translates into a modification of the MH27 pattern dorsally, anteriorly, and posteriorly (Podbilewicz and White, 1994; Fig. 1G,H). Elongation of the spheroid embryo under the control of the hypodermal cytoskeleton and its transformation into a cylindrical worm begin about 350 min after fertilization, which can be viewed by MH27 staining (Priess and Hirsh, 1986; Podbilewicz and White, 1994).

MH27 staining in the intestine primordium is first detected about 240 min after fertilization (Fig. 1I). Intestinal MH27 staining is initially slightly punctate but rapidly becomes contiguous and more intense. The intestinal pattern resembles a ladder, which is located around the prospective position of the intestinal lumen (Fig. 1L). As the embryo elongates, the MH27 antibody stains the connections that link the intestine to the pharynx and to the rectum through the pharyngeal-intestinal and the intestinal-rectal valves, respectively (Fig. 1L). MH27 staining in the pharynx primordium is first detected about 320 min after fertilization (Fig. 1J) as a cloud of small dots and lines. MH27 staining changes dramatically in the pharynx as its organogenesis proceeds (Fig. 1I–L). At the end of embryogenesis, two bulbs and the buccal cavity can be recognized (Fig. 1L and data not shown). The MH27-reactive antigen is located around the presumptive position of the pharyngeal lumen.

Deficiencies Can Be Classified Into Five Classes According to Their MH27 Hypodermal Staining Pattern

I have examined embryos homozygous for 91 chromosomal deficiencies representative of 74–77% of the genome, including approximately 85% of the gene-rich regions (for a discussion on the extent of the genome examined, see Chanal and Labouesse, 1997), by whole-mount immunofluorescence staining with the monoclonal antibody MH27 and an antiserum raised against the LIN-26 protein (Labouesse et al., 1996). These markers allowed me to determine the number, position, and shape of hypodermal cells. I examined the same set of deficiencies as Chanal and Labouesse and included one additional deficiency, qDf16 (LGl), to determine whether it induces the same mutant phenotype as the overlapping deficiency, hDf8 (see below). Embryos homozygous for qDf16 have a normal number of LIN-26+ cells.

Based on their hypodermal MH27 staining pattern, I attempted to classify deficiencies into five categories (Table 1). Most deficiencies appeared to affect the MH27 expression pattern.

Type I deficiencies. Type I deficiencies are the most common deficiencies (Table 1). Three criteria characterize type I deficiencies. First, the embryo generally ruptured before the end of embryogenesis, as seen by Nomarski microscopy, indicating that it was not enclosed normally (Fig. 2C–H), and failed to elongate. Second, the most anterior part of the embryo and/or the ventral part of the embryo were never stained at any embryonic period by MH27 (Fig. 3 and data not shown). In parallel, the positions of hypodermal cells were often abnormal along the anterior/posterior and/or the dorsal/ventral axis. Third, an intense MH27 staining line was often observed (Fig. 3A,F,H), which could correspond to a stalling hypodermal extension/migration front. In
severe cases, the pharynx (when present) and the gut (for deficiencies affecting ventral-directed enclosure) were located abnormally on the external edge of the embryo, as seen by Nomarski optics and by MH27 staining (Figs. 2C–H, 3E,G,J). This observation could be explained by the fact that hydrostatic pressure from the inside caused group of cells or entire organs to be extruded. Such a phenotype has been documented previously by Priess and Hirsh (1986) after killing certain hypodermal cells by laser ablations or in strong lin-26 loss-of-function mutants with defective hypodermal cells (Labouesse et al., 1994). The MH27 and LIN-26 staining pattern changed little after the normal comma stage (except for cell fusion events). Together, these data suggest that ventral- and/or anterior-directed extension/migration processes were defective.

Type I deficiencies can be further subdivided into four categories. In embryos homozygous for deficiencies...
listed in both the “anterior defect” and the “ventral defect” columns of Table 1. MH27 staining was absent both anteriorly and ventrally; hypodermal nuclei did not occupy the characteristic positions that they occupy after the comma stage but, instead, occupied dorsal and posterior positions comparable to their positions at 240 min of development (Fig. 3C). In embryos homozygous for deficiencies listed only in the column “anterior defect” (the overlapping deficiencies hDf8 and qDf16 (LGI), nDf40 (LGII1); see Table 1), MH27 staining was generally absent in the anterior and ventral part of the head (Fig. 3F–H), which ruptured (Fig. 2E,F). An intense staining line could be observed in the head just posterior to the area left uncovered by MH27 (Fig. 3F,H and data not shown). By contrast, the lin-26 and MH27 patterns were essentially normal in the body and the tail (data not shown), which elongated to some extent (Figs. 2E,F, 3F–H). This suggests that hDf8, qDf16, and nDf40 affected mostly anterior-directed migrations. In embryos homozygous for deficiencies listed only in the column “ventral defect” (the overlapping deficiencies hDf9, nDf25, and mnDf111 (LGI), ozDf2 (LGV); see Table 1), there was generally no staining in the ventral part of the embryo, but staining in the anterior part of the head was essentially normal (Fig. 3I,J); hypodermal nuclei occupied rather dorsal positions, and the embryo ruptured from the body region, whereas the head elongated to some extent (Fig. 2G,H). These deficiencies presumably affected only or mostly ventral-directed enclosure. The ventral enclosure defect observed in ozDf2 embryos could be entirely due to deletion of the gene pha-4, because most pha-4 embryos show ventral enclosure defects (Mango et al., 1994; M.L., data not shown). Finally, several deficiencies affected only moderately ventral-directed enclosure of hypodermal cells (column “mild ventral defect” in Table 1). In embryos homozygous for such deficiencies the MH27 rings surrounding the central ventral hypodermal cells failed to connect at the ventral midline, leaving a small ventral area uncovered by hypodermal cells (Fig. 3M); however, hypodermal nuclei occupied their normal positions along the dorsal-ventral axis at the end of embryogenesis.

Type I deficiencies did not necessarily affect lin-26 expression, although most deficiencies that reduced the number of lin-26+ cells in the body (mnDf4, mnDf88, tDf1, cTf2, eDf19, sDf45, sDf40, sDf34, sDf33, and mnDf1; Chanal and Labouesse, 1997) were type I

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**TABLE 1. Classification of Deficiencies Based on Their MH27 Hypodermal Staining and Elongation Defects**

<table>
<thead>
<tr>
<th>Linkage group</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IV</th>
<th>No defect</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGI tDf3, sDf4, hDf8, qDf16, qDf7, qDf8, qDf9, qDf10</td>
<td>tDf3, sDf4, hDf9, qDf11, qDf7, qDf8, qDf9, hDf10, nDf25</td>
<td>hDf15, hDf16, hDf17</td>
<td>hDf10, qDf15, qDf5, eDf3, eDf4</td>
<td>hDf6, sDf6, eDf24</td>
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</tr>
<tr>
<td>LGI maDf4, mnDf68, mnDf61, mnDf29, mnDf57, mnDf83, mnDf87, mnDf189</td>
<td>maDf4, mnDf68, mnDf88, cDf90</td>
<td>cDf1, mnDf30, mDf1, mnDf67</td>
<td>nDf3, mnDf100, mnDf59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGI tDf1, cTf1, qDf2, nDf40</td>
<td>tDf1, cTf2, sDf121</td>
<td>sDf124, nDf11, nDf20, mDf4, mDf18, sDf2</td>
<td>nDf16, sDf110</td>
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<td></td>
</tr>
<tr>
<td>LGIV eDf19, nDf27, sDf23</td>
<td>eDf19, nDf27, sDf23</td>
<td>mDf5, sDf60</td>
<td>sDf17, mDf7</td>
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<td>sDf45, sDf40, sDf33, sDf34, iDf2</td>
<td>sDf124, nDf20, sDf30, yDF12, yDF8, yDF6</td>
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<td>syDf1, mnDf2</td>
<td>mnDf41</td>
<td></td>
</tr>
</tbody>
</table>

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* Deficiencies were classified in five categories (see text) according to the hypodermal MH27 and Nomarski phenotypes observed in at least two-thirds of the embryos. When a deficiency is listed both in the columns “anterior defect” and “ventral defect,” it means that embryos homozygous for that deficiency displayed both anterior and ventral migration defects. For a few deficiencies, about 50% of the embryos could be classified in one category and 50% could be classified in another category (e.g., itDf2), in which cases, the corresponding deficiency was listed in two different categories.

* Deficiencies that caused early cell division arrest.

* Cells fusions that should normally generate the hyp7 syncytium were generally defective; cells fusions also failed to occur in some sDf121 embryos.

* Certain phenotypes are likely to be synthetic.

* Cells fusions were abnormal.

* MH27 staining in the hypodermis showed a forked pattern.

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* MH27 staining in the hypodermis showed a forked pattern.

* Cells fusions have essentially no hypodermal cells. sDTf5 embryos did not express the MH27-reactive antigen at all. 
deficiencies, in accordance with the notion that hypodermal cells are important to preserve the integrity of the embryo. These deficiencies either caused premature cell proliferation arrest (maDf4, ctDf1, tDf1, mnDf1) or eliminated genes that were necessary to specify the fates of all or of a subset of hypodermal cells. A few type I deficiencies reduced the number of LIN-26+ cells in the head (qDf3, qDf4, sDf4, mnDf57, mnDf83, mnDf89, ctDf1). For these, it is not precisely known whether missing LIN-26+ cells are support cells, hypodermal cells, or a mixture of both; lack of hypodermal cells could explain enclosure defects in the head. Two type I

Fig. 2. Nomarski phenotype of a few representative deficiencies. Embryos collected 5–7 hr (A,C,E,G,I,K) or 10–12 hr after egg laying (B,D,F,H,J,L) were photographed under Nomarski optics. Orientation of the embryos and thick and thin solid arrows are as in Figure 1. A,B: Wild type embryos at the one-and-a-half-fold and pretzel stages, respectively. C,D: Embryos homozygous for mnDf68, a type I deficiency. At midembryogenesis, mnDf68 embryos looked almost normal, except that they were not well enclosed ventrally (small arrow in C); at the end of embryogenesis, mnDf68 embryos showed a severely ruptured phenotype marked by extrusion of the pharynx and the gut. E,F: Embryos homozygous for hDf16, a type I deficiency that affected only anterior-directed migrations. The body was enclosed and had partially elongated (open arrow), while the head had ruptured, which extruded the pharynx. G,H: Embryos homozygous for hDf9, a type I deficiency that affected only ventral enclosure. The tip of the tail (arrowhead in G) and the head (open arrow in H) had partially elongated, but the body was not enclosed (small arrows). I,J: Embryos homozygous for hDf16, a type II deficiency. At midembryogenesis, the body had started to elongate, but the head was rupturing; at the end of embryogenesis, the embryo had fully ruptured, filled the entire volume of the eggshell, and it was difficult to distinguish the head from the tail. K,L: Embryos homozygous for syDf1, a type IV deficiency. Some embryos had an abnormal pharynx (see Table 2). Scale bar = 10 µm.
Deficiencies (tDf3 and nDf42) increased the number of hypodermal cells, and three increased the number of support cells (qDf7, qDf8, qDf9). For these last five deficiencies, it is not clear that the changes in the number of LIN-26^+ nuclei could be directly responsible for the enclosure defect.

In summary, type I deficiencies appear to affect enclosure along the dorsal/ventral axis and/or the anterior/posterior axis. Except for the deficiencies listed above, changes in MH27 expression could not be attributed directly to a change in the number of LIN-26^+ cells.

**Type II deficiencies.** Type II deficiencies are particular type I deficiencies characterized by a striking reorganization of the MH27 staining pattern during the second half of embryogenesis. At midembryogenesis (normal comma stage), the hypodermal MH27 pattern was almost normal and covered most of the surface of the embryo (Fig. 4A,G,I,K); at the end of embryogen-
esis, the MH27-reactive antigen could be detected only in a very small area, leaving most of the surface of the embryo uncovered, as if hypodermal membranes had aggregated together in a single position as a clump or as a large ring (Fig. 4E,H,J,L). The ring-shaped arrangement of the MH27-reactive antigen might result from mispositioning of one or more proteins within hypodermal membranes that would have strong affinity for themselves and bring the aggregation; circles or rings often correspond to the most favourable arrangements in terms of free energy. The extent of the embryo covered by the MH27-reactive antigen was observed to become smaller with time (Fig. 4A–F). Most hypodermal nuclei (large LIN-26 nuclei) were not surrounded by the MH27-reactive antigen. In young ctDf3 (G), stDf7 (I), and mDf7 (K) embryos, the hypodermal MH27 pattern did not extend to the anterior part of the head (arrows in G), or it showed a mild ventral migration defect (arrowheads in I and K). In old ctDf3 (H), stDf7 (J), and mDf7 (L) embryos, almost all MH27-reactive hypodermal antigen had withdrawn to a posterior and dorsal position (curved arrow in L).

Fig. 4. Deficiencies that caused hypodermal migration defects followed by severe retraction of hypodermal membranes (type II deficiencies). Embryos collected 4–6 hr (A,B,G,I,K), 6–8 hr (C,D) or 10–12 hr (E,F,H,J,L) after egg laying were costained with the monoclonal antibody MH27 (all but F) and with LIN-26 antiserum (F, which is in the same focal plane as E). Lateral (A,C,E–G,I–K), central (B,D), or dorsal (H,L) focal planes. Arrows, orientation, and enlargement are as in Figure 1. A,B: Young hDf16 embryo; the lateral MH27 pattern was almost normal and showed only a mild enclosure defect; the pharynx was at the beginning of an extrusion process. C,D: hDf16 embryo at midembryogenesis; the hypodermal MH27-reactive antigen had withdrawn from the head (arrow); extrusion of the pharynx was more pronounced. E,F: Old hDf16 embryo; almost all hypodermal MH27-reactive antigen had withdrawn posteriorly (curved arrow); most LIN-26 nuclei were not surrounded by the MH27-reactive antigen. G–L: In young ctDf3 (G), stDf7 (I), and mDf7 (K) embryos, the hypodermal MH27 pattern did not extend to the anterior part of the head (arrows in G), or it showed a mild ventral migration defect (arrowheads in I and K). In old ctDf3 (H), stDf7 (J), and mDf7 (L) embryos, almost all MH27-reactive hypodermal antigen had withdrawn to a posterior and dorsal position (curved arrow in L).
eggshell and in which the MH27 pattern changed very little after midembryogenesis.

I classified four sets of deficiencies as type II: hDf16 and hDf17 (which overlap on LGI), ctDf3 (LGI11), stDf7 (LGIV), and mDf7 (LGIIV). In hDf16, hDf17, stDf7, and mDf7 embryos, which could elongate until the comma or the one-and-a-half-fold stage, enclosure was almost normal, leaving initially only a very small area ventrally and/or anteriorly uncovered (Fig. 4A–I); these embryos had a normal number of LIN-26+ cells or, for stDf7, a slight excess of hypodermal cells (Chanal and Labouesse, 1997). In ctDf3 embryos, enclosure was more strongly affected, and the staining pattern at the time of wild type comma stage was abnormal (Fig. 4G), in part as a result of deleting the gene ale-1 (Chanal and Labouesse, 1997), which is required to generate the normal number of hypodermal cells.

**Type III deficiencies.** In embryos homozygous for type III deficiencies, hypodermal movements seemed normal. Indeed, they could elongate beyond the comma stage (see Table 1 in Chanal and Labouesse, 1997), and the MH27-reactive antigen covered the surface of the embryo, although I cannot exclude some minor enclosure defects. However, as seen by Nomarski optics, embryos homozygous for these deficiencies ruptured before the end of embryogenesis, with internal cells floating between the embryo and the eggshell, or had a very lumpy morphology. This suggests that hypodermal cells were not fully functional and failed to enclose the embryo properly.

**Type IV deficiencies.** In embryos homozygous for type IV deficiencies, the structure of the external epithelium was unaffected, as inferred from their MH27 staining pattern and Nomarski terminal phenotype. However, these embryos could not elongate beyond the comma stage (see Table 1 in Chanal and Labouesse, 1997), and the MH27-reactive antigen covered the surface of the embryo, although I cannot exclude some minor enclosure defects. However, as seen by Nomarski optics, embryos homozygous for these deficiencies ruptured before the end of embryogenesis, with internal cells floating between the embryo and the eggshell, or had a very lumpy morphology. This suggests that hypodermal cells were not fully functional and failed to enclose the embryo properly.

**Type V deficiencies.** Type V deficiencies are deficiencies that allowed embryos to elongate at least to the twofold stage of embryogenesis and did not affect the hypodermis. Most correspond to small deficiencies.

**Aspect of MH27 rings.** I noticed other hypodermal defects using the MH27 antibody that concerned the very existence of MH27 rings, their aspect, or cell fusion events. No deficiency abolished MH27 staining in all parts of the embryo except type III deficiency stDf5 (LGX), as originally noticed by J. Rothman and his colleagues (personal communication; Terns et al., 1997). There was essentially no MH27 staining on the surface of edF19 (LGIIV) embryos; as argued in Chanal and Labouesse (1997), we believe that edF19 deletes a gene involved in specifying the fates of hypodermal cell. The number of MH27 rings was almost null in maDf4 (LGI1) embryos and was strongly reduced in tDf1 (LGI11), tDf2 (LGI11), and mnDf1 (LGX) embryos, which is probably a consequence of their early embryonic arrest (Chanal and Labouesse, 1997), although they could also eliminate a gene required for normal differentiation of hypodermal cells. In most sDf35 (LGV) embryos, the MH27 pattern was punctate in the pharynx, the gut, and the hypodermis (Fig. 3D,E), suggesting an abnormal organization of the MH27-reactive antigen in adherens junctions. A punctate pattern was observed around hypodermal cells in mnDf88 and mnDf89 (LGI1) embryos, but this phenotype is probably synthetic (Chanal and Labouesse, 1997). In sDf124 (LGI11) embryos, the thin lines characteristic of the MH27 staining pattern around each hypodermal cell (the MH27 rings) had an irregular thickness and were often forked or duplicated (Fig. 5P).

Even though MH27 is not the appropriate marker with which to determine in a systematic way whether cell fusion events involved the same cells as in wild type embryos, deficiencies, such as sDf110 (LGI11), nDf40 (LGI11), nDf41 (LGIIV), and sDf1 (LGX), caused a striking increase in the size of some MH27 rings, suggesting that cell fusions were abnormal in these embryos. In nDf41 embryos, the MH27 pattern was already abnormal soon after the MH27-reactive antigen could be detected (Fig. 5F,G). Although the number of LIN-26+ nuclei was normal, the number of MH27 rings was reduced, and most rings appeared to surround more than one nucleus (normally, each hypodermal nucleus should be surrounded by its own MH27 ring at that time; compare with Fig. 1A,E). One possibility could be that hypodermal cells fuse prematurely, immediately after being generated. Alternatively, because LIN-26 nuclei were frequently found by pairs within a single MH27 ring, nDf41 could prevent normal cytokinesis. Later in embryogenesis, MH27 rings were extremely large (Fig. 5H). In nDf40 embryos, the position of hypodermal cells was slightly abnormal early on, although each LIN-26+ nucleus was surrounded by its own MH27 ring (data not shown); at the end of embryogenesis, MH27 rings were larger than normal in the body (Chanal and Labouesse, 1997).

**Type V deficiencies.** Type V deficiencies are deficiencies that allowed embryos to elongate at least to the twofold stage of embryogenesis and did not affect the hypodermis. Most correspond to small deficiencies.

**Aspect of MH27 rings.** I noticed other hypodermal defects using the MH27 antibody that concerned the very existence of MH27 rings, their aspect, or cell fusion events. No deficiency abolished MH27 staining in all parts of the embryo except type III deficiency stDf5 (LGX), as originally noticed by J. Rothman and his colleagues (personal communication; Terns et al., 1997). There was essentially no MH27 staining on the surface of edF19 (LGIIV) embryos; as argued in Chanal and Labouesse (1997), we believe that edF19 deletes a gene involved in specifying the fates of hypodermal cell. The number of MH27 rings was almost null in maDf4 (LGI1) embryos and was strongly reduced in tDf1 (LGI11), tDf2 (LGI11), and mnDf1 (LGX) embryos, which is probably a consequence of their early embryonic arrest (Chanal and Labouesse, 1997), although they could also eliminate a gene required for normal differentiation of hypodermal cells. In most sDf35 (LGV) embryos, the MH27 pattern was punctate in the pharynx, the gut, and the hypodermis (Fig. 3D,E), suggesting an abnormal organization of the MH27-reactive antigen in adherens junctions. A punctate pattern was observed around hypodermal cells in mnDf88 and mnDf89 (LGI1) embryos, but this phenotype is probably synthetic (Chanal and Labouesse, 1997). In sDf124 (LGI11) embryos, the thin lines characteristic of the MH27 staining pattern around each hypodermal cell (the MH27 rings) had an irregular thickness and were often forked or duplicated (Fig. 5P).

Even though MH27 is not the appropriate marker with which to determine in a systematic way whether cell fusion events involved the same cells as in wild type embryos, deficiencies, such as sDf110 (LGI11), nDf40 (LGI11), nDf41 (LGIIV), and sDf1 (LGX), caused a striking increase in the size of some MH27 rings, suggesting that cell fusions were abnormal in these embryos. In nDf41 embryos, the MH27 pattern was already abnormal soon after the MH27-reactive antigen could be detected (Fig. 5F,G). Although the number of LIN-26+ nuclei was normal, the number of MH27 rings was reduced, and most rings appeared to surround more than one nucleus (normally, each hypodermal nucleus should be surrounded by its own MH27 ring at that time; compare with Fig. 1A,E). One possibility could be that hypodermal cells fuse prematurely, immediately after being generated. Alternatively, because LIN-26 nuclei were frequently found by pairs within a single MH27 ring, nDf41 could prevent normal cytokinesis. Later in embryogenesis, MH27 rings were extremely large (Fig. 5H). In nDf40 embryos, the position of hypodermal cells was slightly abnormal early on, although each LIN-26+ nucleus was surrounded by its own MH27 ring (data not shown); at the end of embryogenesis, MH27 rings were larger than normal in the body (Fig. 1H and data not shown). In syDf1 embryos, the MH27 pattern was normal at midembryogenesis; later, what corresponds to the seam cell and P cell MH27 pattern was normal, but there seemed to be only one hypodermal syncytium that included most or all cells that normally contribute to the formation of hyp1-11 syncytia (Fig. 5K,L). Except for syDf1, the nature of the cells that abnormally fused is not clear.

Opposite to the phenotype just described, the deficiencies qDf3 (LGI), sDf23 (LGIIV), nDf42, and itDf2 (LGV; Table 1, footnote a, Figs. 3N,O, 5M–O) generally prevented formation of the hyp7 syncytium and of other syncytia. In embryos homozygous for nDf42 and itDf2, cells in normal positions of intestinal cells expressed LIN-26 (Chanal and Labouesse, 1997), raising the possibility that the hyp7 syncytium did not form,
Fig. 5. A–P: Deficiencies that prevented elongation of the embryo (type IV deficiencies) and that affected cell fusions. Embryos collected 2–4 hr (F,G), 4–6 hr (I,K), or 8–10 hr after egg laying (A–E,H,J,L–P) were costained with the monoclonal antibody MH27 and with LIN-26 antisera (G). Lateral (A–E,H,L–P), or dorsal (F,G,M) focal planes. Arrows, orientation, and enlargement are as in Figure 1. Embryos homozygous for nDf3, (A), mnDf106 (B), sDf75 (C), sDf27 (D), and mnDf2 (E), which are type IV deficiencies that prevented elongation. F–O: Type IV deficiencies that affected cell fusions. F,G:nDf41 embryo (G is a double exposure for LIN-26 and MH27 in the same focal plane as F). MH27 rings did not yet form a contiguous line (arrows in F), and the head was not stained, indicating that this embryo is young; most MH27 rings surrounded two or more LIN-26 nuclei (arrows in G), whereas they should normally surround only one at that stage. J: Old sDf110 embryo; notice the very large MH27 rings. K: Embryo tentatively classified as an syDf1 embryo based on its abnormal pharyngeal MH27 staining (see Table 2); otherwise, the hypodermal MH27 staining appeared normal at that stage. L: Old syDf1 embryo; there appeared to be a single, giant syncytium instead of the usual 11 syncytia hyp1–hyp11 (notice, small arrows, the absence of the MH27 lines that demarcate the hyp7, the hyp6, the hyp5, and the hyp4 syncytia in the head; compare with Fig. 1H or to E in this figure). qDf3 embryo (M), nDf42 embryo (N), and itDf2 embryo (O), three deficiencies that prevented cell fusions (arrows). P: sDf124 embryo (type III); the hypodermal MH27 pattern formed numerous parallel lines (arrows).
because there were cells that ectopically expressed a hypodermal-like cell fate in the body. Alternatively, nDf42 and itDf2 could both delete a gene required for cells to fuse.

**MH27 Staining Defects Within Internal Organs**

Although the main goal of this study was to investigate morphogenesis of the embryo, I noticed that most deficiencies also affected MH27 staining in the pharynx but that very few affected staining in the intestine. In embryos homozygous for the overlap- ing deficiencies qDf3 and qDf4 (LGI), the pharynx was absent or had not differentiated normally. By using the monoclonal antibody MH27, which recognizes an adherens junction protein present in hypodermal cells, the pharynx, and the intestine, and an antiserum against the LIN-26 protein, which is expressed in nonneuronal ectodermal cells, I have carried out a systematic examination of the contribution of the zygotic genome to morphogenesis. Probably the most important outcome of this study is that I have been able to characterize several categories of mutant phenotypes. Second, I have shown that several zygotic genes are essential for enclosure of the embryo, for integrity of the hypodermal membranes and for elongation of the embryo.

**DISCUSSION**

By using the monoclonal antibody MH27, which recognizes an adherens junction protein present in hypodermal cells, the pharynx, and the intestine, and an antiserum against the LIN-26 protein, which is expressed in nonneuronal ectodermal cells, I have carried out a systematic examination of the contribution of the zygotic genome to morphogenesis. Probably the most important outcome of this study is that I have been able to characterize several categories of mutant phenotypes. Second, I have shown that several zygotic genes are essential for enclosure of the embryo, for integrity of the hypodermal membranes and for elongation of the embryo.

**Morphogenesis Depends on the Activity of Zygotic Genes**

The first conclusion is that almost all deficiencies affected in some way morphogenesis of the embryo and/or organogenesis of the pharynx (Fig. 6, Tables 1

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**TABLE 2. Classification of Deficiencies Based on their Pharyngeal MH27 Staining Defects**

<table>
<thead>
<tr>
<th>Linkage group</th>
<th>Absent</th>
<th>Primordium</th>
<th>Pharynx</th>
<th>Variably abnormal</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGI</td>
<td>qDf3, qDf4</td>
<td>df3, df4, mnDf111, qDf7, qDf8, qDf9, hDf15</td>
<td>df10, hDf8, qDf16, hDf6, hDf9, nDf25, qDf10</td>
<td>sDf6, qDf15, qDf17, hDf16, hDf3, eDf4</td>
<td>sDf6, mnDf11, nDf14</td>
</tr>
<tr>
<td>LGII</td>
<td>maDf4, mnDf89, mnDf83</td>
<td>mnDf88, mnDf106, mnDf100, mnDf89, mnDf29, mnDf57, mnDf38, mnDf90, mnDf87</td>
<td>nDf3, ccDf1, mnDf30, mnDf68, mnDf67, mnDf61, mnDf59</td>
<td>sDf6, qDf5, qDf19, hDf6, sDf12, nDf16, sDf20, nDf40, sDf3</td>
<td>sDf124</td>
</tr>
<tr>
<td>LGIII</td>
<td>tDf1, cDf2</td>
<td>sDf110</td>
<td>nDf11, sDf121, nDf16, nDf20, nDf40, sDf3</td>
<td>sDf7, sDf18, sDf60, nDf27</td>
<td>sDf72, sDf74, yDf12</td>
</tr>
<tr>
<td>LGIV</td>
<td>oDf2</td>
<td>mnDf5, mDf4, nDf41, cDf19, mDf17, sDf2, sDf23</td>
<td>sDf75, sDf50, sDf27, sDf30, sDf20, cDf1, yDf6, yDf6, sDf12, yDf12</td>
<td>sDf72, sDf74, yDf12</td>
<td></td>
</tr>
<tr>
<td>LGX</td>
<td>syDf1, mnDf1</td>
<td>sDf40, sDf45, sDf33, sDf34, sDf35, itDf2, nDf42</td>
<td>sDf6, uDf1, nDf19, nDf2, mnDf20</td>
<td>sDf41</td>
<td></td>
</tr>
</tbody>
</table>

*a* A similar classification was made for defects observed in the intestine, the rectum, and the connections between the intestine and the pharynx or the rectum, which is available upon request. sDf5 is not included in this table, because it prevents expression of the MH27-reactive antigen.

*b* Deficiencies were classified according to their pharyngeal MH27 pattern as “absent” (absent or extremely reduced staining); “primordium” (staining reduced to a roundish clump, as observed in wild type embryos when the pharynx is still a primordium; see Fig. 1, K); “variably abnormal” (variable staining depending on the embryo) or “normal” (normal staining).

*c* DF10, sDf2, and syDf1, embryos had almost normal posterior staining but no or strongly reduced anterior pharyngeal staining.

*d* For deficiencies marked with an asterisk in this column, the pharynx was almost normal; for other deficiencies, the pharynx was strongly to slightly abnormal.

*e* In cDf1, mnDf30, mnDf67, nDf20, and nDf40 embryos, staining was essentially normal, except that the pharynx failed to connect to the outside.

*f* Deficiencies that caused early cell division arrest.
and 2). By contrast, early cleavage patterns (Storfer-Glazer and Wood, 1994), the generation of body-wall muscle cells (Ahnn and Fire, 1994), of hypodermal and glial-like cells (Chanal and Labouesse, 1997), and of the pharyngeal primordium and the intestine (this study) were seldom affected. Organogenesis of the intestine...
also seemed little affected, which might be due to the fact that the intestine is set out early during embryogenesis as the unique descendant of the E blastomere (Sulston et al., 1983). In agreement with previous studies in nematodes (Ahnn and Fire, 1994; Storfer-Glazer and Wood, 1994) and flies (Merril et al., 1988; Wieschaus and Sweeton, 1988), these observations suggest that many zygotic genes can be deleted with minimal effects on early embryogenesis and on generation of various cell types. Furthermore, it shows that later aspects of embryogenesis mark an absolute requirement for the activity of zygotic genes.

**Some Deficiencies Affect Only Distinct Steps During Morphogenesis of the Embryo and Are Likely to Define Specific Genes Required for These Steps**

Because the proportion of deficiencies affecting both morphogenesis of the embryo and organogenesis of the pharynx is very high, it raises the question of whether the phenotypes induced by these deficiencies would not result from an indirect effect. For instance, deletion of genes encoding housekeeping proteins could indirectly hinder morphogenesis and organogenesis by making hypodermal or pharyngeal cells sick. Furthermore, the phenotypes induced by certain large deficiencies are likely to be synthetic (the punctate nature of hypodermal MH27 staining in mnDF88 embryos; the late onset of lin-26 expression, and the absence of pharyngeal MH27 staining in the heads of mnDF89 embryos; the early embryonic arrest of mnDF1 embryos; see Chanal and Labouesse, 1997; this study). It is difficult to determine whether or not a given deficiency identifies a gene specifically involved in morphogenesis and/or organogenesis or whether it acts indirectly. One way to do so is to examine Table 1: Deficiencies that affect only one aspect of morphogenesis might be specific. Another criterion is to consider the size of the deficiency: Large deficiencies are more likely to eliminate several housekeeping genes, whereas small deficiencies might be specific.

The second major conclusion from this study is that morphogenesis of the embryo consists of several consecutive steps that are separately mutable and that certain deficiencies affect only specific steps of the morphogenetic process. By using the first criterion outlined above, my suggestions (with all of the limitations raised previously in mind) are that type I deficiencies affecting anterior-directed hypodermal cell migrations, but not ventral-directed movements (hDF8, qDF16, nDF40), type I deficiencies affecting only ventral-directed movements (hDF9, nDF25, mnDF11, ozDF2), type II deficiencies (hDF16, hDF17, tDF3, stDF7, mDF7), and type IV deficiencies that do not also affect cell fusions (nDF3, mnDF100, mnDF106, nDF16, sDF2, mnDF2) define specific loci involved in morphogenesis of the embryo. Some type III deficiencies could also affect in a specific way the function of the hypodermis (for instance, mcDF1 embryos have a terminal phenotype very similar to those of lin-26(null) embryos; see Chanal and Labouesse, 1997). By using the second criterion (size of the deficiency), I would suggest that the smallest type I deficiencies, tDF3 (LG1), qDF4 (LG1), qDF10 (LG1), mnDF90 (LG11), sDF23 (LGIV; although its right border is ill-defined), which delete less than 0.7% of the genome mostly outside of gene-rich areas, possibly mnDF68 and mnDF29 (LG11), sDF60 (LGIV), the next smallest deficiencies, which delete about 1% of the genome, might define genes required for normal hypodermal cell migrations. These suggestions do not imply that deficiencies not listed here do not define genes important for morphogenesis. Systematic study of smaller deficiencies and the use of additional hypodermal markers could provide a means to determine whether any are susceptible to define a gene affecting morphogenesis in a specific way. Along the same rationale, I suggest that deficiencies affecting the aspect of the MH27 ring (sDF124, sDF35) and some of the deficiencies affecting cell fusions (qDF3, sDF23; possibly also sDF110, nDF40, nDF41, nDF42, itDF2, and syDF1, although, for these latter, there are alternative explanations) define specific loci involved in histogenesis of the hypodermal epithelium.

Deficiencies affecting almost exclusively anterior- or ventral-directed hypodermal movements could define loci required to induce certain aspects of hypodermal differentiation (for instance, cells that should migrate could fail to acquire their proper identity) or loci required for the extension and migration process itself (proteins that contribute to reorganization of the cytoskeleton, to membrane ruffling, proteins that constitute migrating cues or that are substrates on which cells migrate, or their receptors). It will be important to determine whether other migration events known to happen during embryogenesis (see Hedgecock et al., 1987; Manser and Wood, 1990) take place in such embryos. Alternatively, the phenotypes conferred by type I deficiencies could result from an early defect in gastrulation, which culminates with closure of the ventral deft (Bucher and Seydoux, 1994). Type II deficiencies, which lead to withdrawal of the hypodermal MH27-reactive antigen from most of the embryonic surface and to its clustering in a small area, could define genes required to maintain the integrity of the external epithelium (adherens junctions or tight junctions), its attachment to the basement membrane, and/or its apical/basal polarity. The phenotypes observed in some type II deficiencies are reminiscent of the phenotypes described for the Drosophila mutants crumbs, stardust, and shotgun, which affect biogenesis of adherens junctions and lead to disintegration of epithelia (for a recent review, see Knust and Leptin, 1996). Type III deficiencies might identify loci important for hypodermal differentiation or for the formation of the cuticle. Type IV deficiencies could identify loci encoding proteins that play an active role during the elongation process, such as proteins that constitute the contractile proteins, which modify the shape of hypoder-
mal cells and bring about elongation of the embryo, or that modify their activity (see Priess and Hirsh, 1986). On the other hand, type IV deficiencies that decrease the number of hypodermal cells (sDf75) or that affect cell fusions (nDf41, sDf110, syDf1) might prevent elongation of the embryo indirectly by some sort of physical hindrance. Indeed, the fact that sDf75 embryos lack many hypodermal cells (see Chanal and Labouesse, 1997) could be incompatible with elongation; similarly, abnormal cell fusions could prevent the abnormal syncytia from changing their shapes and, hence, from mediating elongation of the embryo. Such deficiencies are likely to affect the decision to fuse: For instance, in syDf1 embryos in which there seems to be only one large syncytium, too many cells could have adopted the hyp7 identity, thereby leading to the formation of an extended hyp7 syncytium.

**Genes Involved in Morphogenesis of the Embryo**

Examination of Figure 6, which shows a map of the deficiencies that were analyzed, indicates that overlapping deficiencies generally present a similar phenotype. For instance, hDf8 and qDf16 both affect anterior-directed migrations, whereas hDf9, mnDf111, and nDf25 all affect ventral-directed movements. However, two sets of overlapping deficiencies confer different and conflicting phenotypes (see also the cases in which I suggested that phenotypes were likely to be synthetic). On LGI, qDf5 (type I) and qDf10 (type I) uncover the same loci according to the genetic map and yet have different phenotypes. Because they are small deficiencies, and because qDf7, qDf8, and qDf9 confer the same phenotype as qDf10, I would be inclined to think that the phenotype of qf10 embryos is specific and that the respective endpoints of qDf10 and qDf5 might simply be different. On LGIV, nDf41 (type IV) overlaps with stDf7 (type II) and yet leads to a different but very characteristic phenotype; one possibility could be that nDf41 is a complex deficiency.

Very few zygotic genes have been described that are important for embryonic development after the onset of zygotic transcription, in particular, for morphogenesis. A few have already been mentioned (lin-26, ale-1, emb-29, pha-4). Besides these four genes, is the phenotype of zygotic genes known to be required for embryogenesis consistent with the map shown in Figure 6? The deficiencies that I examined eliminate several lethal mutations, most of which are larval lethal and few of which are zygotic embryonic lethal. Very few among those embryonic lethal mutations have been characterized, such that it is not possible to relate their phenotypes to the phenotypes of the deficiencies that delete them. Some could clearly define the prime genes responsible for certain deficiency mutant phenotypes. The genes lin-12 and glp-1, which are both deleted by sDf110, are redundant during embryonic development and affect cell fate specification of certain precursors that normally contribute to the head hypodermis (Lambie and Kimble, 1991; Moskowitz and Rothman, 1996); deletion of lin-12 and glp-1 explains certain abnormalities in the MH27 pattern of sDf110 embryos (see Fig. 5, legend). The cluster of genes that encodes the SL1 splicing leader (the rrs-1 locus on LGV) is essential for different aspects of embryogenesis starting at gastrulation (Ferguson et al., 1996); it maps between yDf8 and yDf16 and is probably deleted by neither of them, because the phenotypes of embryos homozygous for these type III deficiencies seem quite different from those reported for rrs-1 embryos. Genes that affect muscle development and attachment (pat genes) lead to paralysis and arrest at the twofold stage, but often lead to a lumpy body morphology similar to that observed in certain type III deficiencies (Williams and Waterston, 1994); consistent with the defects observed in pat mutants, deficiencies that delete pat genes (sDf4, sDf3, cDf1, sDf124, nDf11, sDf110, stDf7, stDf1, mnDf2) fail to elongate normally. The only gene known to specifically affect elongation of the embryo is the recently described gene let-502 (LGI), which encodes a Rho-kinase homolog (Wissman et al., 1997). Let-502 maps under hDf6, a deficiency that allows elongation until the twofold stage, and was classified as type V. This classification is only superficially surprising, because severe let-502 mutations are gain-of-function mutations that show both maternal and zygotic effects (Wissman et al., 1997).

This study opens the way for a more detailed investigation of genes involved in controlling different aspects of hypodermal cell functions during morphogenesis of the embryo. Identification of genes important for morphogenesis of the C. elegans embryo will now have to rely on direct screens for lethal mutations that prevent elongation of the embryo.

**EXPERIMENTAL PROCEDURES**

**Strains**

The wild type strain N2 and mutant strains were maintained as described by Brenner (1974) at 20°C. This paper conforms to the standard C. elegans genetic nomenclature described by Horvitz et al. (1979). The deficiencies examined are shown in Figure 6. They were balanced and handled as described in Chanal and Labouesse (1997), where references for the deficiencies can be found (see also the ACEDB database; Eeckman and Durbin, 1995).

**Antibody Staining Experiments**

Embryos were permeabilized, fixed, costained with LIN-26 antiserum (Labouesse et al., 1996) at a 1/2,000 dilution together with the monoclonal antibody MH27 (Francis and Waterston, 1991) at a 1/200 dilution, and analyzed as described in Chanal and Labouesse (1997). Because the vast majority of deficiencies did not allow elongation of the embryos beyond the twofold stage of embryogenesis, staging of the embryos prior to staining and comparison of the terminal Nomarski phenotypes conferred by deficiencies with MH27 staining phenotypes generally allowed me to distinguish homozygous deficiency embryos from heterozygous and balancer
siblings (for details, see Chanal and Labouesse, 1997). I generally examined at least 20 mutant embryos while scoring staining experiments. Distinguishing homozygous deficiency embryos from heterozygous or balancer siblings was difficult when the deficiency allowed elongation beyond the twofold stage and was not conferring any particular phenotype. For such embryos, elongation beyond the twofold stage was strong evidence that hypodermal cells had been generated and were functional (see text). I determined whether they had defects in nonhypodermal organs by examining at least 50 embryos, among which one-quarter should be homozygous mutants.

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