The Zinc Finger Protein DIE-1 Is Required for Late Events during Epithelial Cell Rearrangement in C. elegans

Paul J. Heid,*,1 William B. Raich,†,2 Ryan Smith,† William A. Mohler,†,3 Kristin Simokat,† Steven B. Gendreau,*,4 Joel H. Rothman,*5 and Jeff Hardin†,6

*Department of Biochemistry, †Program in Cellular and Molecular Biology, and ‡Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin 53706

The mechanism by which epithelial cells undergo directed rearrangement is central to morphogenesis, yet the regulation of these movements remains poorly understood. We have investigated epithelial cell rearrangement (intercalation) in the dorsal hypodermis, or embryonic epidermis, of the C. elegans embryo by analyzing the die-1(w34) mutant, which fails to undergo normal intercalation. Dorsal hypodermal cells of die-1(w34) homozygous embryos initiate but fail to complete the process of intercalation. Multiphoton microscopy reveals that intercalating cells extend monopolar, basolateral protrusions in their direction of migration; posterior dorsal hypodermal cells in die-1(w34) mutants appear to extend protrusions normally, but fail to translocate their cell bodies to complete rearrangement. Despite abnormal intercalation, the subsequent morphogenetic movements that enclose the embryo with epithelial cells and the process of dorsal cell fusion still occur. However, elongation of the embryo into a wormlike shape is disrupted in die-1(w34) embryos, suggesting that intercalation may be necessary for subsequent elongation of the embryo. Actin filaments are not properly organized within the dorsal hypodermis of die-1(w34) embryos, consistent with intercalation's being a necessary prerequisite for elongation. The die-1 gene encodes a C2H2 zinc finger protein containing four fingers, which likely acts as a transcriptional regulator. DIE-1 is present in the nuclei of hypodermal, muscle, gut, and pharyngeal cells; its distribution suggests that DIE-1 acts in each of these tissues to regulate morphogenetic movements. die-1(w34) mutants display morphogenetic defects in the pharynx, gut, and muscle quadrants, in addition to the defects in the dorsal hypodermis, consistent with the DIE-1 expression pattern. Mosaic analysis indicates that DIE-1 is autonomously required in the posterior dorsal hypodermis for intercalation. Our analysis documents for the first time the dynamics of protrusive activity during epithelial cell rearrangement. Moreover, our analysis of die-1 shows that the events of epithelial cell rearrangement are under transcriptional control, and that early and later phases of epithelial cell rearrangement are genetically distinguishable. © 2001 Academic Press

INTRODUCTION

Cell rearrangements are known to play a critical role during morphogenesis in both vertebrate and invertebrate systems. There are two main cell types that undergo rearrangement: deep (nonepithelial) cells and epithelial cells. Intercalation of deep cells occurs during epiboly of the animal cap (Keller, 1980; Warga and Kimmel, 1990) and during convergent extension of presumptive axial mesoderm in Xenopus and zebrafish (Keller and Tibbetts, 1989; Wilson and Keller, 1991; Warga and Kimmel 1990), and neural tissue in Xenopus (Ellul and Keller, 2000). Similarly, in Drosophila terminal filament formation involves rearrangement of mesenchymal cells (Godt and Laski, 1995). Several molecular pathways have been implicated in deep
cell rearrangement. The transcription factor BRIC-A-BRAC, a BTB domain transcription factor, is necessary for terminal filament morphogenesis in Drosophila ovaries (Godt and Laski, 1995). Likewise, spadetail, which encodes a T-box transcription factor, is required for convergence movements of ventrolateral deep cells toward the dorsal side of the embryo in zebrafish (Griffin et al., 1998). Paraxial protocadherin (papc) family members also appear to be required in subsets of deep mesodermal cells for proper dorsal convergence movements (Kim et al., 1998; Yamamoto et al., 1998); papc has been implicated as a proximal downstream target of spadetail, suggesting that spadetail may regulate expression of genes directly involved in cell rearrangement.

Recently, it was shown that Wnt-11 and disheveled family members mediate planar polarity signals in both zebrafish (Heisenberg et al., 2000) and Xenopus (Tada and Smith, 2000; Wallingford et al., 2000) that are required for proper convergence of paraxial mesoderm toward the dorsal midline. The mechanisms that regulate cell rearrangement in epithelia are unclear. Gap genes appear to be at least indirectly required for coordination of germ band extension movements in Drosophila (Irvine and Wieschaus, 1994), although it is not known at what level they exert their influence. In the posterior spiracles, the GATAc protein encoded by grain is required for the rearrangement of cells in the stigmaphore (Brown and Castelli-Gair Hombría, 2000); what cell behaviors or processes GATAc regulates are unknown.

The simplicity of the embryonic hypodermis, or epidermis, of the nematode Caenorhabditis elegans makes it a convenient system in which to study epithelial cell rearrangement, because individual epithelial cells can be unambiguously identified from embryo to embryo. The hypodermis forms as a patch of 78 cells on the dorsal/posterior surface of the posterior region of the embryo. The first morphogenetic movement within this tissue involves the intercalation of 20 cells along the dorsal midline that eventually form the dorsal hypodermis (Williams-Masson et al., 1998). Specific cell migrations within the future ventral hypodermis (Williams-Masson et al., 1998) are necessary for the process of enclosure (Williams-Masson et al., 1999), germ band extension (Irvine and Wieschaus, 1994), and posterior spiracle development (Brown and Castelli-Gair Hombría, 2000) in Drosophila. The mechanisms that regulate cell rearrangement in epithelia are unclear. Gap genes appear to be at least indirectly required for coordination of germ band extension movements in Drosophila (Irvine and Wieschaus, 1994), although it is not known at what level they exert their influence. In the posterior spiracles, the GATAc protein encoded by grain is required for the rearrangement of cells in the stigmaphore (Brown and Castelli-Gair Hombría, 2000); what cell behaviors or processes GATAc regulates are unknown.

The simplicity of the embryonic hypodermis, or epidermis, of the nematode Caenorhabditis elegans makes it a convenient system in which to study epithelial cell rearrangement, because individual epithelial cells can be unambiguously identified from embryo to embryo. The hypodermis forms as a patch of 78 cells on the dorsal and ventral surfaces of the posterior region of the embryo. The first morphogenetic movement within this tissue involves the intercalation of 20 cells along the dorsal midline that eventually form the dorsal hypodermis (Williams-Masson et al., 1998). Specific cell migrations within the future ventral hypodermis begin as the process of intercalation is completing, and these movements are necessary for the process of enclosure (Williams-Masson et al., 1999).
al., 1997). Enclosure is followed by the elongation of the embryo into a wormlike shape (Priess and Hirsh, 1986). Once enclosure is complete and elongation is under way, many cells of the dorsal and ventral hypodermis fuse into large multinucleate syncytia (Podbielniak and White, 1994; Mohler et al., 1998). The major events of epithelial morphogenesis in C. elegans are summarized in Fig. 1.

Previous studies of dorsal intercalation in C. elegans showed that dorsal hypodermal cells are initially organized in two rows on the dorsal surface of the embryo (Sulston et al., 1983; Williams-Masson et al., 1998). Cells within each dorsal row rearrange by extending wedge-shaped protrusions between cells in the contralateral dorsal row; the cell tips then migrate until they meet the opposing row of lateral hypodermal cells. The end result of these movements is the formation of a single row of 20 dorsal hypodermal cells. Treatment of wild-type embryos with nocodazole or cytochalasin D prevents intercalation (Williams-Masson et al., 1998), suggesting that intercalation is dependent on both actin filaments and microtubules.

To determine what role dorsal intercalation plays in the subsequent morphogenesis of the organism and to understand how intercalation movements are regulated at the molecular level, we have undertaken a genetic analysis of the process. In this investigation we analyze die-1 (dorsal intercalation and elongation defective), a gene required for cell rearrangement in the dorsal hypodermis of C. elegans. We show that the posterior dorsal hypodermal cells of die-1 mutant embryos initiate, but cannot complete, intercalation. We also show that mutant embryos enclose with hypodermis but fail to elongate. die-1 encodes a putative zinc finger protein expressed in cells that display abnormal morphogenesis in the mutant. Mosaic analysis indicates that DIE-1 is specifically required in dorsal hypodermal cells for intercalation to occur. Our results indicate that DIE-1 regulates directed epithelial cell rearrangement in C. elegans.

**FIG. 2.** Intercalation is defective in homozygous die-1(w34) embryos. Wild-type (left column) and die-1(w34) mutant (right column) embryos at various stages of morphogenesis. Alternating pairs of figures are Nomarski and multiphoton laser scanning microscopy views of different, but comparably staged, embryos. Embryos in this and all figures are presented with anterior to the left. (A, B) Wild-type and die-1(w34) embryos shortly after all hypodermal cells have formed. Arrows indicate dorsal cells that have become wedge shaped (A, B). (C) Wild-type embryo 35 min after the onset of JAM-1:GFP expression. Intercalation has just begun. Dorsal hypodermal cells marked with asterisks have wedged between one another. (D) die-1(w34) embryo 65 min after the onset of JAM-1:GFP expression. Many of the anterior dorsal hypodermal cells have intercalated; however, most of the posterior dorsal cells are wedged and have not completed intercalation (asterisks). Arrows indicate cell boundaries that will fuse prematurely (see H). (E) Same embryo as A, 60 min later. Intercalation is essentially complete. Arrows indicate dorsal cells that have become narrower along the anterior/posterior (A/P) axis, elongated along the left/right (L/R) axis, and have finished intercalation. (F) Same embryo as B, 120 min later. Arrows indicate cells that fail to complete intercalation. Wedged tips have not moved to make contact with the opposing lateral hypodermal cells. Cells have extended along the L/R axis but have not become significantly narrower in the A/P axis. (G) Completely intercalated dorsal hypodermis in a wild-type embryo 100 min after the onset of JAM-1:GFP expression. (H) Same embryo as in B, 80 min after the onset of JAM-1:GFP expression. Arrows indicate cell boundaries that have started to fuse. (I) Same embryo as in A, 200 min later, lateral view. The embryo has elongated to twice its original length. (J) Same embryo as B, 200 min later. Elongation has failed. (K) JAM-1:GFP expression in an eff-1(oj55) embryo comparable to I. Elongation is normal. (L) Reconstruction of JAM-1:GFP expression in a terminal die-1(w34); eff-1(oj55) embryo; for clarity, the gut and intestinal expression has been omitted by “coring” the embryo as in Mohler and White (1998). Unintercalated cells in the posterior are clearly visible (arrows). Siblings had fully elongated. Scale bar = 10 μm.
MATERIALS AND METHODS

C. elegans Strains

The Bristol strain N2 was used as wild-type (Brenner, 1974). Nematodes were grown at 20°C in all experiments and were cultured as described by Brenner (1974). To map and balance die-1, the following mutations and deficiencies were obtained from the C. elegans Genetic Stock Center: LG I, bli-4(e937); LG II, rol-6(e187), unc-4(e120), let-23(mm22), let-31(mm31), let-242(mm90), let-243(mm226), let-244(mm97), let-268(mm189), vab-9(e1744), vab-11(e1879), vab-12, mnDf12, mnDf14, mnDf28, mnDf61, mnDf62, mnDf63, mnDf66, mnDf68, mnDf71, mnDf83, mnDf89; LG III, vab-7(e1562); LG IV, unc-31(e928), him-8(e1489); LG V, dpy-11(e224), him-5(e1467).

Rescue

wDf5 (linkage group II) was isolated in a screen for zygotic lethal mutations deficiencies (Ferguson et al., 1996; our unpublished data). For additional genetic analysis of die-1(w34), eff-1(oj55), LG II, jcs1 (jam-1::gfp), linkage group IV (Mohler et al., 1998), and ojEx3 were used. jcs1 is an integrated version of jam-1::gfp, and was crossed into the die-1 background by standard genetic techniques. The eff-1 mutant is zygotic recessive viable, and lacks cell fusion during embryogenesis (W. A. Mohler, unpublished observations). Heterozygous die-1(w34) males were mated into homozygous eff-1(oj55); jcs1 hermaphrodites. To generate double mutants, recombinants were identified by singling eff-1 homozygous F2 progeny; subsequent matings were performed to obtain eff-1; jcs1; die-1/+ hermaphrodites. ojEx3 was produced by injection of plasmid pK1, which carries a GFP transcriptional reporter for the lbp-1 gene (Plenefisch et al., 2000). This reporter is expressed at high levels in dorsal hypodermal cells.

Genetics

die-1(w34) was isolated from a general, genome-wide screen for ethylmethane sulfonate (EMS)-induced zygotic embryonic lethal mutations (for methods see Ferguson et al., 1996). die-1(w34) was mapped to linkage group II by standard techniques. Recombination between rol-6 and unc-4 placed die-1 very near or to the right of unc-4. Complementation tests against deletion mutants placed die-1 between egf-43 and daf-19.

Rescue

die-1(w34) was mapped to linkage group II between egf-43 and daf-19, a 0.2 map unit interval corresponding to ~300 kb of DNA. die-1(w34) fails to complement the deficiency mnDf71 and complements deficiencies mnDf66 and mnDf83, as well as the lethal mutations let-242(mm90), let-243(mm226), and let-244(mm97). Cosmids spanning the region between egf-43 and daf-19 were obtained from the C. elegans Genome Consortium and were tested for transformation rescue by conjoinment with the dominant marker rol-6(su1006) (Mello et al., 1991). One pool of three clones that rescued die-1 to viability was identified, and subsequently the injection of a 10-kb subclone of cosmid K10A2 was shown to be sufficient for rescue.

The cDNA yk83h8, obtained from the C. elegans cDNA Project (Y. Kohara), was the longest cDNA that mapped to the minimal die-1 rescuing genomic fragment. yk83h8 was sequenced using ABI PRISM dye terminator cycle sequencing (Perkin Elmer Cetus Instruments, Norwalk, CT). Genomic sequence from cosmid C18D1, available from the C. elegans Genome Consortium, agrees with our cDNA sequence and indicates that the die-1 locus consists of six exons included in the 10-kb rescuing fragment.

The genomic region of the die-1 locus was amplified from homozygous die-1(w34) embryos by the single-embryo PCR method (Williams et al., 1992). The following sets of primers were used to amplify the genomic region in three fragments that span the predicted coding sequence: ATCTGAGATAACCCATGTATATTCGAGGATTACGAGG-ATACGGGAC, ATCAAGGACAAGAAGCAACAGGAG; AGTC- TATCCCTTCCTCCTCAC, TTCGATTTGTTCTGGTATG; and ATCTTCGTTCTTCTTATTATTTCCG, GAAAGTAGACTACAAATGGC. In some cases nested internal primers were used to obtain single amplification products. All PCR products were subcloned into either pT7 vector (Novagen, Madison, WI) or PCR-Script (Stratagene, La Jolla, CA). For each amplification product three subclones of two independent samples were sequenced.

Phenotypic Analysis and Live Fluorescence Microscopy

Phenotypic analysis and image acquisition of Nomarski images were performed as previously described (Raich et al., 1998), with the following modifications: 15–24 optical sections of live embryos were recorded every 30–60 s. To monitor GFP expression using jcs1 and ojEx3 in living embryos, mounted embryos were filmed using multiphoton laser scanning microscopy (MPLSM). Data acquisition and stereo-4D processing were performed as previously described (Raich et al., 1999). In brief, Bio-Rad PIC format files were imported into NIH Image using customized Stereo-4D Macros (Mohler and White, 1998). Postimage processing was conducted on a Power Macintosh G3. Time-point projection stacks were converted in 4D Turnaround and 4D Viewer into appended 4D QuickTime movies and replayed as stereo-4D animations using a modified version of NIH Image. All macros, the standard version of NIH Image, and 4D Turnaround/Viewer are available at http://www.rocklabs.wisc.edu/imr/stero4d/stero4d.html. The modified version of NIH Image is available from J. Hardin.

Antibody Production and Immunostaining

Rabbit polyclonal antibodies recognizing DIE-1 were raised and affinity-purified against the following peptide: ALNLTFKNDGT-KEFKEIEE (Quality Controlled Biochemicals, Hopkinton, MA). A modified version of the freeze-crack method (Miller and Shakes, 1995) was used to process embryos for immunostaining essentially as previously described (Raich et al., 1998). Affinity-purified DIE-1 antisera and GFP antisera (Novagen) were added at 1:200 dilution, monoclonal MH27 antibody (kindly provided by Robert Waterston) was added at a 1:500 dilution, and the monoclonal body muscle antibody N68/4C6.3 (Goh and Bogaert, 1991) was added at 1:20 dilution. All primary antibodies were diluted into a solution of nonfat dry milk and applied to fixed specimens for 1 h at 37°C. The samples were washed twice in PBST for 5 min and incubated in FITC (Sigma Chemical Co., St. Louis, MO) or Texas Red conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) diluted 1:100 in PBST + 0.5% nonfat dry milk for 1 h at 37°C. The specimens were rinsed in PBST buffer and sealed in a drop of Slowfade antibleaching solution (Molecular Probes, Eugene, OR).

To analyze the organization of actin filaments within the hypodermis of die-1(w34) mutants, embryos were collected and stained with phalloidin as previously described (Costa et al., 1997). The only modification to this protocol was the use of Alexa 488 phalloidin (Molecular Probes) at a concentration of 10 units/ml. Fluorescent images of fixed samples were obtained using a Bio-Rad MRC1024 confocal laser scanning microscope.
die-1::gfp Translational Fusion

A minimal die-1 rescuing fragment was linearized by digestion with MluI, which cuts uniquely in the first intron of die-1. The GFP intron insertion vector pPD103.75 (courtesy of A. Fire, Carnegie Institution of Washington) was also cut with MluI, which drops out a fragment containing the entire GFP sequence flanked by consensus sequences for intron donor and acceptor splice sites. This fragment was cloned into the linearized die-1 rescuing vector. This construct was co-injected into die-1/mmc1 animals with jam-1::gfp and pRF4 (rol-6). A strain carrying an extrachromosomal array of these constructs (jcEx23) can rescue die-1(w34) homozygous embryos to viability, showing that DIE-1 remains functional with GFP inserted near its amino terminus. This array was subsequently integrated into the genome by crossing it into an N2 background, treating animals with 3000 rad of γ-irradiation, and identifying animals that produce 100% rollers in the F2 generation (Mello and Fire, 1995). GFP expression was visualized in living embryos using standard epifluorescence and MPLSM. Expression was weakly detected in some nuclei but could not be seen consistently or clearly. To enhance the GFP signal, embryos were processed via the freeze-crack method, stained with rabbit polyclonal anti-GFP antibodies (Novagen), and processed for immunofluorescence as described earlier.

Mosaic Analysis

To assess the role of DIE-1 in specific cells, animals expressing die-1::gfp in mosaic fashion were identified and analyzed for proper intercalation. Embryos from the rescued strain carrying jcEx23 were fixed and stained with GFP polyclonal antiserum followed by FITC-conjugated goat anti-rabbit secondary antibody and at the same time stained with monoclonal MH27 antibody followed by Texas Red-conjugated goat anti-mouse secondary antibody. This allowed visualization of all epithelial cell boundaries in the red channel; epithelial junctions could also be seen in the green channel in those cells that expressed the array. Mosaics were identified as embryos lacking epithelial staining in some cells in the green channel (see Results). Mosaic embryos were subsequently analyzed for morphogenetic defects.

Northern Blot

To determine the number and sizes of transcripts produced from the die-1 locus, total RNA was prepared from wild-type worms as previously described (Burdine et al., 1997). Poly(A)+ RNA was then isolated using the PolyATtract kit (Promega Corporation, Madison, WI). RNA (2 μg per lane) was loaded on a 1.2% agarose (w/v), 16% formamide (v/v) gel. The gel was blotted onto Hybond-N filter (Amersham, Buckinghamshire, UK) and RNA was cross-linked to the filter using a UV-Stratalinker 1800 (Stratagene) for 30 s. The probe was generated from linearized die-1::gfp Translational Fusion vector (Qiagen, Santa Clarita, CA). A 100-ng sample of purified cDNA was labeled using random decamers, 1 μl Klenow, 5 μl dCTP32, and 2.5 μl of a mix that contained dA, dT, and dG at 1 mM each. The reaction was incubated for 1 h at room temperature. The Northern blot was prehybridized for 30 min at 65°C in Denhardt’s reagent (Sambrook et al., 1989). The probe was purified by passage over a spin column and hybridizations were performed at 65°C for 24 h. Blots were washed three times for 30 min in 0.1× SSPE and exposed to Scientific Imaging Film (Kodak, Rochester, NY).

RESULTS
die-1 Is Required for Rearrangement of Dorsal Hypodermal Cells and Elongation of the Embryo

The first observable defect in die-1(w34) homozygous embryos is the failure of posterior dorsal hypodermal cells to undergo intercalation (Fig. 2). die-1(w34) embryos produce the normal complement of hypodermal cells and the dorsal cells become wedge-shaped as in wild-type embryos.
(Fig. 2B). However, posterior dorsal cells fail to finish the process of intercalation and remain wedge-shaped until they eventually fuse into syncytia (Fig. 2D). Despite abnormal intercalation, die-1(w34) embryos still enclose with hypodermis (Fig. 2F). Significantly, the process of elongation is also affected in die-1(w34) embryos. Wild-type embryos elongate to almost four times their initial length; die-1(w34) embryos never elongate more than twice their initial length (Figs. 2I–2L).

To visualize hypodermal boundaries more clearly, a jam-1::gfp translational fusion construct was expressed in die-1(w34) homozygous embryos. JAM-1::GFP is expressed at the junctional borders of all epithelial cells in C. elegans, allowing the analysis of hypodermal cell movements in developing die-1(w34) embryos with the use of multiphoton laser scanning microscopy (MPLSM) (Mohler et al., 1998; Raich et al., 1999). Anterior dorsal hypodermal cells derived from the AB founder cell successfully intercalate in mutant embryos (Fig. 2). In contrast, intercalation defects are readily apparent in posterior dorsal hypodermal cells in jam-1::gfp-expressing die-1(w34) embryos (Fig. 2D). The posterior dorsal hypodermis is derived from the C founder cell ( Sulston et al., 1983; Williams-Masson et al., 1998). Based on the position of unintercalated cells in die-1(w34) mutants, the anteriormost pair of C-derived cells consistently intercalate. In 12 out of 12 multiphoton movies in which dorsal hypodermal cells could be unambiguously identified, this pair of cells completed intercalation. In contrast, the remainder of the C-derived dorsal hypodermal cells displays intercalation failure. The posterior C-derived dorsal hypodermis was never observed to progress beyond wedging, consistent with results obtained via anti-JAM-1 immunostaining using the MH27 antibody (data not shown).

Late Steps in Intercalation Are Defective in die-1(w34) Embryos

Previous studies of fixed material indicated that intercalating dorsal hypodermal cells extend basolateral protrusions beneath the level of the adherens junction as they begin to interdigitate (Williams-Masson et al., 1998). To examine these protrusions dynamically in living die-1(w34) embryos, we used a transcriptional reporter for lbp-1 (Pleunefisch et al., 2000) to drive expression of GFP in posterior dorsal hypodermal cells during intercalation. In addition, we used eff-1(oj55) to suppress cell fusion so that the position of individual dorsal cells could be scored in terminal embryos. eff-1(oj55) mutants are homozygous viable, undergo dorsal intercalation normally, and are normal in other respects, but lack cell fusion during embryogenesis (W. A. Mohler, unpublished observations). Within 10 min following the terminal divisions of the dorsal hypodermal precursors, dorsal hypodermal cells in wild-type embryos extend protrusions toward the dorsal midline in the direction in which their cell bodies ultimately translocate (Figs. 3A–3E). Initially, pulsatile activity is detectable on both the medial and lateral edges of cells (Fig. 3A). Shortly after prontrusive activity becomes detectable the preponderance of protrusive activity becomes confined to the medial tips of the cells (Figs. 3B and 3C). There is little deviation of these protrusions along the anterior–posterior axis, that is, the protrusions are extended in a highly directional manner from right to left or left to right. In wild-type embryos, the lateral region of the cell undergoes a change in shape following the extension of a medial protrusion (Fig. 3C), such that the entire cell begins to elongate mediolaterally toward the contralateral side of the embryo until it spans the entire width of the dorsal hypodermis (Fig. 3D). The dynamic analysis of dorsal intercalation shows that (1) intercalating cells are polarized along the right-left (i.e., mediolateral) axis very rapidly after they are born, (2) intercalating cells are protrusively active throughout intercalation, and (3) protrusion extension is rapidly consolidated into a corresponding change in the shape of intercalating cells.

MPLSM analysis of die-1(w34);eff-1(oj55) embryos indicates that C-derived dorsal cells appear normal during the early phase of intercalation. C-derived dorsal cells extend dynamic protrusions that appear indistinguishable from those in wild-type embryos and appear correctly polarized by the time the cells’ apical surfaces become wedge-shaped [n = 6 embryos examined for both eff-1(oj55) and die-1(w34);eff-1(oj55); Figs. 3A, 3B, 3F, and 3G]. However, the cell bodies of C-derived posterior dorsal cells fail to translocate (Figs. 3H and 3I). These results indicate that C-derived dorsal hypodermal cells are polarized in die-1(w34) embryos and that they can extend protrusions that are normally associated with cell rearrangement prior to later defects in cell translocation.

die-1(w34) embryos appear to undergo premature fusion of the dorsal hypodermis, based on timing of fusion relative to the onset of jam-1::gfp expression (Fig. 2H). Measure-
ments from multiphoton recordings indicate fusion occurs approximately 45 min early in die-1(w34) embryos. In contrast, ventral enclosure occurs at the same relative time in both mutant and wild-type embryos (Table 1). Although fusion occurs early in die-1(w34) homozygotes, we were able to show that the precocious fusion phenotype does not account for the intercalation defects in die-1(w34) mutants by producing die-1(w34);eff-1(oj55);jam-1::gfp embryos. Although eff-1(qj55) single mutants undergo normal intercalation, we found that intercalation still fails to occur in die-1(w34);eff-1(qj55) double-mutant embryos, even though dorsal cells do not fuse (Fig. 2L). The posterior dorsal cells did not intercalate by the time muscle activity was observed (data not shown), more than 2 h after intercalation begins in wild-type embryos. This indicates that the process of intercalation is not simply delayed in die-1(w34) embryos and that precocious fusion is not masking dorsal cell rearrangement in die-1(w34) mutants.

die-1(w34) Mutant Embryos Display Additional Morphogenetic Defects

We were able to identify other morphogenetic defects within die-1(w34) embryos. In wild-type embryos the lateral hypodermis consists of two linear rows of 10 cells per side. (B) die-1(w34) embryos typically have lateral cells pinched out of the row (arrow). (C, D) Ventral views of wild-type and die-1(w34) embryos expressing JAM-1::GFP, as viewed with multiphoton microscopy. Both embryos are enclosed with hypodermis and are approximately the same age. Asterisks indicate the same cells in both embryos. (C) Hypodermal cells in a wild-type embryo meet at the ventral midline in a pairwise manner. The arrow indicates a junction at the midline between a specific pair of cells. (D) A die-1(w34) embryo; cells have met at the midline out of register. Small arrowheads indicate cells that failed to reach the midline. The arrow indicates the missing junction between the same pair of cells highlighted in C, which have fused prematurely. (E, F) Wild-type(E) and die-1(w34) (F) embryos expressing JAM-1::GFP, as viewed with multiphoton microscopy. (E) An embryo displaying normal attachment of the pharynx to the buccal cavity (arrow). (F) die-1(w34) embryo in which the pharynx has failed to attach to the buccal cavity. Arrow indicates absence of JAM-1-expressing cells between the pharynx and buccal cavity. (G, H) Wild-type (G) and die-1(w34) (H) embryos stained with the body wall muscle-specific antibody NE8/4C6.3. (G) Body muscle cells separate into distinct quadrants prior to elongation of the animal. The two dorsal quadrants can be seen in this image. (H) die-1(w34) embryos also display four muscle quadrants but often exhibit crossover of tissue between rows (arrows). (I, J) 3D reconstructions of wild-type (I) and embryos derived from a die-1(w34) germline mosaic mother (J) stained with MH27. The exterior focal planes have been removed and the focal planes containing the gut have been reconstructed. Arrows in J indicate sites at which the posterior intestine has detached from the anterior intestine (upper arrow) and the anal region (lower arrow). Scale bar = 10 μm.
tently enclosed with hypodermis, indicating that intercallation is not a prerequisite for ventral enclosure.

die-1(w34) embryos also display nonhypodermal defects in tissues that are known to undergo rearrangement along the anterior–posterior axis. In wild-type animals, the pharynx develops as a cluster of cells in the anterior half of the animal. The pharynx eventually elongates, connecting to the surface of the embryo via the buccal cavity (Fig. 4E). Nine of 33 die-1(w34) embryos analyzed using jam-1:GFP were determined to have defects in pharynx attachment (Fig. 4F). To examine body wall muscle, immunostaining experiments were performed with NE8/4C6.3, a body muscle-specific antibody (Goh and Bogaert, 1991). Body muscle cells appeared to be present in normal quantities and were generally organized into four quadrants, as is the case in wild-type. In wild-type embryos, muscle cells organize into tight rows within each quadrant and muscle tissue is never observed to cross between quadrants (Fig. 4G). However, in die-1(w34) mutants, crossing between quadrants was observed (Fig. 4H). Crossing of muscle was primarily confined to the posterior half of the embryo. Finally, in wild-type embryos, the intestinal rudiment is attached to the posterior region of the pharynx at its anterior end and the anus at its posterior (Fig. 4I). In contrast, in die-1(w34) mutants we observed cases in which the intestine appeared severed from either the posterior pharynx or the anus, or both, resulting in a straight intestine, rather than the typical curved rudiment at the comma stage (n = 11 out of 22 embryos examined; similar results were obtained from the offspring of die-1(w34) germline mosaic mothers; Fig. 4J). These results suggest that die-1 is required for multiple cell rearrangement events within the embryo, in addition to those associated with dorsal intercalation.

**Cytoskeletal Arrays Form, but Do Not Organize Properly, within the Dorsal Hypodermis of die-1(w34) Embryos**

Actin and microtubule inhibitors (Pries and Hirsh, 1986; Williams-Masson et al., 1998) and loss-of-function mutations in the actin-binding protein α-catenin (Costa et al., 1998; Raich et al., 1999) are known to disrupt elongation in *C. elegans* embryos. To address whether actin organization is disrupted in die-1(w34) mutants, we stained embryos from die-1(w34) heterozygous hermaphrodites with phalloidin. Actin forms into parallel, circumferentially oriented arrays within the dorsal hypodermis of wild-type animals (Fig. 5A). As elongation proceeds these arrays become uniformly spaced, thicker, and more distinct (Fig. 5B). Constriction of these filaments is thought to result in the subsequent elongation of the embryo (Pries and Hirsh, 1986). In die-1(w34) embryos the dorsal midline region of the dorsal hypodermal syncytium is often devoid of organized actin. In contrast, small regions of organized actin can be seen in lateral regions of the dorsal hypodermis (Fig. 6C). Actin organization often appears relatively normal in the anterior dorsal hypodermis of die-1(w34) embryos (Fig. 5C).

This region corresponds to the region in which intercalation is unaffected in die-1(w34) mutant embryos. Terminal die-1(w34) mutant embryos display thickened bundles of actin filaments, similar to those in elongating wild-type embryos, but these filaments are discontinuous and nonuniform in appearance (Fig. 5D). Although the defects are less pronounced, we observed similar midline abnormalities in the organization of microtubules in die-1(w34) embryos (Figs. 5E and 5F). These data suggest that die-1, or the process of intercalation, may be required for cytoskeletal elements to form a continuous, parallel array spanning the dorsal syncytium.

**die-1 Encodes a C2H2 Zinc Finger Protein**

We mapped die-1 and determined its molecular identity using standard methods (Figs. 6A and 6B; see Materials and Methods). Comparison of genomic and cDNA sequences revealed that the die-1 locus consists of six exons (Fig. 6C). The largest cDNA appears to be full length, based on identification of a poly(A) tract at the end of the 3’ UTR, the presence of a GeneFinder-predicted start codon, and the presence of stop codons in all three frames of the sequence upstream of the predicted start codon. In addition, the only transcript detected on a Northern blot is 2.9 kb, the size predicted if the cDNA is assumed to be full length (Fig. 6D). Unlike many transcripts in *C. elegans* (Blumenthal, 1995), die-1 transcripts do not appear to be trans-spliced.

**die-1 encodes a predicted 645 amino acid protein containing four C2H2 zinc fingers (Fig. 7A).** Two hallmarks of C2H2 transcriptional regulators can also be identified (Ptashne, 1988): serine/threonine-rich regions in the amino terminal half of the protein, which are approximately 50% serine or threonine (underlined in Fig. 7A), and two short glutamine-rich regions (amino acids 58–90 and 229–251) upstream of the zinc fingers, in which 7 out of 23 residues are glutamine. All four fingers contain the conserved cysteine and histidine residues associated with C2H2 zinc finger proteins. Three out of four fingers have the conserved leucine residue at position 16, whereas only one of the four fingers has the typically conserved phenylalanine residue at position 10 (Fig. 7B). Typically, C2H2 zinc finger proteins have a well-conserved 7 amino acid linker region between the individual fingers (Klug and Rhodes, 1987; Schuh et al., 1986). The DIE-1 protein has much longer linker regions. Other examples of long linker regions are known, although their significance is uncertain (Wilson et al., 1994; and unpublished sequences submitted to GenBank). The zinc finger domain is located in the carboxy-terminal half of the protein and there is no significant homology to other proteins outside the zinc finger domain.

We amplified and sequenced the die-1 region from die-1(w34) homozygous embryos. The die-1(w34) sequence contains a C to T transition in exon 2 that results in a premature stop codon following amino acid 89 (Fig. 6C). The premature stop codon in die-1(w34) truncates the protein prior to the zinc finger domain of DIE-1 and is therefore predicted to destroy its ability to bind DNA. Two
other lines of evidence suggest that die-1(w34) is a null allele: (1) the similarity in phenotype of die-1(w34)/mnDf71 embryos to that of die-1(w34) homozygotes (data not shown) and (2) the phenotypic similarity between die-1(w34) homozygotes and die-1(RNAi) embryos (data not shown).

DIE-1 Is Expressed in Dorsal Hypodermal Cells prior to and during Intercalation and Also in Other Cells That Display Morphogenetic Defects in die-1(w34) Mutants

We employed two methods to determine when and where DIE-1 protein is expressed during embryonic development. First, we generated anti-DIE-1 polyclonal antibodies. Second, we produced a translational fusion in which gfp was inserted in-frame between exons one and two in the smallest rescuing die-1 clone. die-1::gfp rescues die-1(w34) embryos to viability, indicating that the fusion protein is functional and expressed in all required cells. Furthermore, DIE-1::GFP faithfully reproduces the wild-type die-1 expression pattern as determined by immunostaining.

Consistent with its role as a putative transcriptional regulator, DIE-1 is expressed in the nuclei of cells in tissues that display phenotypes in die-1(w34) mutants. Maternally expressed DIE-1 can be detected in all blastomeres of the early embryo (data not shown). We infer that such expression represents translation of maternal mRNA based on two pieces of evidence. First, extrachromosomal arrays are not typically well expressed in the C. elegans germline (Kelly and Fire, 1998); that die-1::gfp is not expressed until the ~50-cell stage suggests that zygotic expression begins at this time. Second, immunostaining of cross-progeny from matings of wild-type males with die-1(w34) germline mosaic hermaphrodites indicates that the first detectable expression of zygotic DIE-1 occurs at the ~50-cell stage. Moreover, viable embryos result in either case, which indicates that the early expression of DIE-1 is not required for rescue of all die-1(w34) phenotypes.

FIG. 5. Actin and microtubule organization in the hypodermis of die-1(w34) embryos. Insets are ×3 enlargements of the boxed area in each panel. Intense phalloidin staining running horizontally across the embryo indicates the underlying dorsal muscle in A–D (asterisks). (A) A wild-type embryo shortly after dorsal cells have fused (embryo approximately twofold its original length); dorsal view. Fine actin filaments can be seen within the hypodermis in a mediolaterally oriented pattern (arrowheads). Arrows indicate the boundary between dorsal and lateral hypodermis that runs horizontally across the image. (B) Dorsal view of a die-1(w34) embryo shortly after fusion has occurred. This embryo is equivalent in age to the embryo in A. Small regions of organized actin can be discerned (arrows); however, the central region of the dorsal hypodermis does not display organized actin filaments. (C) Dorsal view of a completely elongated wild-type embryo. Actin filaments are thinner and organized into distinct fibrils within the dorsal hypodermis and display uniform spacing. Arrows indicate the boundary between dorsal and lateral cells. (D) Terminal die-1(w34) embryos display regions of actin filament organization within the hypodermis; however, filaments often appear discontinuous (arrowheads). (E) Microtubule organization in a wild-type embryo. Partially parallel arrays of microtubules are visible. (F) Microtubule organization in a die-1(w34) mutant. Similar arrays are visible. Scale bar = 10 μm.
expression is prominent in the posterior half of the embryo, in the nuclei of muscle and hypodermal precursors (Fig. 8A). When jam-1::gfp expression first becomes visible, shortly after the hypodermal cells undergo their terminal divisions, DIE-1 can be detected very strongly in the dorsal hypodermis (Fig. 8B). As intercalation proceeds, DIE-1 expression becomes weaker in these cells. Interestingly, the “pointer” cells, which are the last dorsal hypodermal cells to intercalate (Williams-Masson et al., 1998), maintain elevated levels of DIE-1 longer than other dorsal hypodermal cells. DIE-1 is undetectable in the nuclei of C-derived dorsal cells by the time intercalation is complete (Fig. 8C).

In addition to expression in dorsal hypodermal cells, DIE-1 is present in ventral hypodermal cells during the process of enclosure (Fig. 8D); expression ceases in the hypodermis after enclosure has completed, prior to the process of elongation (see Fig. 8G). The absence of expression during elongation suggests that DIE-1 may not play a direct role in elongation and that the elongation defect is a secondary result of other morphogenetic abnormalities. DIE-1 is also present in pharyngeal cells and in the gut primordium (Figs. 8E and 8F). DIE-1 is also expressed in muscle cells, as confirmed by double-staining die-1::gfp-expressing embryos with anti-GFP and muscle-specific antibodies. Nuclei containing GFP indicative of DIE-1 expression were consistently observed within the rows of muscle (Fig. 8G). After enclosure is complete, DIE-1 expression completely disappears in all tissues until late in embryogenesis (Fig. 8H), at which time ~15 cells between the anterior and posterior bulbs of the pharynx express die-1::gfp (data not shown). DIE-1 is also present in numerous cells along the ventral midline in L2–L4 larvae (data not shown).

Mosaic Analysis Suggests That DIE-1 Acts within the C Lineage to Promote Intercalation of Posterior Dorsal Hypodermis

To determine whether DIE-1 acts in the C-derived dorsal hypodermis in a cell-autonomous or cell-nonautonomous fashion, we employed two methods. First, we asked whether intercalation movements might be dependent on the presence of underlying C-derived muscle cells in the posterior by ablating Cap and Cpp in wild-type animals. These ablations eliminate 32 of the 81 body muscle cells that are present in the two quadrants that underlie the dorsal hypodermis. Three embryos were ablated and intercalation was observed to pro-

FIG. 6. Mapping and cloning of die-1. (A) Genetic map of the region surrounding die-1. Three factor-mapping and complementation tests against deletion mutants place die-1 between egl-43 and daf-19. (B) Cosmids spanning the die-1 region were injected, and a 10-kb rescuing fragment was isolated (pPH31). (C) Top: intron/exon structure of the only predicted ORF in the smallest rescuing fragment. The arrow indicates the location of a premature stop codon. Bottom: intron/exon structure of the die-1::gfp fusion construct generated by insertion of gfp coding sequence (represented by gray box) into the first intron of the predicted gene. (D) Northern blot probed with die-1-specific probe. A single band was detected at approximately 2.9 kb (arrow).
ceed normally in each case (Figs. 9A–9C). These results suggest that intercalation occurs independently of the underlying muscle tissue.

Second, we used a die-1(w34) homozygous strain containing the rescuing die-1::gfp and jam-1::gfp in an extrachromosomal array (jcEx23) for mosaic analysis. We identified cells that lacked the array by scoring for the loss of jam-1::gfp expression; we monitored the shape and position of epithelial cells in such mosaic animals via anti-JAM-1 immunostaining using the MH27 antibody. Given that the dorsal hypodermal defects in die-1(w34) mutant embryos are confined to the posterior, we focused on identifying rare losses in the C lineage. Because our ablation results indicated that C-derived dorsal muscle is dispensable for dorsal intercalation, losses in C-derived hypodermis should provide information about DIE-1 requirements in posterior dorsal hypodermal cells. We were able to identify two embryos in which expression of the array was lost from the C lineage, and one in which the array was lost from its precursor P2. In all of these embryos, intercalation defects were apparent in C-derived posterior dorsal cells, with the exception of the anteriormost pair of C-derived hypodermal cells (Fig. 9D). The phenotype of these embryos is consistent with a requirement for DIE-1 function within individual intercalating cells in the posterior.

In contrast, DIE-1 does not appear to be required in ventral hypodermal cells. We identified 11 mosaic embryos with losses in lineages derived from ABp, which generates ventral hypodermal cells, among other cell types. All of these embryos appeared to elongate normally (Fig. 9E), suggesting that DIE-1 function within the ABp lineage is not critical for morphogenesis of the embryo. As an additional by-product of this analysis, other mosaic losses in epithelial cells were identified. In two embryos complex mosaic losses were sustained in cells that generate the gut (one in EMS, another in E), as well as cells in AB lineages; both exhibited misshappen guts with an abnormally wide lumen and abnormal organization (data not shown).

**DISCUSSION**

Dorsal Hypodermal Cells Display Monopolar, Basolateral Protrusive Activity during Intercalation

Dorsal intercalation in *C. elegans* is a simple example of directed epithelial cell rearrangement. Because it involves the rearrangement of a small number of cells in a predictable, alternating pattern that is invariant from embryo to embryo (Williams-Masson et al., 1998), dorsal intercalation should be particularly amenable to cellular and genetic analysis. Although dorsal intercalation is reminiscent of convergent extension of dorsal deep cells in vertebrates (reviewed in Keller et al., 2000), it involves the rearrangement of epithelial cells, and hence the cellular and molecular mechanisms that regulate it may differ significantly...
from those operating in deep cells. Our previous work (Williams-Masson et al., 1998) showed that intercalating cells extend basolateral protrusions in the direction of rearrangement. Our dynamic analysis of dorsal intercalation using gfp translational fusions and multiphoton microscopy here confirms and extends this finding. As intercalation proceeds, basolateral protrusions become highly elongated medially; in contrast, we observed little protrusive activity at the lateral edges of intercalating cells.

Such highly asymmetric protrusive activity during intercalation is reminiscent of the “monopolar” protrusive activity exhibited by rearranging deep neural cells in the lateral neural plate of Xenopus (Elul and Keller, 2000). The signals that regulate such monopolar behavior are unclear. Monopolar protrusive activity in Xenopus deep neural cells requires persistent vertical interactions with the underlying mesoderm. In addition, monopolar protrusions are unable to cross the boundary between lateral and medial (“notoplete”) neural cells (reviewed in Keller et al., 2000). Whether similar stimulatory or repulsive cues exist in C. elegans is unclear. It is possible that local cell–cell signals mediate the initial polarization of dorsal hypodermal cells. If such signals operate, they must do so immediately after the terminal divisions of dorsal hypodermal precursors, given that monopolar behavior ensues within minutes after dorsal hypodermal cells are born. In contrast to Xenopus deep neural cells, our laser ablation studies and genetic mosaic analysis suggest that, once polarized, dorsal hypodermal cells perform many of the subsequent events of intercalation in a cell-autonomous manner (see below).

FIG. 8. DIE-1 expression. Confocal images were obtained of fixed wild-type embryos stained with anti-DIE-1 polyclonal antibodies (E, F) or of fixed homozygous die-1(w34) embryos expressing DIE-1::GFP; JAM-1::GFP from an extrachromosomal array, which were subsequently immunostained with anti-GFP antibodies (A–D, G, H; the signal represented by each channel is shown for each panel). (A) Middle focal plane of an embryo immediately prior to intercalation. Strong staining is observed in presumptive hypodermal precursors and muscle precursors. (B) An embryo during the early phase of dorsal intercalation. Note the elevated levels of DIE-1 in dorsal hypodermal nuclei (arrows). (C) An embryo near the completion of dorsal intercalation. DIE-1 expression has declined in posterior dorsal hypodermal cells (arrowheads), but is now higher in the “pointer cells,” which are the last dorsal cells to intercalate (arrows). (D) Expression of DIE-1 (green) and JAM-1 (MH27 immunostaining, red) in lateral (arrowhead) and ventral (arrow) hypodermal cells (lateral view). (E) Expression of DIE-1 (green) in the pharyngeal (ph) and intestinal (int) primordia. DIE-1 is present in the nuclei of all gut cells; the lumen is identifiable by MH27 immunostaining (red, arrow). (F) Pharyngeal expression of DIE-1. An enclosed embryo immunostained for DIE-1 (green) and JAM-1 (red). Middle focal plane, lateral view. Arrow indicates JAM-1 expression associated with the epithelium lining the pharyngeal lumen. (G) An unenclosed embryo expressing DIE-1::GFP; JAM-1::GFP double stained with anti-GFP antibody (green) and muscle-specific antibody (red). DIE-1::GFP is detected in nuclei of muscle cells (arrows). (H) An enclosed embryo expressing DIE-1::GFP; JAM-1::GFP double stained with anti-GFP antibody (red) and a muscle-specific antibody (green). Epithelial cell junctions are observed in both channels (detecting JAM-1::GFP), but no nuclear GFP expression is observed, indicating DIE-1::GFP expression has ceased. Scale bar = 10 μm.

Defects in die-1(w34) Embryos Show That the Steps of Intercalation Are Genetically Separable into Early and Late Phases

die-1 is the first cloned gene that has been shown to be directly required for cell rearrangements within the dorsal hypodermis, and provides several insights into how dorsal intercalation is regulated, both temporally and spatially. Based on our multiphoton microscopic analysis, C-derived dorsal hypodermal cells appear normal in die-1(w34) mutants in several respects. First, they are correctly polarized along the right-left axis because they can extend polarized protrusions that initially appear indistinguishable from those of wild-type siblings. Second, because basolateral protrusions are initially extended in a normal fashion by C-derived mutant cells, the cytoskeletal machinery required for this process must function normally, at least initially. Third, the apical junctional domains of C-derived mutant cells become wedge-shaped, indicating that in-
tially the cells are capable of remodeling their apical junctions. However, the subsequent translocation of the cell body, which is crucial for successful completion of cell rearrangement, is defective in the mutants. How cell translocation occurs during dorsal intercalation is currently unknown. It is known that dorsal intercalation depends on both actin filaments and microtubules (Williams-Masson et al., 1998); DIE-1 could regulate proteins required for cytoskeletal reorganization. It is also possible that cell-substrate or cell-cell interactions are affected in the mutant. It is known that the classical cadherin–catenin complex is not required for dorsal intercalation to occur (Costa et al., 1998; Raich et al., 1999), but little else is known about the changes that occur on the surface of migrating dorsal cells as they rearrange. Whatever molecules are regulated by DIE-1, our results demonstrate that

![Figure 9](image1.png)

**FIG. 9.** Tissue-specific requirements for DIE-1. (A–C) Time course of development of a single wild-type embryo in which the muscle precursor cells Cap and Cpp were ablated (dorsal view). Despite the absence of about one-third of the body muscle (primarily posterior and dorsally positioned cells), intercalation occurs normally. Intercalating dorsal cells in A and B are indicated by arrows. (A) Start of intercalation. (B) Intercalation complete. (C) Interior focal plane; arrowheads indicate undifferentiated ablated material. Times indicate amount of time elapsed from the beginning of intercalation. (D) DIE-1 is required within progeny of C for their intercalation. Embryos from a homozygous die-1(w34) strain rescued by an extrachromosomal array expressing DIE-1::GFP; JAM-1::GFP were analyzed. Embryos were stained with anti-GFP antibody (green) and MH27 (red). Mosaic embryos were identified as embryos that showed normal MH27 staining in the red channel, but expressed JAM-1::GFP only in a subset of the epithelial cells in the green channel. No JAM-1::GFP expression is observed in the posterior dorsal hypodermis, which is derived from the C lineage and includes several unintercalated cells (arrows). AB-derived cells have intercalated normally. (E) Mosaic loss in ABp. This embryo elongated normally. Arrows indicate ventral hypodermal cells that are not expressing the array, based on the absence of signal in the green channel. Arrowhead indicates a lateral hypodermal cell that does not contain the array. Thorough analysis of the embryo showed that none of the ABp-derived hypodermal cells that could be analyzed contained the array, whereas all ABA-derived and non-AB cells that could be analyzed expressed the array. Scale bar = 10 μm.

![Figure 10](image2.png)

**FIG. 10.** A model for DIE-1 function during intercalation and elongation. Hypodermal cells produce arrays of cytoskeletal elements (red: actin; blue: microtubules) that become progressively aligned circumferentially as intercalation is completed and elongation begins. During elongation, loss of DIE-1 function results in incomplete intercalation, leading to failure of cytoskeletal elements to span the entire dorsal hypodermal array. After fusion, the cytoskeletal network is discontinuous at the dorsal midline, leading to elongation failure.
the process of epithelial cell rearrangement can be subdivided into several distinct steps in C. elegans. Moreover, since mutations in die-1 affect only the later stages of intercalation, it should be possible to genetically dissect these temporally distinct phases of intercalation in C. elegans.

**die-1 Encodes a Cell-Autonomous Regulator of Intercalation in Posterior Dorsal Hypodermal Cells**

die-1 encodes a C2H2 zinc finger nuclear protein containing four zinc fingers. Elucidation of transcriptional targets of DIE-1 awaits further experiments. However, based on the lack of overt patterning defects in die-1(w34) homozygotes, we favor the hypothesis that DIE-1 regulates structural genes whose products are required for intercalation. In these embryos, with the exception of the anteriormost pair of C-derived dorsal cells, cells lacking DIE-1 fail to intercalate. Previous laser ablation experiments also support the view that dorsal cells, once polarized, intercalate in a predominantly autonomous fashion. Dorsal cells can intercalate in the absence of underlying muscle cells (our results), and our previous studies have shown that intercalation can occur in the absence of half of the lateral hypodermal cells or among anterior dorsal cells in the absence of posterior dorsal cells (Williams-Masson et al., 1998). We infer from these experiments that the observed DIE-1 expression within the posterior muscle and lateral hypodermis is not required for dorsal intercalation because the cells themselves are dispensable.

Because DIE-1 is present in both posterior and anterior dorsal hypodermal cells, an obvious question arises: why are anterior dorsal hypodermal cells unaffected in die-1(w34) mutants? One possibility is that a lineage-restricted difference in regulatory pathways exists between AB- and C-derived hypodermal cells. If a second regulatory process operates in parallel with DIE-1 within the anterior, AB-derived dorsal cells, then intercalation could still occur in these cells despite the absence of DIE-1. That the boundary between dorsal hypodermal cells that can successfully intercalate and those that cannot corresponds to the boundary between AB- and C-derived dorsal hypodermis makes such lineage-specific regulatory differences a distinct possibility.

**DIE-1 Is Required in Other Tissues That Undergo Directed Cell Rearrangement and Extension**

In addition to defects in dorsal intercalation, die-1(w34) mutant embryos also display abnormalities in the processes of ventral enclosure, pharynx attachment, muscle organization, and gut formation. The presence of multiple morphogenetic defects in die-1(w34) mutants and the appearance of DIE-1 in each of the affected tissues suggest that DIE-1 may act to regulate multiple morphogenetic processes within the embryo. Our mosaic analysis is consistent with this view in the case of dorsal hypodermis (see above). However, it is also possible that some of the additional defects are secondary defects that arise because the process of intercalation has been perturbed. Our mosaic analysis identified one case in which such indirect defects may occur. We identified mosaic embryos that elongated completely normally yet displayed losses in the ABp lineage, which generates ventral hypodermal cells. This suggests that the ventral hypodermal defects associated with die-1(w34) mutants do not result from an autonomous requirement for DIE-1 within the ventral cells but, rather, are indirect.

In contrast to the ventral hypodermis, DIE-1 appears to be autonomously required in the gut. It is interesting to note that the cells within the C. elegans gut primordium undergo a type of intercalation (Leung et al., 1999), although the process is somewhat different from that in the dorsal hypodermis. Failure of gut progenitors to intercalate properly could lead to a subsequent morphogenetic defect, including the detachment phenotype we observed in die-1(w34) mutants and offspring of die-1(w34) germ line mothers. Although we did not recover mosaic animals in which a clean loss of DIE-1 function occurred within E, the gut progenitor cell, we did recover mosaics in which complex losses occurred that included E. In each case gut abnormalities were observed, consistent with an autonomous role for DIE-1 in the gut.

It is intriguing that the other tissues in which defects are observed in die-1(w34) mutants (i.e., pharynx and body wall muscle) also undergo coordinated changes in cell shape and position along a preferred axis. Muscle cells organize into quadrants and elongate along the anterior-posterior axis (reviewed in Moerman, 1997). Similarly, pharyngeal precursors, which arise as a ball of cells attached to the forming midgut, elongate anteriorly to connect to the buccal cavity (Portereiko and Mango, 2001). Although motile events in these tissues have not been examined carefully, they may involve processes that resemble intercalation. If this is the case, then DIE-1 may be a regulator of several similar morphogenetic processes in C. elegans.

**Elongation Defects in die-1 Embryos May Result from Defects in Actin Filament Formation**

Our analysis of the morphogenetic defects in die-1(w34) mutants sheds light on the consequences of intercalation for subsequent morphogenetic events. We have demonstrated defects in the actin cytoskeleton in die-1(w34) mutants. The defects in the cytoskeleton we observed could arise in several ways. One possibility is that DIE-1 indirectly regulates the reorganization of actin and microtubules in rearranging cells. A second possibility is that cytoskeletal disorganization in die-1(w34) mutants is a secondary result of the failure of dorsal hypodermal cells to intercalate. Although our results are consistent with either...
possibility, we favor the latter. Elongation of C. elegans embryos is dependent on actin filaments that organize into a circumferential, uniformly spaced array within the hypodermis; distribution of actin-based contractile forces is thought to be mediated in part by microtubules (Priess and Hirsh, 1986; reviewed in Simske and Hardin, 2001). In die-1(w34) mutant embryos we observed organized actin and microtubules around the periphery of the dorsal syncytium, whereas the central region of the dorsal syncytium did not display organized, circumferentially organized filaments. That the cytoskeleton can organize to some extent in mutant embryos suggests that DIE-1 may not directly regulate filament formation per se; instead, the failure of cells to intercalate may prevent the cytoskeleton from aligning across the entire dorsal hypodermis. In this model, once intercalation is complete in wild-type embryos, cells span the width of the dorsal hypodermis, allowing the forming actin filaments and microtubules to stretch across the entire dorsal surface. In die-1(w34) mutants, individual polymers may be able to span the length of a cell, but if the cell fails to intercalate the individual filaments may not be able to span the entire dorsal hypodermis. In this case, the central area where the tips of cells were located might be expected to lack organized actin and microtubules (Fig. 10). Once the dorsal cells fuse, the actin and microtubule arrays would be interrupted by gaps at the dorsal midline; the lack of organized actin in turn could lead to defects during elongation.

The model we propose provides a role for intercalation in morphogenesis in C. elegans and suggests that intercalation events may be generally required for the production of columns of cells with aligned arrays of cytoskeletal elements that bear mechanical loads anisotropically. In this sense, the dorsal hypodermis is similar to the dorsal involuting marginal zone of Xenopus. Marginal zone cells that have undergone convergent extension are measurably stiffer along the anterior–posterior axis than those that have not (Moore et al., 1995). Moore et al. (1995) proposed that tension-resisting elements (i.e., actin filaments) reinforce converging and extending cells medially (i.e., perpendicular to the axis of extension) as they rearrange, and that microtubules may bear compressive loads as the cells extend. We have provided direct evidence for defects in similar tension-resisting and compression-bearing elements within the hypodermis of C. elegans when directed cell rearrangement is disrupted. Thus, such mechanical changes in rearranging cells may be a feature common to both deep (nonepithelial) and epithelial cells. As this and other morphogenetic processes regulated by DIE-1 are unraveled, significant insights will be gained into the process of epithelial cell rearrangement. This in turn should deepen our understanding of this ubiquitous morphogenetic process.

ACKNOWLEDGMENTS

This work was supported by NSF Grant DBI97-24515 and NIH Grant GM58038 awarded to J.H., and NIH Grant GM48137 awarded to J.H.R. W.A.M. was supported by a NIH postdoctoral fellowship and a Development Grant from the Muscular Dystrophy Association. W.B.R. and P.J.H. were supported by a UW Molecular Biosciences Predoctoral Training grant (NIH T32-GM0721). We thank S. Segal for assistance with initial mapping experiments, K. Kopish for help with ojEx3, and J. Squirrel and J. G. White for access to the multiphoton optical workstation at the University of Wisconsin. We also thank members of the Hardin laboratory, especially T. Lindblom and J. Simske, for helpful discussion and critical reading of the manuscript.

REFERENCES


