ISOLATION AND GENETIC CHARACTERIZATION OF CELL-LINEAGE MUTANTS OF THE NEMATODE *CAENORHABDITIS ELEGANS*

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ABSTRACT

Twenty-four mutants that alter the normally invariant post-embryonic cell lineages of the nematode *Caenorhabditis elegans* have been isolated and genetically characterized. In some of these mutants, cell divisions fail that occur in wild-type animals; in other mutants, cells divide that do not normally do so. The mutants differ in the specificities of their defects, so that it is possible to identify mutations that affect some cell lineages but not others. These mutants define 14 complementation groups, which have been mapped. The abnormal phenotype of most of the cell-lineage mutants results from a single recessive mutation; however, the excessive cell divisions characteristic of one strain, CB1322, require the presence of two unlinked recessive mutations. All 24 cell-lineage mutants display incomplete penetrance and/or variable expressivity. Three of the mutants are suppressed by pleiotropic suppressors believed to be specific for null alleles, suggesting that their phenotypes result from the complete absence of gene activity.

THE relationship between genes and development is unknown. It appears that development and differentiation are based on differential gene activity, and considerable effort has been dedicated to determining the mechanisms used in eliciting this differential gene activity (*e.g.*, GURDON 1974; DAVIDSON 1976). Nonetheless, little is understood about the genetic control of gene expression during development. Perhaps the most likely candidates for genes that play fundamental regulatory roles in development are those that, when mutated, result in a switch from one fate to another; examples of this class include homeotic mutants, in which one body part is replaced by another normally found elsewhere in the organism (*e.g.*, MORATA and LAWRENCE 1977) and transformer mutants, in which sexual development is opposite to that defined chromosomally (*e.g.*, STURTEVANT 1945; CATTANACH, POLLARD and HAWKES 1971; HODGKIN and BRENNER 1977). It would be of interest to identify other mutations that lead to similar switches in developmental fate.

The free-living soil nematode *Caenorhabditis elegans* is well suited for studies concerning the genetic control of the development of a multicellular organism. *C. elegans* is small, easily cultured and readily amenable to genetic manipulations (BRENNER 1974; HERMAN and HORVITZ 1980). This nematode has relatively few cells—there are about 950 somatic nuclei in the adult—of many different types; both the cellular anatomy and the pattern of cell divisions from the single-celled zygote to the adult are essentially invariant among individuals (SULSTON and HORVITZ 1977; DEPPE *et al.* 1978; KIMBLE and HIRSH 1979). Mutations affecting this rigidly determined cell lineage offer one way of answering a variety of questions about the partitioning of the genetic program for *C. elegans* development. For example, is it possible to isolate mutants altered in a specific cell division or set of cell divisions? If so, how many genes are involved in each cell division? In how many cell divisions is each such gene involved? What other features are common to that set of cell divisions affected by a given gene? Can mutants be isolated in which cell fate is transformed so that a cell follows a lineage that is normally that of another cell?

Cell-lineage mutants would also be useful in other ways. First, animals lacking a particular cell or set of cells would help identify cell function, as well as help characterize regulative potential during development; such genetic ablation experiments would complement physical ablation experiments that have been performed using a laser microbeam to identify the functions of specific cells in *C. elegans* (WHITE and HORVITZ 1979; KIMBLE, SULSTON and WHITE 1979; SULSTON and WHITE 1980). Second, cell-lineage mutants may generate cells abnormal in lineage history and/or position, allowing the relative influence of these two factors on cell fate to be examined.

The nongonadal post-embryonic cells lineages of *C. elegans* are particularly promising for the isolation and characterization of cell-lineage mutants. These lineages are completely known, easy to study and generate readily recognizable components of the neuronal, muscular, hypodermal and digestive systems (SULSTON and HORVITZ (1977). One way to seek cell-lineage mutants is to isolate strains defective in these structures. We have used this approach to obtain 24 cell-lineage mutants of *C. elegans*. This paper describes the isolation and genetic characterization of these mutants.

MATERIALS AND METHODS

Strains and genetic nomenclature: *Caenorhabditis elegans var.* Bristol strain N2 and most of the mutant strains used for genetic mapping were obtained from BRENNER (1974). N2 is the wild-type parent of all nematode strains used in this work. Mutant genes and alleles utilized are listed below:

LGI: *unc-35(e259)*; *lin-6(e1466)*; *dpy-5(e61)*; *unc-15(e73)*; *unc-59(e261,e1005)*; *unc-54(e190)*.

LGII: *cat-2(e1112)*; *lin-8(n111)*; *unc-85(e1414)*; *lin-4(e912)*; *dpy-10(e128)*; *lin-5(e1348, e1457)*; *unc-4(e120)*; *unc-52(e444)*; *lin-7(e1413)*.

LGIII: *dpy-17(e164)*; *lon-1(e185)*; *lin-9(n112)*; *sup-5(e1464)*; *unc-32(e189)*; *unc-86(e1416, e1507)*; *unc-69(e587)*; *tra-1(e1099)*; *dpy-18(e364)*.

LGIV: *lin-1(e1026,e1275,n176)*; *dpy-13(e184)*; *unc-5(e53)*; *lin-3(e1417)*; *dpy-20(e1282)*.

LGV: *unc-46(e177)*; *dpy-11(e224)*; *unc-83(e1408,e1409)*; *unc-23(e25)*; *unc-42(e270)*; *him-5(e1467)*.

LGX: *lon-2(e678)*; *sup-7(st5)*; *dpy-7(e1324)*; *lin-2(e1309,e1424,e1453)*; *unc-9(e101)*; *unc-84(e1174,e1410,e1411,e1412)*; *unc-3(e151)*.

This paper conforms to the standardized nomenclature for *C. elegans* genetics (HORVITZ *et al.* 1979).

Sources of cell-lineage mutants: All but 3 of the cell-lineage mutants described in this work were obtained after mutageneis with ethyl methanesulphonate (EMS), as described by BRENNER (1974). *lin-4(e912)* was obtained by BABU (personal communication) after P^{32} decay, and *unc-84(e1174)* was obtained by D. RIDDLE (personal communication) after treatment with ICR-191. The origin of *unc-86(e1416)* is unclear. A mutant strain, CB192, isolated by S. BRENNER (personal communication) after EMS treatment was crossed with wild-type males, and strains carrying 3 distinct mutations were isolated from F_2 progeny of this mating: *unc-86(e1416) III*, *lin-3 (e1417) IV* and *dpy-20(e192) IV*. By a variety of criteria, it has been established that the mutations *e192* and *e1417* are present in the original strain, CB192. However, *e1416* appears not to be: CB192 has none of the phenotypic characteristics associated with *e1416*, and a second attempt to extract *e1416* from CB192 failed.

S. BRENNER isolated *unc-59(e261,e1005)*, *lin-1(e1026,e1275)*, *lin-2(e1309)* and CB1322. BABU isolated *lin-4(e912)*; M. CHALFIE, *unc-86(e1507)*; I. GREENWALD, *lin-1(n176)*; and D. RIDDLE, *unc-84(e1174)*.

Genetic techniques: General techniques for culturing and genetically studying *C. elegans* have been described by BRENNER (1974). Genetic data usually have been derived from complete progeny counts obtained according to HODGKIN, HORVITZ and BRENNER (1979). Most experiments, including many of the mutant hunts, were done at 25°.

A number of mutations that affect cell lineages in hermaphrodites do not affect males. In these cases, complementation tests were performed by including an additional marker in the hermaphrodite strain, which allowed hermaphrodite cross progeny to be scored. Also, a number of cell-lineage mutants display incomplete penetrance; hence an animal that is phenotypically wild-type may be genotypically mutant. Linkage was determined in these cases by constructing a double heterozygote (*e.g.*, $a\,+/+\,b$ or $a/+;b/+$, where "*a*" is a cell-lineage marker and "*b*" is a mapping marker) and picking progeny that expressed the cell-lineage phenotype (*i.e.*, are a/a); the frequency of such animals that segregate progeny carrying the mapping marker is $2p/(1+p)$, which is 2/3 for unlinked markers and about $2p$ for closely linked markers.

Suppression studies: Most of the cell-lineage mutants were tested for suppressibility by 2 pleiotropic suppressors, *sup-5 III* and *sup-7 X* (WATERSTON and BRENNER 1978; WATERSTON, submitted for publication).

For *sup-5*, we first constructed *mut*; *sup* double mutants by employing the visible marker *lon-1*, which is closely linked to the suppressor. If *mut*/+; *lon-1 sup-5*/+ + hermaphrodites readily generated Lon Mut progeny (directly or as segregants from either Lon or Mut animals), the suppression test was scored as negative. If Lon Mut progeny were not obtained, suppression was suggested, but not proved; it was necessary to establish that the *mut*; *lon-1 sup-5* strain was viable, for example, by finding Mut animals that generated Lon non-Mut progeny. (This test is possible only in cases of recessive suppression, *i.e.*, when *sup/sup* suppresses but *sup*/+ does not.) Candidates for suppressibility by *sup-5* were then tested using an alternative scheme developed by R. WATERSTON (personal communication) in which an additional marker known to be suppressed by *sup-5* was included to allow direct verification of the *sup-5* genotype. Specifically, *dpy-18 (e364)*, for which *sup-5* is a semi-dominant suppressor, was utilized: *e364/e364* animals are Dpy: *sup-5 e364/sup-5 e364* animals are non-Dpy; and *sup-5 e364*/+ *e364* animals are of an intermediate phenotype, "Dpy-ish." Thus, in an animal homozygous for *e364*, the *sup-5* genotype can be determined directly.

Most *sup-7* suppression studies were done using *e364* to indicate the *sup-7* genotype. The effects of *sup-7* are similar to those of *sup-5*: *e364*; *sup-7* animals are non-Dpy; *e364*; *sup-7*/+ animals are Dpy-ish; and *e364*; +/+ animals are Dpy. *e364*/+; *sup-7/O* males were mated with either *mut* or *e364*; *mut* hermaphrodites and Dpy-ish F_2 progeny examined. If Mut Dpy-ish

animals were present, these animals were picked and the Mut phenotype of their non-Dpy (*sup-7/sup-7* homozygotes) and Dpy-ish (*sup-7/+*) progeny examined; if a Mut non-Dpy strain (genotype *e364*; *mut*; *sup-7*) could be generated, the suppression test was scored as negative. For the *unc-86* alleles, *e1416* and *e1507*, strains of genotype *unc-86/e364*; *sup-7/+* were constructed; the appearance of approximately 1/4 Unc F_1 progeny indicated a lack of dominant suppression; such Unc animals failed to generate non-Unc progeny, indicating a lack of recessive suppression. For *lin-5(e1348, e1457)* and *lin-6(e1466)*, both of which produce sterile adults, Lin strains could not be established; instead, appropriate non-Dpy strains that segregated Lin progeny (genotype *lin/+*; *e364*; *sup-7*) were taken to indicate a lack of suppression. Because the multivulva mutant *lin-8(n111) II*; *lin-9(n112) III* requires 2 mutations for the expression of its phenotype (see below), slightly modified protocols were employed to test these mutations for suppressibility by *sup-7*. *e364/+*;*sup7/O* males were mated with *lin-8*; *lin-9 e364* hermaphrodites and non-Dpy cross progeny (*lin-8/+*; *lin-9 e364/++*; *sup-7/+* picked. From these animals, multivulva Dpy-ish progeny were obtained and found to segregate multivulva non-Dpy progeny of genotype *lin-8*; *lin-9 e364*; *sup-7*, indicating that neither *lin-8* nor *lin-9* is suppressed by *sup-7*. *lin-8* and *lin-9* were also tested separately with *sup-7* to determine if either could be "suppressed" to a multivulva phenotype. *e364/+*; *sup-7/O* males were mated with either *lin-8 dpy-10* or *dpy-17 lin-9* hermaphrodites and F_2 and F_3 Dpy-10 and Dpy-17 progeny examined for multivulva animals; none were found.

sup-5 suppression studies were done at 20°; *sup-7* studies were done at 25°.

Microscopy and anatomical nomenclature: The microscopical and histological techniques employed have been described previously: Nomarski differential interference contrast optics for the observation of living nematodes (SULSTON and HORVITZ 1977), bright-field optics for Feulgen-stained animals (SULSTON and HORVITZ 1977), fluorescence microscopy for animals stained with Hoechst 33258 (ALBERTSON, SULSTON and WHITE 1978) and for dopaminergic neurons stained by the technique of formaldehyde-induced fluorescence (SULSTON, DEW and BRENNER 1975). Cells are named as in SULSTON and HORVITZ (1977).

RESULTS

Identification of mutants: To identify mutants with abnormal post-embryonic cell lineages, we sought strains defective in structures known to be generated by post-embryonic cell divisions. A variety of strains have been screened in three ways: (1) Feulgen or Hoechst-stained whole mounts were examined for possible defects in the ventral cord and vulva; (2) formaldehyde-induced fluorescence was used to check for postdeirid dopaminergic neurons; and (3) Nomarski optics was used to confirm apparent cell-lineage defects by the direct observation of cell divisions and to examine other lineages and structures. The strains we examined can be divided into three categories.

(1) *Random screening:* Not knowing what phenotypes to expect of cell-lineage mutants, we screened the progeny of individual F_1 and F_2 animals derived from mutagenized hermaphrodites; each of these F_1 or F_2 animals had been transferred to a separate Petri dish. Three mutants obtained in this way were uncoordinated and defective in both vulva and ventral cord development: *unc-83(e1409)* and *unc-84(e1411, e1412)*. A number of individuals segregated sterile, uncoordinated animals with abnormal development of both the gonad and the ventral nerve cord; three of these mutations have been characterized: *lin-5(e1348, e1457)* and *lin-6(e1466)*.

(2) *Egg-laying defective:* Because post-embryonic cell divisions generate most components of the egg-laying system of the hermaphrodite (the vulva, the vulval

and uterine muscles, and neurons that innervate these muscles; SULSTON and HORVITZ 1977), it seemed likely that certain cell-lineage mutants should be unable to lay eggs. Many egg-laying-defective mutants have been isolated, either by selecting animals that are bloated in appearance or that contain internally hatched larvae, or by picking random F_2 progeny of mutagenized hermaphrodites and identifying fertile populations with no or very few free eggs (R. HORVITZ, unpublished results). When examined microscopically as described above, 11 such mutants proved to have abnormal post-embryonic cell lineages: *unc-59(e1005)*, *unc-83(e1408)*, *unc-84(e1410)*, *unc-85(e1414)*, *unc-86(e1416)*, *lin-2(e1309, e1424, e1453)*, *lin-3(e1417)*, *lin-4(e912)* and *lin-7(e1413)*.

(3) *Other morphological and/or behavioral abnormalities:* "Multivulva" mutants display up to five ventral protrusions that look like ectopic pseudovulvae. Examination of multivulva mutants with Nomarski optics revealed that excess divisions of ventral hypodermal cells lead to the multivulva phenotype. Multivulva mutants include three alleles of *lin-1—e1026, e1275* and *n176*—and the strain CB1322, which requires the presence of two unlinked mutations for expression of its phenotype (see below). Two mutants isolated because of their uncoordinated phenotypes, *unc-59(e261)* and *unc-84(e1174)*, proved abnormal in ventral cord development. One strain, *unc-86(e1507)*, isolated because of its failure to respond to mechanosensory stimuli, was shown by M. CHALFIE (personal communication) to be allelic with a known cell lineage mutant, *unc-86(e1416)*.

Phenotypes: The phenotypes visible in the dissecting microscope that were used in genetic studies and our initial bases for identifying these strains as cell-lineage mutants are listed in Table 1. Figures 1, 2 and 3 indicate some of the relevant characteristics of these strains. These cell lineage mutants fall into six phenotypic classes: (1) blocks in the divisions of the ventral hypodermal cells that generate the vulva ("vulvaless" mutants) (*lin-2, lin-3, lin-4, lin-7*); (2) abnormal proliferation of ventral hypodermal cells, leading to the formation of multiple pseudovulvae along the ventral side of the ("multivulva" mutants (*lin-1* and the synthetic double mutant *lin-8*; *lin-9*); (3) extra cell division generating excess dopaminergic neurons (*unc-86*); (4) blocks in development that prevent normal formation of both the vulva and the ventral nervous system (*unc-83, unc-84*); (5) defects in multiple, but not in all, post-embryonic cell divisions (*unc-59, unc-85*); and (6) blocks in virtually all post-embryonic somatic-cell lineages (*lin-5, lin-6*). More detailed descriptions of the phenotypes of these mutants will be presented elsewhere (SULSTON and HORVITZ 1980; CHALFIE, HORVITZ and SULSTON, in preparation).

All 24 of these cell-lineage mutants display incomplete penetrance and/or variable expressivity. Only strains with reasonably high penetrance were selected for study; thus, for all mutants described, most individuals are clearly abnormal. Some mutants (*lin-2, lin-3, lin-7*) produce a proportion of phenotypically wild-type individuals. Other strains (*unc-59, unc-83, unc-84, unc-85*) produce some individuals that are superficially normal in morphology and behavior, but have developmental lesions that are revealed by Feulgen staining or

TABLE 1

Phenotypes of cell lineage mutants

1 Gene	2 Egg-laying?	3 Morphology	4 Coordinated?	5 Fertile?	6 Male mating?	7 Ventral cord	8 Vulva	9 Postdeirid DN cells	10 Other
wild-type	+	+	+	+	+	+	+	+	
unc-59(e261, e1005)I	—	+	—	+	—	d	h	—	
*unc-83(***e1408***, e1409)V*	—	+	—	+	c	e	—	+	k
unc-84(e1174, **e1410***, e1411, e1412)X*	—	+	—	+	c	e	—	+	k
unc-85(e1414)II	—	+	—	+	—	d	+	—	
unc-86(e1416, e1507)III	—	+	Mec	+	c	+	+	j	
*lin-1(***e1026***, e1275, n176)IV*	+	Muv	+	+	—	+	i	+	l
*lin-2(***e1309***, e1424,* **e1453***)X*	—	+	+	+	+	+	—	+	
lin-3(e1417)IV	—	+	+	+	+	+	—	+	
lin-4(e912)II	—	a	+	+	—	+	—	—	
lin-5(e1348, e1457)II	—	b	—	—	—	f	—	—	
lin-6(e1466)I	—	b	—	—	—	g	—	—	
lin-7(e1413)II	—	+	+	+	+	+	—	+	
lin-8(n111)II; lin-9(n112)III	+	Muv	+	+	+	+	+	+	

Columns 2 through 6 indicate characteristics visible in the dissecting microscope. Columns 7 through 9 indicate characteristics visible at higher magnification in the light microscope. All of these mutants display incomplete penetrance and/or variable expressivity, so that the abnormal characteristics listed are not necessarily visible in all individuals. For genes with multiple alleles, if all alleles do not have essentially equivalent phenotypes, alleles with higher penetrance and or greater expressivity are indicated in boldface; phenotypes listed refer to those alleles. Phenotypes described by "+" or "—" are qualitative indications of mutant characteristics; for example, many mutant strains less fertile than wild-type are nonetheless indicated by "+" and for some mutants strains indicated by "—" males can mate, although poorly. +, normal; —, abnormal; Muv, multivulva; Mec, mechanosensory abnormality; (a) elongated, flaccid body; (b) thin; (c) low efficiency (see HODGKIN, HORVITZ and BRENNER 1979); (d) somewhat low in number with occasional polyploid nuclei; (e) reduced in number, with occasional subventral neuron-like cells; (f) juvenile cells and cells with elongated, polyploid nuclei; (g) juvenile cells only; (h) protruding vulva; (i) malformed vulva; (j) multiple dopaminergic cells; (k) all alleles are temperature sensitive sensitive; (l) *e1275* is weakly temperature sensitive.

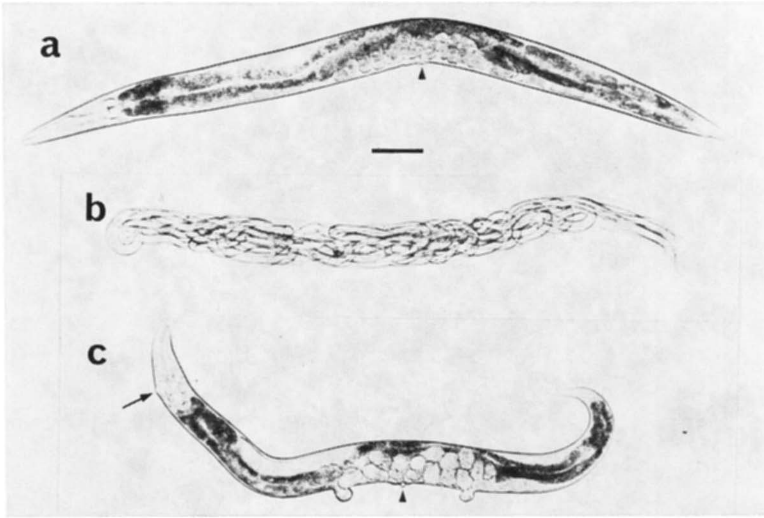

FIGURE 1.—Adults anesthetized with 0.5% 1-phenoxy-2-propanol and slightly compressed with a cover slip to display internal features and improve planarity. Bright-field, left lateral views. Bar = 100μ. (a) Wild type; arrowhead points to vulva. (b) Vulvaless, *lin-3(e1417)*. Progeny have hatched internally and devoured the body of their parent so that only the cuticle remains. (c) Multivulva, *lin-8(n111)*; *lin-9(n112)*. Arrowhead points to true vulva, flanked by two pseudovulvae; arrow points to protruding excretory pore.

with Nomarski optics. All individuals of still other mutants (*unc-86, lin-1, lin-4, lin-5, lin-6* and the double mutant *lin-8; lin-9*) are obviously abnormal, but nonetheless vary from individual to individual in the precise nature of their defects. One consequence of this phenotypic variability was that vulvaless mutants that had been isolated as homozygotes (*lin-2, lin-3, lin-4, lin-7, unc-83* and *unc-84*) could be mated, hence studied genetically, by picking individuals with vulvae. *lin-4*, however, makes a vulva very rarely and for convenience in genetic manipulations was maintained as a balanced heterozygote *lin-4/dpy-10*.

All isolates of *unc-83* and *unc-84* are temperature sensitive, showing more mutant phenotypes at 25° then at 20° or 15°; *lin-1(e1275)* is weakly temperature sensitive.

Mapping and complementation: Each mutant was crossed with wild-type males to test for sex-linkage and/or dominance and to re-isolate a homozygous mutant stock from the resulting heterozygous hermaphrodites in an attempt to eliminate extraneous mutations introduced by mutagenesis. The linkage group of a new mutation was identified by trying to construct double mutants with a set of standard markers, usually *dpy-5 I, dpy-10 II, dpy-18 III, dpy-13 IV, dpy-11 V* and *lon-2 X*. In most cases, one and only one double mutant was difficult to construct, indicating linkage. More precise map locations, shown in Figure 4, were defined by the experiments described below. All of the cell-lineage mutations described are recessive, suggesting that the phenotypes of these mutants may result from the absence or reduction of gene activity. Complementa-

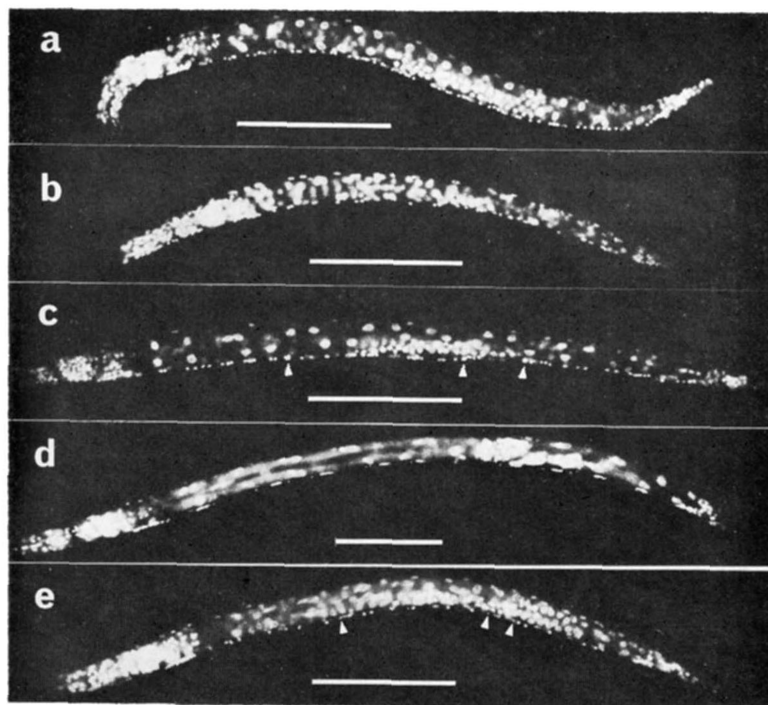

FIGURE 2.—Nuclear-stained whole mounts. Animals mounted in M9 buffer containing 0.5% 1-phenoxy-2-propanol and 5 μg/ml Hoechst 33258 (ALBERTSON, SULSTON and WHITE 1978; S. WARD and M. CHALFIE, personal communication). Photographed after one to five hr on Kodak PanX film, using a Zeiss Standard microscope with UG1 exciter and 41 barrier filters. Left lateral views. Bar = 100μ. (a) Wild type, L2. Note chain of motor neuron nuclei in ventral cord (lower edge). (b) *unc-84(e1410)*, L2. Number of nuclei in ventral cord greatly reduced. (c) *unc-85(e1414)*, L3. Number of nuclei in ventral cord somewhat reduced; polyploid nuclei can be seen (arrowheads). (d) *lin-5(e1348)*, L4. Few neuronal nuclei in ventral cord, but a series of elongated polyploid nuclei are seen. Very large polyploid nuclei in posterior half of body are gonadal and mesodermal. (e) *lin-6(e1466)*. Few nuclei in ventral cord. Subdiploid nuclei can be seen (arrow heads).

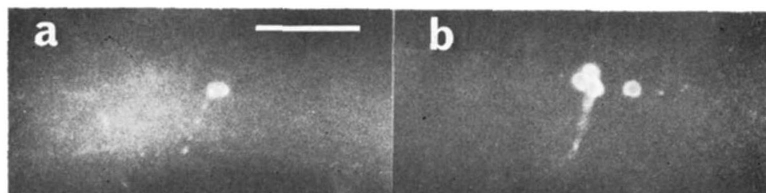

FIGURE 3.—Dopaminergic cells in the postdeirid, visualized with the technique of formaldehyde-induced fluorescence (SULSTON, DEW and BRENNER, 1975). Lateral views displaying subdorsal superficial regions about midway along the anterior-posterior axis from the vulva to the anus. Bar = 20μ. (a) Wild type. Varicosities can be seen in the neuronal process that runs ventrally from the cell body into the ventral nerve cord. (b) *unc-86*. Four dopaminergic cell bodies and an increased intensity of the neuronal process(es) are apparent.

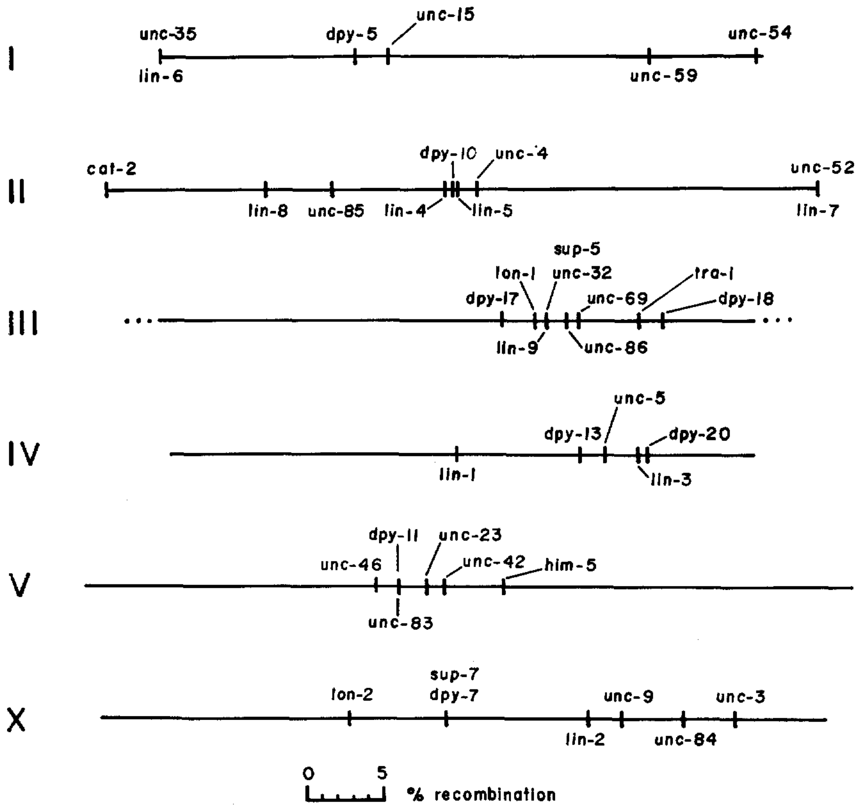

FIGURE 4.—Partial genetic map of *C. elegans*, showing the positions of markers used in this study. Genes affecting cell lineages are indicated below the line of each linkage group; other markers are indicated above the line. The known extents of all linkage groups except *LGIII* are shown.

tion tests were routinely performed with alleles of genes of similar map positions and phenotypic effects. For all cell-lineage mutants but one, simple Mendelian inheritance was observed; the exception, CB1322, was a synthetic mutant and contained mutations in two unlinked genes.

Most of our mapping data are summarized in Tables 2, 3 and 4. Additional data, in particular those for CB1322, and a few specific comments are presented below. The alleles used in mapping are indicated in parentheses.

unc-59(e1005) I: *e1005* fails to complement *e261*; although *e261* has been reported to map to the right of *unc-54* (BRENNER 1974), the original data are also consistent with the position assigned to *e1005* (S. BRENNER, personal communication).

unc-83(e1408) V: The expressivity of mutations in *unc-83* (as well as in *unc-84*) is reduced by many different *C. elegans* alleles, including certain *dpy*, *unc* and *lon* mutations (E. J. HESS, personal communication); however, in all cases studied so far, suppression is incomplete, and double mutants can still be

TABLE 2

Two-factor crosses in cis

Genotype of heterozygote	Progeny: Parental phenotypes		Progeny: Recombinant phenotypes	Percent recombination (100 p)
unc-59 unc-54/+ +	1209 WT	390 Unc-59 Unc-54 (or Unc-54)	71 Unc-59	8.7%
dpy-5 unc-59/+ +	1308 WT	490 Dpy Unc (or Dpy)	160 Unc	18.0%
lon-2 unc-84/+ +	393 WT	85 Lon Unc	64 Lon 94 Unc	29.0%
unc-85 dpy-10/+ +	515 WT	164 Unc Dpy	28 Unc 15 Dpy	6.2%
unc-86 dpy-18/+ +	642 WT	218 Unc Dpy	22 Unc 23 Dpy	5.1%
lon-1 unc-86/+ +	773 WT	227 Lon Unc	5 Lon 11 Unc	2.1%
lin-1 dpy-13/+ +	282 WT	71 Lin Dpy	13 Lin 17 Dpy	8.0%
lin-6 dpy-5/+ +	445 WT	116 Lin Dpy	25 Lin 38 Dpy	10.0%

The recombination frequency (p) was calculated from the total proportion of recombinant progeny (R) according to the formula $p = (1 - 2R)^{1/2}$. When only one of the two recombinant classes was counted, as in the *unc-59 unc-54* and *dpy-5 unc-59* experiments, the value determined was considered as $R/2$. WT, wild type.

recognized, allowing mapping experiments to be interpreted with reasonable confidence. No *dpy-11 unc-83* animals were generated in a number of experiments involving *unc-83/dpy-11* heterozygotes, *unc-83/unc-46 dpy-11* heterozygotes and *unc-83/dpy-11 unc-42* heterozygotes, suggesting either that *unc-83* maps very close to *dpy-11* or that *dpy-11 unc-83* zygotes are inviable. (Phenotypic suppression of *unc-83* by *dpy-11* would not explain the failure to obtain Dpy animals from the Unc segregants of *unc-83/dpy-11*.) That each of eight

TABLE 3

Two-factor crosses in trans

Genotype of heterozygote	Phenotype of selected progeny	Genotypes of selected progeny (with respect to unselected markers)	Percent recombination (100 p)
unc-83/dpy-11	Unc	0/36 *dpy/+*	
	Dpy	0/24 *unc/+*	Less than 1%
lin-2/unc-3	Lin	4/20 *unc/+*	10%
lin-2/unc-9	Lin	5/110 *unc/+*	2.1%
lin-6/unc-35	WT	22/23 *lin/unc*, 1/23 *unc/+*	2%
lin-7/unc-52	Lin	0/36 *unc/+*	
	WT	17/17 *lin/unc*	Less than 1%

Progeny from heterozygous parents were selected and their genotypes determined from the phenotypes of their progeny. For example, of 20 Lin progeny derived from *lin-2/unc-3* parents, four segregated Lin Unc progeny and hence were heterozygous for *unc-3*. The recombination frequency (p) was estimated from the frequency of recombinant chromosomes obtained. For phenotypically "A" animals derived from $a\,+/+\,b$ parents, $2p/(1+p)$ should be heterozygous for b (*i.e.*, $a\,+/a\,b$); for phenotypically wild-type (WT) animals derived from $a\,+/+\,b$ parents, approximately $2\,p$ (for small p) will not be of genotype $a\,+/+\,b$.

TABLE 4

Three-factor crosses

Genotype of heterozygote	Phenotypes of selected recombinants	Genotypes of selected recombinants (with respect to *trans* marker)
+ + *unc-59/dpy-5 unc-15* +	Dpy	1/10 *unc-59/unc-59*, 9/10 *unc-59*/+
dpy-5 + +/+ *unc-59 unc-54*	Unc-59	1/20 *dpy*/+
unc-83/unc-46 dpy-11	Dpy	0/10 *unc-83*/+
unc-83/dpy-11 unc-42	Dpy	0/27 *unc-83*/+
unc-83/dpy-11 unc-23	Unc-23	8/8 *unc-83*/+
	Dpy	0/13 *unc-83*/+
+ + *unc-84/dpy-7 unc-9* +	Dpy	6/6 *unc-84*/+
	Unc-9	0/6 *unc-84*/+
+ *unc-84* +/*dpy-7* + *unc-3*	Unc-3	4/20 *unc-84*/+
unc-85 + +/+ *dpy-10 unc-4*	Unc-4	5/5 *unc-85*/+
	Dpy	0/6 *unc-85*/+
cat-2 + +/+ *unc-85 dpy-10*	Unc	2/6 *cat*/+, 4/6 +/+
	Dpy	2/5 *cat/cat*, 3/5 *cat*+
+ *unc-69* +/*unc-86* + *dpy-18*	Unc-86	1/5 *unc-69*/+
	Dpy	9/10 *unc-69*/+
lin-1 + +/+ *unc-5 dpy20*	Dpy	6/6 *lin*/+
	Unc	0/7 *lin*/+
+ *lin-2* +/*dpy-7* + *unc-9*	Unc	4/20 *lin*/+
	Dpy	17/20 *lin*/+
+ *lin-3* +/*unc-5* + *dpy-20*	Dpy	3/15 *lin*/+
	Unc	12/15 *lin*/+
+ *lin-5* +/*dpy-10* + *unc-4*	Dpy	1/8 *lin*/+
	Unc	6/6 *lin*/+
+ + *unc-59/lin-6 dpy-5* +	Dpy	1/9 *unc*/+
lin-6 + +/+ *dpy-5 unc-15*	Dpy	1/15 *lin*/+
	Unc	5/6 *lin*/+
+ + *lin-7/dpy-10 unc-4* +	Dpy	2/8 *lin/lin*, 6/8 *lin*/+
	Unc	5/12 *lin*/+, 7/12 +/+

Three-factor crosses were performed as described by BRENNER (1974): from heterozygotes *a/bc*, B non-C and C non-B recombinants were selected and their genotypes determined by progeny testing; the frequency of recombinants of each class carrying the *trans* marker *a* indicates the relative position of *a* with respect to *b* and *c*. For example, 6 of 6 Dpy recombinants and 0 of 6 Unc-9 recombinants from *unc-84/dpy-7 unc-9* segregated Unc-84 progeny, indicating that *unc-84* is either to the right of, or very close to, *unc-9*; 4 of 20 Unc-3 recombinants from *unc-84/dpy-7 unc-3* segregated Unc-3 Unc-84 progeny, indicating that *unc-84* is between *dpy-7* and *unc-3* and probably closer to *unc-3*. In a few cases, some of the recombinants selected were homozygous for the nonselected *trans* marker.

Unc-23 recombinants from *unc-83/dpy-11 unc-23* heterozygotes segregated Unc-83 progeny confirmed the linkage of *unc-83* to the cluster of *LGV*.

unc-84(e1410, e1411) X: The presence of Unc-84 animals among the progeny of Unc-9 or Unc-3 recombinants from *unc-84/dpy-7 unc-9* or *unc-84/dpy-7 unc-3* heterozygotes was determined by Feulgen staining. Males of genotype *tra-1; unc-84*/+ (HODGKIN and BRENNER 1977) were used in constructing *unc-84/dpy-7 unc-3*. *unc-84* mutants display complex complementation patterns. The four *X*-linked mutations, *e1174*, *e1410*, *e1411* and *e1412*, all fail to comple-

ment three newly isolated *X*-linked cell-lineage mutants (*n323, n369, n371*) (W. FIXSEN and R. HORVITZ, unpublished results) and at this point will be considered to define a single genetic locus, *unc-84*.

unc-85(e1414) II: The presence of Unc-85 animals among the progeny of Unc-4 recombinants from *unc-85/dpy-10 unc-4* heterozygotes was determined by Feulgen staining. The *cat* genotypes of progeny of recombinants from *cat-2/ unc-85 dpy-10* heterozygotes were determined by formaldehyde-induced fluorescence.

unc-86(e1416) III: *e1416* and *e1507* were shown to be allelic in a complementation test performed by M. CHALFIE (personal communication); *e1416* and *e1507* fail to complement in all aspects of their complex phenotypes examined, except in the production of males (HODGKIN, HORVITZ and BRENNER 1979).

lin-1(e1275) IV: *n176* was shown to be allelic to *e1275* in a complementation test performed by C. FERGUSON (personal communication).

lin-2(e1309) X.

lin-3(e1417) IV.

lin-4(e912) II: *lin-4* was mapped by HODGKIN (1974) as 0.3% left of *dpy-10*.

lin-5(e1348) II: Wild-type males were crossed with *e1457/+* hermaphrodites and single male progeny were crossed each with a single *e1348/dpy-10* hermaphrodite. The progeny from one such animal consisted of 46 Lin, 13 Dpy, 68 wild-type hermaphrodites and 43 wild-type males. The excess of Lin over Dpy progeny indicated the allelism of *e1348* and *e1457*. Feulgen staining of the Lin animals confirmed the presence of *lin-5* males.

lin-6(e1466) I.

lin-7(e1413) II: Of 30 Lin progeny segregated from *lin-7/dpy-10 unc-4* heterozygotes, nine segregated Dpy Unc Lin progeny, two segregated Dpy Lin non-Unc progeny and none segregated Unc Lin non-Dpy progeny.

lin-8(n111) II; lin-9(n112) III: When a homozygous multivulva ("Muv") strain was re-isolated from heterozygous hermaphrodites obtained by mating wild-type males with Muv hermaphrodites of strain CB1322, only 20 Muv animals were observed among a total of 386 progeny; this ratio is close to that of 1:16 expected if mutations in two unlinked genes were required to produce the Muv phenotype. To test this possibility, non-Muv progeny of "CB1322"/+ hermaphrodites were picked. If a single mutation ("*a*") were involved, 1/3 of the non-Muv progeny (+/+) would segregate no Muv animals and 2/3 (*a*/+) would segregate 1/4 Muv animals (or fewer, if reduced viability, incomplete penetrance and/or a maternal effect were responsible for the deficiency of Muv animals). On the other hand, if two mutations ("*b*" and "*c*") were involved, 4/15 of the non-Muv progeny (*b/b;c/+* and *b/+;c/c*) would segregate 1/4 Muv animals, 4/15 (*b/+;c/+*) would segregate 1/16 Muv animals and 7/15 (all other genotypes) would segregate no Muv animals. Sixteen non-Muv progeny from "CB1322"/+ were cloned and yielded the three classes expected in the ratios of 4:4:8, consistent with the two-mutation hypothesis.

These two mutations were mapped to chromosomes *II* and *III* by crossing "CB1322"/+ males with hermaphrodites carrying standard mapping markers

and picking F_2 Muv progeny; the frequency with which such Muv animals segregate a given mapping marker is $2p$ in the case of close linkage and 2/3 for unlinked markers. One of 12 Muv progeny from "CB1322"/*dpy-10 II* segregated Dpy progeny, indicating that one mutation, named *lin-8(n111)*, maps not far from *dpy-10*. Three of 12 Muv progeny from "CB1322"/*dpy-18 III* segregated Dpy progeny, and one of 12 Muv progeny from "CB1322"/*dpy-17 III* segregated Dpy progeny, indicating that the second mutation, named *lin-9(n112)*, maps near *dpy-17*.

More precise map positions were determined from three-factor crosses. *lin-8 II; lin-9 III; him-5 V* males were crossed with *unc-85 dpy-10 II; unc-32 III* hermaphrodites and phenotypically wild-type F_2 progeny were picked. Progeny from individuals that did not segregate Unc-32 animals or F_1 or F_2 males, but did segregate both Muv and Unc-85 Dpy animals (*i.e.*, from parents of genotype *lin-8/unc-85 dpy-10; lin-9*) were utilized. None of four Unc-85 recombinants and 10 of 10 Dpy recombinants segregated Muv progeny, indicating that *lin-8* is close to or left of *unc-85*. Five of 26 Muv progeny from *lin-8/unc-85 dpy-10*; *lin-9* parents segregated Dpy animals, indicating that *lin-8* maps about 10% from *dpy-10*; for all five, the Dpy animals segregated were Muv Dpy Unc-85, indicating that *lin-8* is not between *unc-85* and *dpy-10*. *dpy-17 unc-32 III/+ +* males were mated with *lin-8 dpy-10 II; lin-9 III* hermaphrodites and from cross progeny that segregated Dpy-17 Unc animals, Dpy-10 non-Unc non-Muv F_2 progeny (genotype *lin-8 dpy-10*; *lin-9/dpy-17 unc-32*) were cloned. None of six Unc non-Dpy-17 and five of six Dpy-17 non-Unc recombinants segregated Muv progeny, indicating that *lin-9* is close to *unc-32* and probably between *dpy-17* and *unc-32*.

To determine the phenotypes of animals carrying the separated *lin-8* and *lin-9* mutations, Muv Dpy strains constructed in the experiments described above were employed. Wild-type males were mated with *dpy-10 lin-8; lin-9* hermaphrodites and an F_2 Dpy animal that failed to segregate Muv progeny was saved as *dpy-10 lin-8*. *dpy-17 lin-9* was similarly constructed from *lin-8*; *dpy-17 lin-9*. Neither *dpy-10 lin-8* nor *dpy-17 lin-9* could be distinguished from its "parental" *dpy* strain, indicating that *lin-8* and *lin-9* are apparently wild-type in phenotype. To confirm the genotypes of *dpy-10 lin-8* and *dpy-17 lin-9* and to confirm that the Muv phenotype is caused by two mutations each of which alone results in a wild-type phenotype, *dpy-10 lin-8/+ +*; *dpy-17 lin-9/+ +* hermaphrodites were constructed; almost all Dpy-10 Dpy-17 progeny were Muv and almost all Muv progeny were Dpy-10 Dpy-17.

Suppression studies: As mentioned above, all 24 cell-lineage mutants display incomplete penetrance and/or variable expressivity. Such characteristics might result from partial gene activity; another phenotype, such as embryonic death, might result from a total loss of gene function. To examine this possibility, we tested cell-lineage mutants for suppressibility by two recently identified suppressors, *sup-5 III* and *sup-7 X* (WATERSTON and BRENNER 1978; WATERSTON, submitted for publication). *sup-5* and *sup-7* have the characteristics of informational suppressors: they suppress some, but not all, alleles of many different

genes. For the two *C. elegans* genes with known products, *sup-5* and *sup-7* suppress only alleles that result in no detectable polypeptide product. Thus, the phenotype of an allele suppressed by *sup-5* and/or *sup-7* may well result from the complete absence of gene activity.

The 24 cell-lineage mutants were tested for suppressibility by *sup-5 III* and/or *sup-7 X*. Mutations on LGI, LGII, LGIV and LGV were tested with both *sup-5* and *sup-7*: those on LGIII were tested with *sup-7* only; those on LGX were tested with *sup-5* only. The details of these experiments are described in MATERIALS AND METHODS.

Twenty-one of the 24 mutants studied showed no signs of suppression when examined with a dissecting microscope. *unc-85 (1414) II*, *lin-7(e1413) II* and *lin-2(e1453) X* were suppressed in strains homozygous for *sup-5* and/or *sup-7*; *e1413* and *e1453* also were suppressed in suppressor heterozygotes.

unc-85(e1414) II: Unc progeny from *e1414/+*; *lon-1 sup-5/+ +* hermaphrodites were isolated and their Lon progeny picked. Scoring the Unc phenotypes of these animals was difficult; it appeared that these presumptive *e1414*; *lon-1 sup-5* strains were of lower penetrance with respect to the Unc phenotype than is *e1414* alone. Lon progeny from *e1414/+*; *lon-1 sup-5/+ +* segregated very few Unc progeny; these Lon Unc animals seemed to produce relatively few Unc progeny, again suggesting that *sup-5* partially suppresses *e1414*. *sup-5 e364/+ +* males were mated with *e1414*; *e364* hermaphrodites and Dpy-ish cross progeny (genotype *e1414/+ +*; *sup-5 e364/+ e364*) were picked. These animals segregated Unc Dpy-ish progeny (*e1414*; *sup-5 e364/+ e364*), indicating that a suppressor heterozygote does not suppress *e1414*. From these animals, non-Dpy progeny (*e1414*; *sup-5 e364*), most of which were Unc, were cloned. Most (but not all) animals in subsequent generations of strains of *e1414*; *sup-5 e364* were non-Unc. Thus, *sup-5* appears to be a recessive suppressor of *e1414*. An Unc strain similar in phenotype to the original *unc-85(e1414)* strain was derived from an F_2 progeny of a mating between wild-type males and *e1414*; *sup-5 e364* hermaphrodites.

Similarly, *e364/+*; *sup-7/0* males were crossed with *e1414*; *e364* hermaphrodites and Dpy-ish cross progeny (*e1414/+*; *e364*; *sup-7/+*) were picked. These animals segregated Unc Dpy-ish progeny (*e1414*; *e364*; *sup-7/+*), which produced Dpy-ish progeny, almost all of which were Unc, and non-Dpy progeny, many of which were non-Unc. These non-Dpy animals (*e1414*; *e364*; *sup-7*) produced mostly non-Unc progeny. Thus, *sup-7* appears to be a recessive suppressor of *e1414* and may be a slightly better suppressor than *sup-5*. Wild-type males were crossed with *e1414*; *e364*; *sup-7* hermaphrodites; an Unc strain of phenotype similar to that of the original *unc-85(e1414)* strain was derived from the F_2 progeny of this mating.

In these experiments there is a suggestion of a maternal effect. *e1414*; *sup* progeny from *e1414*; *sup/+* parents were mostly Unc, whereas *e1414*; *sup* progeny from *e1414*; *sup* parents were mostly non-Unc. Thus, the Unc phenotype of an *e1414*; *sup* animal seems to depend upon the *sup* genotype of its parent. This result indicates that one must be cautious when interpreting suppres-

sion studies; if strains of *e1414*; *sup* animals had not been established, it would have been difficult to detect the suppression of *e1414*. One possible explanation for the maternal effect is that the *sup*(+) gene product might be present in oocytes.

lin-7(e1413) II: None of six Lon progeny from *e1413/+*; *lon-1 sup-5/+ +* hermaphrodites segregated Lin animals, suggesting that in a *sup-5/sup-5* background *e1413* is not expressed. None of six Lin progeny from *e1413/+*; *lon-1 sup-5/+ +* segregated Lon animals, suggesting that Lin is expressed only in a *sup-5*(+)/*sup-5*(+) background. *sup-5 e364/+ +* males were mated with *e1413*; *e364* hermaphrodites and Dpy-ish progeny (*e1413/+*; *sup-5 e364/+ e364*) picked. From these animals, 23 Dpy-ish progeny, presumably of three genotypes (*e1413*; *sup-5 e364/+ e364* and *e1413/+*; *sup-5 e364/+ e364* and +/+; *sup-5 e364/+ e364*) were picked. All 23 were phenotypically non-Lin (*i.e.*, all laid eggs). Many of these Dpy-ish animals segregated some Lin progeny, all of which were Lin Dpy. Some of these Dpy-ish animals segregated many Lin progeny, all of which were Lin Dpy. These animals were presumably *e1413*; *sup-5 e364/+ e364*, and since both non-Dpy and Dpy-ish progeny were non-Lin, it can be concluded that *sup-5* is a dominant suppressor of *e1413*. Non-Dpy progeny of these animals (genotype *e1413*; *sup-5 e364*) were mated with wild-type males and Lin animals were isolated from the F_2 progeny of this mating.

Similarly, *e364/+*; *sup-7/0* males were mated with *e1413*; *e364* hermaphrodites and Dpy-ish progeny picked. Their Dpy-ish progeny, again presumably of three different genotypes, were picked and three classes identified: those with no Lin progeny, those with some Lin progeny and those with many Lin progeny. All Lin animals were Dpy Lin. Thus, *sup-7* is a dominant suppressor of *e1413*. Putative *e1413*; *e364*; *sup-7* non-Dpy non-Lin hermaphrodites were crossed with wild-type males, and Lin animals were isolated from the F_2 progeny of this mating.

lin-2(e1453) X: From *lon-1 sup-5/+ +*; *e1453/+* hermaphrodites, none of six Lon progeny segregated Lin animals and none of six Lin animals segregated Lon progeny, suggesting that *sup-5* could be a dominant suppressor of *e1453*. *sup-5 e364/+ +* males were mated with *e364*; *e1453* hermaphrodites and Dpy-ish progeny (*sup-5 e364/+ e364*; *e1453/+*) picked. From these animals, 24 Dpy-ish progeny were picked, all of which were phenotypically non-Lin; some segregated no Lin progeny, many segregated some Lin progeny, and some segregated many Lin progeny. All Lin animals were Dpy Lin. Thus, *sup-5* is a dominant suppressor of *e1453*. Putative *sup-5 e364*; *e1453* non-Dpy non-Lin hermaphrodites were mated with wild-type males and Lin animals were isolated from the F_2 progeny of this mating.

DISCUSSION

We have described the isolation and genetic characterization of 24 mutants defining 14 genes that affect the normally invariant post-embryonic cell lineages of *C. elegans*. The existence of these mutants demonstrates that it is possible

to isolate strains defective in post-embryonic cell divisions. The spectrum of phenotypes suggests that different genes are involved in different cell lineages.

Most of the cell-lineage mutants that we have isolated affect the ventral hypodermal cells involved in the development of the vulva. Both mutations causing excessive ventral hypodermal cell divisions and mutations preventing these divisions have been identified. The ease of isolation of these mutants indicates that it may be possible to characterize all genes specifically involved in vulval cell divisions. It should be noted that the apparently relatively high frequency of vulval mutants reflects our focusing on egg-laying defective strains in our screenings. We believe that many other classes of cell-lineage mutants could be identified by utilizing other approaches; mutants defective in any cell or structure produced post-embryonically might be sought. For example, the copulatory apparatus of the male tail is generated from post-embryonic cell divisions (SULSTON and HORVITZ 1977; SULSTON, ALBERTSON and THOMSON 1980). Some mutant strains that produce morphologically and behaviorally abnormal males have proved to be defective in post-embryonic cell lineages (HODGKIN 1974 and personal communication).

In all but one of the cell-lineage mutants described in this manuscript, the abnormal phenotype appears to result from a single mutation. However, the multivulva phenotype of strain CB1322 requires the presence of two unlinked mutations. The requirement for both *lin-8(n111)* and *lin-9(n112)* for the phenotypic expression of CB1322 suggests that these two genes might code for equivalent functions; for example, J. CULOTTI (personal communication) has observed that mutations in both *ace-1* and *ace-2*, two genes controlling distinct forms of nematode acetylcholinesterase, are necessary for the expression of a behavioral abnormality.

Some of these cell-lineage mutants have already been utilized in studies of *C. elegans* development. ALBERTSON, SULSTON and WHITE (1978) used *lin-5* to explore the relationships among DNA replication, cell division and cell fate. They reported that although post-embryonic cell divisions fail in *lin-5*, DNA replication continues. The polyploid cells of *lin-5* display characteristics of at least some of the different types of progeny they would produce ordinarily, indicating that at least certain aspects of differentiation do not require cell division and, furthermore, are not mutually exclusive within a single cell. WHITE, ALBERTSON and ANNESS (1978) used *lin-6* to explore the basis of a neuronal reorganization that occurs during *C. elegans* development. They determined that in *lin-6*, which fails in post-embryonic cell divisions, this reorganization is essentially normal, indicating that it is independent of post-embryonically generated cells.

The contrast between the invariance of the post-embryonic cell lineages in wild-type animals and the variability (manifested as incomplete penetrance and/or variable expressivity) of the cell-lineage mutants is striking. This variability occurs despite the probable isogenicity of individuals of a given strain. Variability could result from "leaky" alleles that reduce gene activity to near the minimal level required for normal function; relatively minor fluctuations

in activity around a threshold value could lead to all-or-none variations in phenotype. The suppression studies with *sup-5* and *sup-7* argue against this hypothesis for the three genes with identified suppressible alleles, which are likely to be null alleles. Perhaps an alternative function (such as an isozyme or another member of the same multigene family) or an alternative pathway exists, with the alternative activity near a threshold level. It is possible that, by selecting mostly viable and fertile cell-lineage mutants, we have systematically identified "leaky" alleles and genes for which there are alternative activities. Perhaps mutants in which neither residual gene activity nor an alternative route exists would fail in all, or at least all post-embryonic, cell divisions; however, *lin-5* and *lin-6*, which do fail in all postembryonic divisions, nonetheless display variability. It should be noted that variability in mutant phenotype need not result from variabilty in either the activity of the mutated gene or in that of an alternative route. For example, *unc-86* and *lin-4* mutants display variable reiterations of parts of specific cell lineages (SULSTON and HORVITZ 1980; CHALFIE, HORVITZ and SULSTON, in preparation); such phenotypic variability could be caused by variability in the levels of any activity needed for cell growth and/or cell division among different daughter cells: if a cell that normally does not divide embarks upon a series of divisions, any cellular component could become diluted beyond a critical level. Furthermore, phenotypic variability need not reflect a threshold event; variability could result from truly stochastic biological processes, such as the interaction of an effector molecule with one of a number of similar genomic sites.

To understand how a given gene functions in development, it is important to know the phenotype that results from the complete absence of gene activity. For example, it is possible that some of these cell-linkage mutants have partial gene activity and that another phenotype, such as embryonic death, would result from a total loss of gene function. For this reason, we examined the 24 cell-lineage mutants for possible suppression by *sup-5* and *sup-7*, which appear to suppress only alleles that result in no detectable polypeptide product (WATERSTON and BRENNER 1978; WATERSTON, submitted for publication). Three mutants were suppressed—*unc-85(e1414) II*, *lin-2(e1453) X* and *lin-7(e1413) II*—suggesting that their phenotypes result from a complete absence of gene activity. However, two reservations must be noted: (1) the specificity of *sup-5* and *sup-7* for null alleles is based upon studies of only two genes (*unc-15* and *unc-54*); (2) null alleles of these genes are defined by the absence of detectable polypeptide product on polyacrylamide gels, but some residual activity may remain. If *sup-5* and *sup-7* act at or after translation, then *unc-85*, *lin-2* and *lin-7* must have polypeptide products.

The frequency with which alleles of different genes have been isolated may also indicate the nature of the mutational lesion involved: mutations that reduce or eliminate gene function are likely to be more common than mutations that increase or specifically alter gene activity. Most of the 14 genes affecting cell lineages described in this paper are now represented by multiple isolates with similar phenotypes; besides those listed in Table 1, additional alleles of *unc-83*,

unc-84, *unc-85*, *unc-86*, *lin-1*, *lin-2*, *lin-3* and *lin-7* have been recently identified (R. HORVITZ *et al.*, unpublished results). Thus, mutants in most of these genes may display null phenotypes, although a unique isolate such as *lin-4(e912)* (which was obtained after P^{32} mutagenesis) may reflect a rarer mutational event.

Of all alleles tested with both suppressors (*i.e.*, all alleles except *n111* and those on LGIII and LGX), only *e1414* and *e1413* were suppressed; *sup-5* and *sup-7* are dominant suppressors of *e1413* and recessive suppressors of *e1414*. This identity of suppression patterns, also observed in other studies using *sup-5* and *sup-7* (WATERSTON, submitted for publication), suggests that similar mechanisms of suppression are probably involved. WATERSTON also noted that suppression by *sup-7* seems to be somewhat stronger than that by *sup-5*.

The absence of visible suppression of all alleles of a given gene by *sup-5* and *sup-7* could occur even if "suppressible" alleles have been tested. For example, a low level of restoration of gene activity might be insufficient to alter a visible phenotype, particularly when assayed only crudely at the level of the dissecting microscope; at 25°, *sup-5* and *sup-7* restore less than 20% of the normal level of *unc-15* gene product (WATERSTON and BRENNER 1978; WATERSTON, submitted for publication). Another ambiguity in suppression studies is the possibility of either enhancer or suppressor effects resulting from other markers or from effects of *sup-5* or *sup-7* other than informational suppression. For example, mutations in *unc-83* and *unc-84* appear to be either enhanced or suppressed by many different *C. elegans* mutations, including *lon-1(e185)* and, probably, *dpy-18(e364)* (E. J. HESS, personal communication), both of which were utilized in these suppression studies; the apparent failure of suppression for alleles of these genes is particularly difficult to interpret. Similarly, examples of weak suppression, particularly for mutants that vary considerably in expressivity, such as *unc-85(e1414)*, must be regarded with caution.

Of the three identified suppressible alleles, both *e1413* and *e1453* were well suppressed by suppressor heterozygotes (*i.e.*, in *sup/+* animals), whereas *e1414* was at most partially suppressed, even in *sup/sup* homozygotes. The degree of phenotypic suppression with *sup-5* and *sup-7* appears to be gene specific and most likely reflects gene function as opposed to the efficiency of restoration of gene product. Structural functions seem to be poorly suppressed, whereas putative enzymatic functions appear to be well suppressed (WATERSTON and BRENNER 1978; WATERSTON, submitted for publication). Since gene products that function catalytically require relatively lower quantities of activity to restore normal phenotype than do products that function stoichiometrically (SNUSTAD 1968), it may be that the products of *lin-2* and *lin-7* function catalytically, whereas the product of *unc-85* functions stoichiometrically. Stoichiometric functions are likely to be structural and hence present at high concentrations; they are reasonable candidates for which to seek polypeptide products. The availability of alleles likely to produce no (or little) gene product, identified by their suppressibility with *sup-5* and/or *sup-7*, should be particularly useful in such attempts.

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