Effects of Chromosomal Deficiencies on Early Cleavage Patterning and Terminal Phenotype in Caenorhabditis elegans Embryos

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ABSTRACT

We have analyzed pregastrulation cleavage patterns in Caenorhabditis elegans embryos homozygous for various chromosomal deficiencies. By two different estimates these deficiencies represent between 37 and 49% of the genome, including the entire X chromosome and substantial portions of each of the five autosomes. Among these genomic regions, we find none whose absence causes defects in pregastrulation cleavage patterns. We can conclude that there are at most very few genes whose transcription after fertilization is required for normal early patterning of cell divisions. We also scored terminal phenotypes of the homozygous deficiency embryos for stage of arrest and for expression of three tissue-specific differentiation markers. Based on these phenotypes, we have identified regions of the genome that are required for completion of cell proliferation, expression of gut differentiation and entry into morphogenesis. Somewhat surprisingly, embryos in which cell proliferation is arrested at less than 20% of the normal cell number can nevertheless initiate morphogenesis and undergo elongation to the twofold stage. Our results are consistent with the view that many early events in C. elegans embryogenesis are controlled exclusively by maternally produced gene products. However, they are also consistent with the likely possibility that, at least in some deficiency embryos, although cleavage patterns may be normal, blastomere identities are not. In this respect the early cleavages may differ from later lineages, in which cell division patterns appear to be characteristic of cell identity.

Previously genetic studies of embryogenesis in Caenorhabditis elegans have favored the view that early embryonic patterning is largely if not exclusively under maternal control. Early genetic screens for temperature-sensitive (ts) mutants identified about 55 genes defined by maternal-effect lethal mutations that result in embryonic arrest (Hirsh and Vanderslice 1976; Vanderslice and Hirsh 1976; Miwa et al. 1980; Schierenberg et al. 1980; Wood et al. 1980; Cassada et al. 1981; Isenghi et al. 1983; Denich et al. 1984). More recent screens for ts and nonconditional maternal-effect lethals have identified several additional genes (Kemphues et al. 1988a,b; Mains et al. 1989; Bowerman et al. 1992; Mello et al. 1992), and it is clear that such screens are still far from saturation. Many of these mutations cause altered early cleavage patterns in addition to altered cell fates. In contrast, early screens for nonmaternal-effect lethals identified primarily mutations that result in larval arrest (Herman 1978; Meneely and Herman 1979, 1981; Rogalski et al. 1982; Sigurdson et al. 1984; Rosenbluth et al. 1988); only 9 of the 160 genes identified in these studies were represented by alleles that cause embryonic lethality. These results suggested that little embryonic gene expression may be required for early patterning in C. elegans.

Earlier molecular analysis appeared to confirm this view, suggesting that substantial embryonic transcription began only after the onset of gastrulation (Hirsh et al. 1981). However, more recent work has shown that transcription is detectable by the 8-cell stage (Edgar et al. 1994) and that between 21 and 30 genes are probably transcriptionally active preferentially in the pregastrulation embryo (Schafer and Wood 1990). Regarding the genetic evidence, Kemphues et al. (1988a) demonstrated that the preponderance of maternal effects among embryonic lethals in the early ts screens can be explained by a high representation of hypomorphic alleles of genes whose null phenotype would be nonmaternal-effect lethality. In addition, more recent screens for nonmaternal-effect mutants have identified a substantial number of mutations that result in embryonic lethality (Bucher and Greenwald 1991; J. Rothman, personal communication; our unpublished results). These findings suggest that early expression of embryonically transcribed genes could be more important than previously suspected, as it is in embryos of Drosophila, where both maternally and embryonically expressed genes are clearly essential for pregastrulation patterning (for review see St. Johnston and Nüsslein-Volhard 1992).

To further explore this possibility, we have analyzed the embryonic phenotypic effects of removing substantial portions of the genome, using a collection of previously mapped deficiencies. We have characterized the
homzygous deficiency embryos produced by self-fertilization of hermaphrodites heterozygous for single deficiencies, as well as nullo-X embryos produced by nondisjunction during parental gametogenesis in *him* (high incidence of males) mutant hermaphrodites. (Throughout the remainder of this report, reference to the set of deficiencies we have analyzed includes the nullo-X condition.) In aggregate, based on both genetic and physical estimates described under "RESULTS" below, the regions removed by these deficiencies plus the X chromosome include between 37 and 49% of the *C. elegans* genome. Since the parental hermaphrodites (*Df/+* or *XX*) provide their embryos with a complement of maternal gene products that is sufficient to permit wild-type development, this strategy enabled us to look specifically at contributions of the embryonic genome to early development.

In similar studies with Drosophila, embryos deficient for entire chromosome arms displayed the early defects and the terminal phenotypes characteristic of the earliest acting genes in the deleted regions (MERRILL *et al.* 1988; WIESCHAUS and SWEETON 1988; VAARA and CARROLL 1989). Therefore, we anticipated that the earliest observed defect in a homozygous deficiency embryo should generally result from absence of the earliest required gene removed by the deficiency, rather than from loss of several genes. The Drosophila studies were successful in identifying two previously unknown, embryonically active loci necessary for cellularization, an early event in Drosophila embryogenesis.

Our primary interest was in determining the effects of deficiencies on early events in embryogenesis. To this end, we analyzed homozygous deficiency embryos for the patterning of pregastrulation cleavages and gastrulation onset. In addition, to identify regions of the genome required for later specific aspects of embryogenesis, we recorded attributes of the terminal arrest phenotype for each deficiency, including number of nuclei, extent of morphogenesis, and expression of a small set of tissue-specific differentiation markers.

### MATERIALS AND METHODS

**C. elegans strains:** All strains derived from the wild-type *C. elegans* var. Bristol strain (N2) originally described by BRENNER (1974). Nomenclature is according to HORTVITZ *et al.* (1979).

The homozygous deficiency strains used, listed by linkage group (LG) of deficiencies, are given in Table 1.

#### General methods:

Maintenance and breeding of *C. elegans* were carried out according to BRENNER (1974), HERMAN (1988) and SUNSTON and HODGGIN (1988). Stocks were maintained at 16°C and crosses were carried out at 20°C unless otherwise indicated.

**Maintenance of deficiency chromosomes:** All deficiency strains used in this study are homozygous inviable. Balancer chromosomes that do not affect the viability of nonrecombinant animals (mncI II (HERMAN 1978) and qCI III (AUSTIN and KIMBLE 1989)) were used for deficiencies on LGII and LGIII, respectively. Reciprocal translocations were not used as balancers in this study because hermaphrodites heterozygous for these translocations segregate a high proportion of inviable aneuploid embryos. Deficiencies on the other three autosomes were each maintained in trans to a chromosome bearing two visible mutations that closely flank the deficiency. A deficiency on the X chromosome was maintained in the presence of a complementing free duplication.

**Percent inviabilities of deficiency strains:** Individual hermaphrodites heterozygous for a deficiency were picked to separate plates, allowed to lay eggs for approximately 24 hr and

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**Table 1:** Heterozygous deficiency strains used in this study

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGI</td>
<td>MT2181</td>
</tr>
<tr>
<td>LGII</td>
<td>BW1345</td>
</tr>
<tr>
<td>LGIII</td>
<td>CB681</td>
</tr>
<tr>
<td>LGIV</td>
<td>BW1062</td>
</tr>
<tr>
<td>LGV</td>
<td>BW1298</td>
</tr>
<tr>
<td>LGX</td>
<td>SP1007</td>
</tr>
</tbody>
</table>

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*Strain BW707 was used to produce nullo-X embryos (see text).*
removed. The following day, unhatched embryos and total progeny (N > 1000) were counted. One-quarter of the progeny should be homozygous for the deficiency; \( \chi^2 \) tests were used to determine whether the fraction of inviable animals differed significantly from 25%. If no unhatched embryos were observed, plates were scored for arrested larvae. All heterozygous hermaphrodite strains used in these experiments produced 25% of either unhatched embryos or arrested larvae within 95% confidence limits.

**Nullo-X embryos:** Nullo-X embryos were obtained from *him-8(e536); X* hermaphrodites mated to N2 males. The *him-8* mutation results specifically in LGX non-disjunction (Hodgkin et al. 1979). The predicted frequency of nullo-X embryos among outcross progeny is 19%, based on the measured frequency of nullo-X ova (Hodgkin et al. 1979; this study) produced by these hermaphrodites.

**Estimates of deficiency sizes and numbers of genes:** Deficiency sizes were estimated as described under RESULTS. Recent genetic and physical map data were obtained online from ACeDB (A. C. elegans Database, compiled by R. Durbin and J. Tierny-Mee, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom).

**Nomarski microscopy of embryos:** Embryos were mounted in Nonidet P-40 (NIP) or agar pads (3% agar in S basal medium) essentially as described by Sulston and Hodgkin (1988). Gravid hermaphrodites were dissected in 1% Agar and 1- and 2-cell embryos were transferred to an agar pad by means of a drawn-out pipette. Embryos were gently moved together using an eyelash. The coverslip was sealed on three sides with molten Vaseline to prevent evaporation. Embryos were observed, photographed (Tmax 400 film at ASA 400), and videotaped (see below) at room temperature (about 22°C) using a Zeiss Universal microscope with Nomarski optics. When not being observed, slides were stored in a wet box at 20°C.

**Analysis of early embryonic lineages and terminal phenotypes:** Developing embryos, produced by self-fertilization of heterozygous deficiency hermaphrodites or by mating of N2 males to *him-8* hermaphrodites, were videotaped with an MTI tube camera and a Panasonic AG-6050 time-lapse video cassette recorder set for 1/6 normal speed, using an automatic electric drive on the microscope to focus continuously from the top of the embryo to the bottom and back again approximately every 20 sec. Development was recorded from first cleavage for about 2 hr until the second E cell division, which occurs after the E cell daughters Ea and Ep have moved internally to begin gastrulation. Slides were then stored overnight and again videotaped briefly on the following morning to record which embryos had hatched (about 75%), indicating that they carried at least one functional copy of each chromosome, and which had arrested with mutant terminal phenotypes; the latter were presumed to be deficiency-homozygous or nullo-X embryos. The percentage of unhatched embryos on slides was determined in all cases to be similar to the percentage of unhatched embryos on plates, indicating that little or no nonspecific embryonic arrest occurred as a result of the mounting and observation procedures.

To analyze early cleavage patterns, tapes were viewed on a Comrac monitor at normal speed. For each deficiency, at least two non-hatching embryos from each of two slides were analyzed for early cleavages to minimize chances of scoring artificially damaged normal embryos. Embryos were analyzed up to the 30-cell stage (gastrulation onset). Cell divisions during the first four rounds of cleavage were scored for time of occurrence, spindle position and orientation, and relative sizes of daughter cells. Cell divisions in the fifth cleavage were scored for timing only.

Following the final brief videotaping to determine viability, 6–12 of the arrested embryos for each deficiency were analyzed for a few characteristics of terminal phenotype. Stage of morphogenesis was estimated as degree of elongation, which normally proceeds during the second half of embryogenesis from 1 to approximately 3 egg lengths (3-fold stage) just prior to hatching. Twitching of the embryo was used as a crude indicator of functional muscle differentiation. Presence of rhodamine granules, visualized as characteristic refractile or autofluorescent bodies using polarization optics or ultraviolet epi-illumination, respectively, was taken as an indication of gut differentiation (Bass 1974). If granules were present, the arrangement of cells containing them provided an additional measure of morphogenesis by indicating whether they had formed a normal elongated primorium or remained in a spherical clump. A characteristic flattening or smoothing of the outer cells of the embryo was taken as a crude indication of hypodermal differentiation (Press and Hirsh 1986).

To determine total number of nuclei, arrested embryos after scoring as above were gently lifted off the agar pad with a polysine-coated coverslip and floated on a drop of 2.5% paraformaldehyde in 125 mM phosphate buffer, pH 7.4. After about 3 min, they were squashed to a monolayer of cells by pressure applied to the coverslip with a tungsten needle. The squashed embryos were washed with three changes of the same buffer containing 1 mg/ml of 4',6-diamidino-2-phenylindole (DAPI) and then stained in this solution for 10 min at room temperature, washed with the same buffer minus DAPI and photographed using epifluorescence optics. For the class 1 phenotype, nuclear counts were carried out for 4 embryos, and the remainder were estimated to include about 550 cells. For the class 2, 3 and 4 phenotypes, counts were carried out on 24 embryos each.

**Nomarski microscopy of L1 larvae:** Larvae were mounted in S basal medium on agar pads as described above. L1 larvae homozygous for a deficiency were scored for the following nuclei (Sulston and Horvitz 1977): P (12 nuclei), V (12), M (1), T (2), E (20), B (1), gonad (4 nuclei enclosed in a characteristic sheath), HSN (2), Q1 (1), H1 (2), H2 (2), U (1), Y (1), K (1), F (1) and coelomocytes (4). Nuclei were scored for correct number, position and morphology. Organization of dorsal and ventral nerve cords and muscle bands were also scored.

**RESULTS**

**Extents of deficiencies:** The locations and extents of the deficiencies used in this study are shown in the *C. elegans* genetic and physical maps of Figure 1. As first observed by Brenner (1974), gene distribution on the genetic map is distinctly nonuniform. Genes appear to cluster in one or two regions of each autosome, with less apparent clustering on the X chromosome. This clustering does not reflect physical distance relationships, as shown by steadily accumulating evidence that in general, the amount of DNA per map unit is 5–10-fold greater in cluster regions than in regions outside of the clusters (Greenwald et al. 1987; Starr et al. 1989; Sulston et al. 1992). The nearly complete physical map of *C. elegans* is based on linkage of overlapping contigs of cosmid and yeast artificial chromosome (YAC) clones (Coulson et al. 1986, 1988; Sulston et al. 1992) (current information from the *C. elegans* database ACeDB; see MATERIALS AND METHODS). It includes an increasing num-
A. Genetic Map

I

\[ \text{lin-10} \rightarrow \text{unc-29} \rightarrow \text{unc-54} \]

II

\[ \text{egl-26} \rightarrow \text{lin-10} \rightarrow \text{dpy-10} \rightarrow \text{egf-11} \rightarrow \text{rol-6} \rightarrow \text{unc-4} \rightarrow \text{bli-1} \rightarrow \text{sqt-1} \]

III

\[ \text{dpy-10} \rightarrow \text{dpy-9} \rightarrow \text{unc-45} \rightarrow \text{unc-36} \rightarrow \text{sub-7} \rightarrow \text{unc-64} \]

IV

\[ \text{unc-29} \rightarrow \text{unc-20} \rightarrow \text{dpy-7} \rightarrow \text{unc-42} \rightarrow \text{unc-51} \]

V

\[ \text{unc-1} \rightarrow \text{dpy-7} \rightarrow \text{stDf1} \rightarrow \text{null-X} \]

B. Physical Map

I

\[ \text{mnDf17(1)} \rightarrow \text{mnDf20(1)} \rightarrow \text{mnDf20(1)} \]

II

\[ \text{mnDf88(1)} \rightarrow \text{mnDf88(1)} \]

III

\[ \text{mnDf51(1)} \rightarrow \text{mnDf51(1)} \]

IV

\[ \text{mnDf88(1)} \rightarrow \text{mnDf88(1)} \]

V

\[ \text{mnDf17(1)} \rightarrow \text{mnDf20(1)} \rightarrow \text{mnDf20(1)} \]

X

\[ \text{null-X} \rightarrow \text{null-X} \]

FIGURE 1.—Genetic and physical maps of the C. elegans genome showing positions and extents of deficiencies. (A) Genetic map. Lengths of chromosome lines indicate relative genetic sizes of the six linkage groups. Open boxes indicate the positions of gene clusters on each chromosome. Only a few markers have been included for orientation. Extents of deficiencies are indicated below the corresponding chromosomes, except that for LGII, the deficiency line (\(\#\)) indicates the extent of the entire region removed by the several overlapping deficiencies tested. The blowup of the LGII cluster region shows extents of all these deficiencies except \(\text{mnDf98}\), which removes a small region between \(\text{lgy-11}\) and the right end of the large deletion \(\text{mnDf88}\). The number in parentheses following the name of each deficiency indicates its phenotypic class, as described in the text and Table 3. (B) Physical map. Because there remain gaps in the YAC and cosmid contigs on which the map is based, precise relative physical sizes of the chromosomes cannot yet be estimated, although cytologically all appear approximately the same in length (ALBERTSON and THOMSON 1982). Here all six are represented as equal, and positions are indicated as best estimates for percentages of total chromosome length, shown by the scale at the bottom of the figure. Open boxes show approximate physical extents and positions of the gene clusters from the genetic map in panel A, to give some impression of the distortion inherent in the genetic map. Physical regions known to be deleted by the deficiencies tested for each chromosome are shown by solid lines; the considerable uncertainty in the end points of several regions are shown by dashed lines. Asterisks indicate the locations of genes shown to be transcribed preferentially in the pregastrulation embryo by SCHAUER and WOOD (1990). For two of these genes, one on LGV and the other on LGX (*), the contigs that include them are not yet positioned on the physical map.
The number of genetically defined marker genes that allow at least rough comparison of the physical and genetic maps, as discussed further below.

Attempts were made to estimate both the fraction of total genes that the deficiencies in aggregate remove and the fraction of the physical genome that they represent. The first estimate involves gene counting, using the fraction of known marker genes uncovered as an approximation of the fraction of total genes removed. Combined, the deficiencies remove a total of 49% (156/318) of mapped marker genes. This total includes all genes on the X chromosome and 39% (103/265) of marker genes on the autosomes. The genes counted include only those positioned reliably on the genetic map (the same subset used originally in defining deficiency endpoints) using either three-factor cross data or physical data (see below). Genes designated let and mel were excluded because their distribution is expected to be nonrandom; most of them were identified in saturation screens for essential genes in specific regions of the genome (Rogalski et al. 1982; Sigurdson et al. 1984; Clark et al. 1988; Rosenbluth et al. 1988; Kempfues et al. 1988a). The marker genes used, by contrast, were identified primarily in genome-wide screens for various phenotypes and are assumed to be distributed more randomly. It is possible that a disproportionate fraction of the available marker genes have been identified in the cluster regions, on which more attention has been focussed. However, the assumption that marker genes are randomly distributed is supported by the observation that the six linkage groups, which appear to be roughly equal in genetic (Herman et al. 1976) as well as physical size (Albertson and Thomson 1982), include similar numbers of these genes.

The second estimate involves summing the physical extents of the regions that the deficiencies remove. On the genetic map (Figure 1A), these regions total 55 map units, only 18% of the estimated total map length of 310 map units. However, because most of the deficiencies used either occur in or span cluster regions on the autosomes, the genetic map grossly underrepresents their physical sizes. On the physical map (Figure 1B), the extents of the deficiencies can be only roughly estimated as shown, because most of the genetic markers used to define deficiency endpoints have not yet been physically located. According to these estimates, the deficiencies in total remove about 37% of the physical genome. This number is undoubtedly an underestimate (see Discussion), and, therefore, in reasonable agreement with the estimate of 49% based on the number of marker genes removed.

Pregastrulation cleavage patterns: In wild-type embryos, the pregastrulation cleavages proceed with a characteristic spatial and temporal pattern of spindle orientations, cell sizes and cell divisions, which are essentially invariant from one embryo to another (Deppe et al. 1978; Lauper et al. 1980; Sulston et al. 1983). Early cleavage patterns were analyzed in embryos homozygous for each of the deficiencies shown in Figure 1, using time-lapse video microscopy as described in Materials and Methods. These patterns were compared to those of normal embryos on the same slide. Minor pattern variations seen among embryos that subsequently hatched were disregarded. None of the deficiencies tested showed reproducible dominant effects on embryonic development, based on the finding that no Df/+ heterozygotes produced significantly more than 25% arrested embryos, either on plates or among embryos mounted for microscopy.

Results of these analyses are summarized in Table 2 for nullo-X and homozygous deficiency embryos of 13 different genotypes, together representing substantial portions of each linkage group and estimated to include about half of the known C. elegans genes. Strikingly, none of these embryos showed significant differences in the patterning of cleavage when compared to wild-type embryos.

Terminal arrest phenotypes: Terminally arrested embryos from each of the deficiency strains were analyzed as described in Materials and Methods for number of nuclei (normally 550 at hatching), degree of morphogenesis and expression of differentiated characteristics in three different tissues: gut, muscle and hypodermis. For reference, several stages of normal embryogenesis subsequent to gastrulation onset are shown in Figure 2.

Results of the terminal phenotype analysis are summarized in Table 2 for the 14 genotypes mentioned above plus four more, homozygous for smaller deficiencies on LGII, which were not analyzed for early cleavage pattern. Embryos homozygous for a single emb-29 mutation are also included for comparison. Defective terminal phenotypes resulting from these 19 genotypes could be grouped somewhat arbitrarily into five classes, whose properties are summarized below and in Table 3. Examples of the different phenotypes are shown in Figure 3.

Class 1 embryos, the largest group, were found to result from deficiencies on each of the six linkage groups (Figure 1A). These embryos generally arrested at the transition between cell proliferation and morphogenesis and exhibited normal differentiation. However, their terminal phenotypes were somewhat variable, both among embryos of different strains and sometimes among embryos of the same genotype (Figure 3, panels A–D). The embryos included approximately 550 nuclei, sometimes progressed through morphogenesis as far as the twofold stage, sometimes twitched, always displayed gut granules (sometimes in an elongated and sometimes in a spherical pattern), and always displayed a smooth surface indicative of hypodermal differentiation.
Class 2 embryos, resulting from one deficiency on LGIII and three overlapping deficiencies that define at least two loci on LGII, failed to complete normal cell proliferation (Figure 3, panels E and F). They arrested at 150–300 nuclei, depending on the deficiency, yet occasionally initiated morphogenesis (never progressing beyond the comma stage). In addition, all embryos displayed a spherical array of gut granules and a smooth surface, providing evidence for gut and hypodermal differentiation. Only a few twitched, indicating general lack of muscle function.

Class 3 is the designation given to the nullo-X embryos, which like those of class 1 exhibited arrest at the end of proliferation and before the onset of morphogenesis but showed a different pattern of differentiation marker expression (Figure 3, G and H). These embryos contained approximately 550 nuclei, did not twitch or display gut granules, but did display a smooth surface, suggesting hypodermal but no gut or muscle differentiation.

Class 4 is represented by only the single deficiency sD/28, which caused arrest earlier in proliferation than the class 2 deficiencies. Arrested embryos contained only 95–140 cells, yet differentiated to a surprising degree (Figure 3, panels I and J). These embryos, including those of <100 cells, underwent morphogenesis as far as the twofold stage; they also twitched, displayed gut granules in an elongated array and exhibited a smooth surface, indicating muscle, gut and hypodermal differentiation, respectively. The same terminal phenotype was observed in embryos homozygous for a single mutation (s1666) at the emb-29 locus, which is removed by the sD/28 deficiency.

Class 5 deficiency embryos, resulting from two non-overlapping deficiencies on LGII, hatched and arrested as first-stage larvae (L1s). These animals were analyzed for presence and arrangement of nuclei from several embryonic lineages. In L1s homozygous for one of the deficiencies, the anatomy appeared normal except for enlarged hypodermal nucleoli. In L1s homozygous for the other, no anatomical abnormalities were observed.

### TABLE 2

Summary of deficiency strain characteristics and defective phenotypes of homozygous embryos

<table>
<thead>
<tr>
<th>Linkage group</th>
<th>Deficiency</th>
<th>Marker genes removed</th>
<th>Heterozygous deficiency strain</th>
<th>Percent inviable embryos (χ²; P)</th>
<th>Pregastrulation cleavage defects</th>
<th>Terminal phenotype</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>nDf24</td>
<td></td>
<td>MT3181</td>
<td>26.0 (0.56; 0.45)</td>
<td>None</td>
<td>±M; G; sm</td>
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<td>II</td>
<td>nDf20</td>
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<td>MT3022</td>
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<td>None</td>
<td>±M; G; sm</td>
<td>1</td>
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<tr>
<td></td>
<td>ndf2</td>
<td></td>
<td>CB4118</td>
<td>27.0 (1.8; 0.18)</td>
<td>None</td>
<td>G; sm</td>
<td>1</td>
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<tr>
<td>IV</td>
<td>nDf27</td>
<td></td>
<td>BW1062</td>
<td>29.2 (25; 0.00)</td>
<td>None</td>
<td>M(2-f); T; G; sm</td>
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<tr>
<td>V</td>
<td>sDf28</td>
<td></td>
<td>BW1298</td>
<td>27.2 (7.2; 0.01)</td>
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<td>95–140 cells; M(2-f); T; G; sm</td>
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<tr>
<td></td>
<td>emb-29</td>
<td></td>
<td>BW1215</td>
<td>25.3 (0.28; 0.60)</td>
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<td>95–140 cells; M(2-f); T; G; sm</td>
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<td></td>
<td>sDf35</td>
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<td>BW1307</td>
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<tr>
<td>X</td>
<td>nullo-X</td>
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<td>BW707</td>
<td>18.4 (0.07; 0.79)</td>
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<td></td>
<td>sDf1</td>
<td></td>
<td>SP1007</td>
<td>80 (80)</td>
<td>None</td>
<td>±M; ±T; ±G; sm</td>
<td>1</td>
</tr>
</tbody>
</table>

*a* Based on the May 1992 genetic map, *Caenorhabditis* Genetics Center, Department of Genetics and Cell Biology, 250 Biological Sciences Center, University of Minnesota, St. Paul, Minnesota 55108-1095. Genes counted include only non-let and non-mel genes positioned on the genetic map from 3-factor mapping data or physical data (see text).

*b* See MATERIALS AND METHODS for genotypes.

*c* χ² and P values are calculated based on prediction of 25% homozygous deficiency embryos, unless otherwise indicated. If P ≥ 0.05, the observed percentage of inviable embryos is not significantly different from 25%.

*d* Abbreviations: M, morphogenesis; 24, twofold stage; T, twitching; G, gut granules; sm, hypodermal differentiation; ±, at least half the arrested embryos displayed the differentiation marker in question; nd, not determined.

*e* See text and Table 3.

*f* No inviable embryos were produced. Numbers given are for arrested L1 larvae.

*g* E cells are arranged in a spherical, rather than linear, array.

*h* Embryos were produced by BW707 hermaphrodites [*him-8(e1489); lon-2(e678)] that had been mated to N2 males. The expected frequency of nullo-X embryos is 19% (see MATERIALS AND METHODS).

*i* No basis for predicting frequency of homozygous deficiency embryos.
DISCUSSION

How many embryonically expressed genes are required for cleavage patterning? We have shown that embryos deficient for substantial regions of any of the five autosomes or for the entire X chromosome exhibit normal pregastrulation cleavage, indicating that embryonic transcription of few if any genes is required for the correct patterning of early cell divisions. We can conclude that there are no such genes on the X chromosome, which represents about one sixth of the genome physically and carries about one-sixth of the mapped marker genes. To estimate the possible number of such genes on the autosomes that might have been missed in our analyses, we would need to know what fraction of autosomal genes the tested deficiencies remove. Estimates of this fraction at present are imprecise. By counting marker genes uncovered, we estimate that 39% of autosomal genes are removed; this figure is accurate only to the extent that the distribution of counted marker genes reflects that of genes affecting the early embryo, which of course is unknown. By positioning deficiencies on the physical map based on the most distal physical markers uncovered, we estimate that they remove in aggregate at least 25% of autosomal DNA. This is certainly an underestimate, since in several cases genetic markers distal to the physical markers used are known to be removed. Even if the two estimates could be made more precise, they would agree only if genes are randomly distributed on the physical map, which is also not yet certain. If for the sake of a rough calculation we assume that the deficiencies tested remove in aggregate one third of the autosomal genes, and that embryonically required early patterning genes, if any, are randomly distributed on the 5 autosomes, then a Poisson distribution would predict with 95% confidence that there are no more than 9 such genes. If such genes were randomly distributed among all 6 linkage groups, and we assume that the deficiencies and the nullo-X condition together represent half the genome, then the distribution would predict that there are no more than 6 such genes. At least two possibilities might require these estimates to be revised upward. First, if there is functional redundancy among embryonically expressed genes required for early patterning, then some such genes might have been missed in our analysis. Second, functionally related genes in this class might be clustered rather than randomly distributed as we have assumed.

Interpretation of terminal phenotypes: Conclusions regarding the functions of missing genes based on interpreting terminal phenotypes of mutant embryos are subject to at least four general caveats, apart from the fact that we have carried out only a very limited characterization of arrested embryos. First, the primary effects that result from a deficiency, occurring at some unknown time during embryogenesis, may lead to additional defects prior to arrest that are not directly related to the functions of the missing genes. Second, an
observed phenotype may result from the combined effects of lacking two or more genes, despite the evidence in Drosophila that homozygous deficiency phenotypes were generally the same as those resulting from null mutations in the earliest acting uncovered gene (MERRILL et al. 1988; WIESCHAUS and SWEETON 1988; VAVRA and CARROLL 1989). Third, a deficiency that causes failure to exhibit a differentiation marker may do so either by preventing normal determination of the affected lineage, or simply by preventing expression of the marker itself. For example, nullo-X embryos, which do not display gut granules, nevertheless stain for gut-specific esterase (F. STORFER-GLAZER and L. EDGAR, unpublished), suggesting that absence of the X chromosome blocks rhabditin granule formation but not gut cell determination. Fourth, since the embryos in our experiments were not observed continuously throughout development, it is possible that a differentiation marker scored as absent at the terminal arrest stage, for example twitching as an indicator of muscle differentiation, was in fact transiently present at some time prior to 12 hr post-fertilization. More extensive analyses of deficiency embryos would need to be carried out in order to draw more specific conclusions regarding functions of the removed genes.

With these caveats, we can summarize and tentatively interpret our results for the five phenotypic classes. The 8 or more essential loci on all 6 linkage groups defined by the class 1 deficiency embryos, which arrest near the onset of morphogenesis, could include genes that encode collagens required for cuticle formation or any of the cytoskeletal machinery required for elongation of the embryo (PIRES and HIRSH 1986), as well as genes that control cell interactions essential for morphogenesis. The 3 or more loci on LGII and LGIII defined by the class 2 embryos are apparently required for completion of cell proliferation, and the absence of these regions generally leads to defective muscle function as well. The class 3 phenotype suggests that the X chromosome does not include, surprisingly, any genes that are uniquely required in the embryo for completion of cell proliferation, but does include genes essential for gut differentiation, muscle differentiation, and initiation of morphogenesis. The class 4 phenotype of arrest at 95-140 nuclei, found only in embryos homozygous for sDf28(V), must result primarily from removal of the emb-29 gene, since mutations in this gene alone cause embryonic defects virtually identical to those resulting from the deficiency (CASSADA et al. 1981; ISNENGI et al. 1983; DENICH et al. 1984; R. HECHT, personal communication; and see Table 2). The emb-29 gene product could be required only after about the 100-cell stage for a step in cell division that initially utilizes maternally supplied components. The two or more loci on LGII defined by the class 5 phenotype, characterized by arrest as an anatomically normal L1 larva, appear to be required for the initiation of larval development. Such phenotypes have been observed to result from single-gene mutations isolated in previous screens for nonmaternal-effect mutants (see Introduction). They could also reflect lack of "housekeeping" gene products that are provided maternally in sufficient supply to complete embryogenesis but not to proceed with larval cell divisions.

We can also make three more general conclusions in addition to those cited above. First, completion of cell proliferation is not required either for onset of morphogenesis or for expression of any of the differentiation markers scored, as shown by the phenotype of class 4 embryos with less than 20% of the normal number of cells. It is striking that embryos of <100 cells can undergo morphogenesis to the twofold stage. Second, expression of genes in at least four regions of the genome (defined by the class 2 and class 4 deficiencies) is required embryonically for the completion of cell proliferation, and such genes in three of these regions may represent previously unidentified loci. Third, many genes are probably required for successful morphogenesis, based on our finding that deficiencies of any of at least 8 different genomic regions result in failure of this process. As men-

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**TABLE 3**

<table>
<thead>
<tr>
<th>Class</th>
<th>Deficiencies</th>
<th>Cells at arrest</th>
<th>Morphogenesis</th>
<th>Twitching</th>
<th>Gut granules</th>
<th>Smooth hypodermis</th>
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<td>10</td>
<td>550</td>
<td>Variable</td>
<td>Variable</td>
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<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>150-300</td>
<td>Rarely</td>
<td>Rarely</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>1 (nullo-X)</td>
<td>550</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>1 (sDf28 V)</td>
<td>95-140</td>
<td>Yes (2-fold)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>550</td>
<td>Complete</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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a See text.
b See Table 2 for listing of the deficiencies comprising classes 1, 2 and 5.
c Some genotypes.
d Embryos homozygous for sDf89 appeared to arrest slightly earlier than embryos homozygous for the other deficiencies in this class, with 150-200 cells rather than 200-300 cells.
e L1 larval arrest.
Possible effects of deficiencies on early cell fate determination: None of the genes corresponding to pre-gastrulation transcripts identified by Schauer and Wood (1990) have now been located on the C. elegans physical map as shown in Figure 1B. At least two of these genes are removed by the deficiencies we have tested. The striking observation that none of these deficiencies affect pre-gastrulation cleavage patterns could be explained in either of two ways. All the essential genes removed by these deficiencies could be required only after gastrulation onset, and any early transcripts from these regions could have no essential early functions. Alternatively, some of these genes, though not required for normal elaboration of the pre-gastrulation cleavage pattern, could nevertheless be essential for early events such as determination of founder cell fates, which affect later development. This latter alternative initially seemed unlikely in light of the general finding that in postembryonic lineages, cell fates and lineage patterns appear to be coupled; that is, mutations that result in cell fate changes generally also cause changes in the orientation and timing of the divisions that produce the affected cells as well as the divisions of their progeny cells (reviewed by Horvitz 1988). However, recent evidence supports the view that early embryonic cleavage may be different from postembryonic cell divisions in this regard. Maternal-effect mutations in the genes skn-1, pie-1 and mex-1, which have profound effects on determination of founder cell fates, apparently altering the first three cleavages, do not substantially alter the normal morphological pattern of pre-gastrulation cleavage divisions. Cleavage of skn-1 embryos is normal to the 28-cell stage (Bowerman et al. 1992), and cleavage in mex-1 and pie-1 embryos is normal except that P, divides equally instead of unequally at the 24-cell stage (Mello et al. 1992) (such a defect would have been seen in our analyses). Moreover, early cleavage patterns also appear to be normal in permeabilized α-amanitin-treated embryos, which show no detectable embryonic poly(A)⁺ RNA synthesis (Edgar et al. 1994). Thus it is not unlikely that some of the deficiencies tested here include embryonically expressed genes required for early cell determination.

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LITERATURE CITED