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Lethal mutations defining 112 complementation groups in a 4.5 Mb sequenced region of *Caenorhabditis elegans* chromosome III

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Abstract The central gene cluster of chromosome III was one of the first regions to be sequenced by the Caenorhabditis elegans genome project. We have performed an essential gene analysis on the left part of this cluster, in the region around *dpy-17111* balanced by the duplication sDp3. We isolated 151 essential gene mutations and characterized them with regard to their arrest stages. To facilitate positioning of these mutations, we generated six new deficiencies that, together with preexisting chromosomal rearrangements, subdivide the region into 14 zones. The 151 mutations were mapped into these zones. They define 112 genes, of which 110 were previously unidentified. Thirteen of the zones have been anchored to the physical sequence by polymerase chain reaction deficiency mapping. Of the 112 essential genes mapped, 105 are within these 13 zones. They span 4.2 Mb of nucleotide sequence. From the nucleotide sequence data, 920 genes are predicted. From a Poisson distribution of our mutations, we predict that 234 of the genes will be essential genes. Thus, the 105 genes constitute 45% of the estimated number of essential genes in the physically defined zones and between 2 and 5% of all essential genes in C. elegans.

Key words Caenorhabditis elegans  $\cdot$ Essential genes  $\cdot sDp3 \cdot$  Genome organization

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## Introduction

Understanding the mechanism by which contiguous blocks of genes contribute to the development and maintenance of an organism requires knowledge of the identity, organization and function of genes within the genome. Toward this goal, the genomes of a number of genetically amenable organisms are being sequenced in their entirety. The first metazoan to be completely sequenced will be the nematode, Caenorhabditis elegans. The present estimated completion date is 1998 (Wilson et al. 1994; Waterston and Sulston 1995). To date, almost all of the 100 Mb genome has been sequenced and 16400 protein-encoding genes have been predicted from sequence data (Waterston et al. 1997; S. J. M. Jones, personal communication). While sequence data may allow for the identification of many of the genes, the sequence alone is not enough to determine their biological relevance. Approximately 40% of the predicted genes share significant sequence similarities with genes of known function in other organisms. Such comparative sequence analysis provides useful information but does not provide a complete understanding of how the information encoded in sequence data correlates with biological function. In addition, the remaining 60% of predicted genes either share no significant similarity with genes in the database or have similarity to genes of unknown function. To understand fully how the information encoded in the nucleotide sequence contributes to the development and maintenance of an organism, functions must be attributed to these genes. The analysis of genetic mutations in genes positioned within sequenced regions supplies this functional information. The information is useful not only for understanding the functions of the genes in the C. elegans genome but also provides insight into the functions of homologous genes in other organisms.

Such functional analysis has already begun on the sequenced genome of *Saccharomyces cerevisiae* (Goffeau et al. 1996). The techniques that are employed in func-

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tional analysis in yeast are not available for the C. elegans genome as homologous recombination techniques have not been developed for use in *C. elegans*. However, many other techniques are available with which to perform functional analyses in C. elegans. One approach is the use of RNA interference to manipulate gene expression (Fire et al. 1998). This involves the microinjection of double-stranded RNA (dsRNA) complementary to the coding region of the gene to be studied. The dsRNA injection results in a decrease in production of the protein. However, this method does not always result in a detectable phenotype, and it is difficult to establish whether such negative results indicate that the gene, when interfered with, gives no obvious phenotype or the RNA interference method does not knockout the protein product of this gene. A second approach is mutational analysis, either random mutagenesis and subsequent positional cloning, or gene-selected mutagenesis. The selected mutagenesis of specific genes is often referred to as reverse genetics. Gene-selected mutagenesis employs the polymerase chain reaction (PCR) to identify deletions or Tc1 transposon insertions into a specific gene in DNA pooled from a library of mutagenized animals (Jansen et al. 1997). The PCR-based screening process allows the isolation of putative null mutants of specific genes. However, this method will likely be applied preferentially to genes that share sequence similarity with known genes. The random mutagenesis approach, on the other hand, does not rely on the identification of sequence homologies or similarities to known genes. The screen criterion determines what types of gene mutations will be isolated. In addition, random mutagenesis can identify novel, non-null mutations in genes. These mutations can provide functional information beyond that available if the function of the gene is eliminated.

To correlate the sequence data with biological function, we performed a mutagenesis screen for lethal mutations using the duplication *sDp3* as a balancer. Lethal mutations comprise the largest class of easily identified mutations in C. elegans. It is estimated that 25–33% of C. elegans genes are essential (Johnsen and Baillie 1997). Large collections of lethal mutations in essential genes exist for many regions of the C. elegans genome: the unc-22 region of chromosome IV (Rogalski et al. 1982; Rogalski and Baillie 1985; Clark et al. 1988), the dpy-10 to rol-5 region of chromosome II (Sigurdson et al. 1984), the left portion of chromosome V balanced by the translocation eT1 (III;V) (Rosenbluth et al. 1988; Johnsen and Baillie 1991), and the left portion of chromosome I balanced by the duplication *sDp2* (Howell and Rose 1990; McKim et al. 1992). In this paper we report the generation of 151 lethal mutations defining 112 essential genes, as well as six new deficiencies, within a 7 map unit (mu), 4.5 Mb region around dpy-17 on chromosome III. Only two of the genes have been previously identified genetically. The lethal mutations allow us to estimate the total number of essential genes in the C. elegans genome by extrapolation.

The *dpv-17* region was chosen for study because it was one of the first regions to be sequenced by the C. elegans Genome Sequencing Consortium and is now almost entirely sequenced. According to the 1997 C. elegans Genetic Map (Hodgkin et al. 1997), 75 genetic markers were available for the region, of which only 31 were correlated with the physical map. We have mapped the new lethal mutations to 14 distinct zones and have anchored 13 of these zones to the physical map using PCR deficiency mapping. This will facilitate the assignment of genetically identified genes to predicted gene sequences. Analyses of the mutant phenotypes will then assist in correlating biological function with sequence data. The mutational identification and physical positioning of 112 genes reported here is a significant contribution toward such a correlation in the 4.5 Mb region around dpy-17.

## Materials and methods

Nematode strains and culture conditions

Nematodes were maintained and mated on petri plates containing Nematode Growth Medium, seeded with *Escherichia coli* OP50 (Brenner 1974). Nomenclature follows the uniform system adopted for *C. elegans* (Horvitz et al. 1979). The wild-type N2 strain var. Bristol and the mutant strains used were obtained from our own laboratory or through the *C. elegans* Genetic Stock Center unless otherwise stated. We define a recessive lethal mutation as one that when homozygous prevents the worm from developing into a fertile adult producing viable progeny [lethals, steriles, maternal-effect lethals (Mels)]. Maternal-effect lethal mutations are those that have no obvious effect on the homozygote, but that result in the progeny of the homozygote exhibiting a lethal phenotype. All experiments were carried out at 20° C. The duplications *sDp3* (Rosenbluth et al. 1985) and *sDp8* (Stewart et al. 1991) were obtained in previous studies.

## Isolation of lethal mutations

Our mutagenesis and screening protocol makes use of the fact that sDp3 covers dpy-17 but not *unc*-32 and that it does not recombine with the normal LGIII chromosome. Thus homozygous lethal mutations in the covered region can be maintained in strains without being lost by recombination, i.e., they are "balanced" by sDp3.

 $P_o$  dpy-17(e164) unc-32(e189)/dpy-17(e164) lin-12(n941)III; sDp3(III;f) hermaphrodites were phenotypically wild type since sDp3 covers dpy-17. They were exposed to one of the following mutagen treatments. For ethyl methanesulfonate (EMS) mutagenesis, worms were suspended in 0.018 M EMS in M9 buffer for 4 h [as by Brenner (1974), but with a lower EMS concentration]. For ultraviolet (UV) irradiation mutagenesis, worms were irradiated with 110 J/m<sup>2</sup> UV irradiation in uncovered petri plates, from a distance of 43 cm using a 30 W Germacidal lamp (254 nm) preequilibrated for 15 min (Stewart et al. 1991). For gamma-ray mutagenesis, worms were irradiated with 2000 R, using a Cobalt-60 source for 8 min 26 s. In some cases of EMS mutagenesis, the dpy-17 unc-32 marked chromosome included the mutation ncl-1(e1865) between the two markers.

After exposure to a mutagen, the worms were allowed to recover for 1–2 h before picking young gravid hermaphrodites (one per plate) and allowing these to lay eggs for 24 h. They were then transferred to fresh plates and after another 24 h the  $P_o$  worms were removed. The recovery period was used to ensure that the in utero embryos present during mutagen treatment were expelled, and not included in the F1 samples. For screening, wild-type F1s were individually allowed to selffertilize. If no lethal mutation had been induced on the *dpy-17 unc-32* marked chromosome, they should have produced fertile Dpy-17 Unc-32s among their F2 progeny (i.e., *dpy-17 unc-32* homozygotes not carrying *sDp3*). Plates on which no more than two gravid Dpy Unc F2s were detected were retained, and Unc-32s were picked to fresh plates (i.e., *dpy-17 unc-32; sDp3*, with a putative lethal mutation on the marked chromosome). The presence of a lethal mutation was established if these failed to produce any fertile Dpy Uncs. These lethal mutations ("lethals") were presumed to be within the region covered by *sDp3*. They were maintained as homozygotes with *sDp3*, by picking Unc-32s.

Lethals to the left of dpy-17 could have crossed off in the F1 heterozygote to produce some gravid Dpy Unc F2s (*let-x dpy-17 unc-32*/+ *dpy-17 unc-32*). Thus, those mutations that were more than 4 mu to the left were not expected to be recovered.

#### Positioning lethal mutations relative to dpy-17

Lethal-bearing Unc-32 hermaphrodites were crossed to wild-type (N2) males and the F2 progeny from wild-type F1 hermaphrodites were scored. Recovery of fertile Dpy non-Unc recombinants indicated the lethal was to the right of dpy-17, while fertile Dpy Unc recombinants indicated it was to the left.

#### Complementation tests

Tests between lethal mutations "sx" and "sy" isolated in this study were performed by first crossing Unc-32 hermaphrodites from the stock plates carrying lethal sx to N2 males. Phenotypically wildtype F1 males were then crossed to Unc-32s carrying lethal sy. The presence or absence of fertile Dpy Unc F2 hermaphrodites established either complementation or failure to complement, respectively. Other appropriate protocols were devised to test our lethals for complementation with mup-4(ar60) (Gatewood and Bucher 1997) and let-972(ar65) (both received from E. A. Bucher), visible morphological mutations and nDf20, as well as for coverage by sDp8, since these were in a variety of genetic backgrounds.

If two mutations isolated in this study complemented, the ratio of fertile Dpy Unc hermaphrodites/wild-type males was expected to be 0.5. However, the low viability of some of the deficiency heterozygotes often gave lower ratios even with EMS mutations mapping far away. Therefore establishing failure to complement required that there be no fertile Dpy Unc hermaphrodites in the presence of at least 30 wild-type males. On the other hand, in the case of lethals mapping to the left of *dpy-17*, crossing over could occur in the male heterozygote. Thus, + dpy-17 unc-32/+ + +sperm would give Dpy Unc hermaphrodite progeny even if the mutations failed to complement and appear as a false-positive complementation. Based on the recombination experiments with *dpy-17*, we calculated the ratio of fertile Dpy Unc hermaphrodites/ wild-type males expected, for a given mutation, simply due to recombination. Complementation, then, was recorded as positive only if the ratio of Dpy Uncs hermaphrodites/wild-type males was larger than this ratio.

### Identification of deficiencies

UV- and gamma ray-induced lethals failing to complement two or more mutations that complement each other, were defined as deficiencies (*sDf*) deleting at least two genes.

### Establishing zones and identifying essential genes

Positioning with respect to dpy-17, together with complementation tests, mapped the lethal mutations with respect to chromosomal rearrangements (*sDfs*, *nDf20* and *sDp8*). The rearrangement breakpoints, thus determined, were refined relative to the physical

map by PCR mapping (see below). This, as well as left-right positioning with respect to dpy-17, was used to divide the sDp3-covered region into separate zones (Fig. 1). Inter se complementation tests between lethals mapping to the same zone identified individual genes.

#### Phenotypic analysis of EMS-induced lethal mutations

Unless otherwise noted in Table 1, animals were staged as follows: adult parental animals (*let-x dpy-17 unc-32; sDp3* or *let-x dpy-17 unc-32/+* males  $\times$  *let-x dpy-17 unc-32; sDp3*) were placed on fresh plates. Three to four days later Dpy Unc animals were selected and prepared for observation under Nomarski optics as described by Sulston and Horvitz (1977). The animals were staged based on the extent of gonad development (Kimble and Hirsh 1979), and the presence or absence of cuticular alae, which are present in L1 and adult cuticle (Cox et al. 1981). The most mature animals were assumed to represent the mutant arrest stage.

#### PCR mapping of deficiency endpoints

As homozygotes, in the absence of sDp3, the sDf deficiencies are embryonic lethals. Fifteen hermaphrodites of the genotype dpy-17(e164) sDf x unc-32(e189); sDp3(III;f) were transferred to a seeded plate and allowed to lay eggs for 8 h. Adult hermaphrodites were removed and the plates kept at 20°C for 48 h to allow viable eggs (carrying sDp3) to hatch. Eggs that did not hatch were considered to be of the genotype dpy-17 sDf x unc-32. Template DNA was extracted from homozygous deficiency eggs according to Barstead et al. (1991) with the modifications of Williams et al. (1992). For each reaction one or two eggs were treated for 3 min with chitinase and transferred to worm lysis buffer (50 mM KCl, 10 mM TRIS-HCl, pH 8.2, 2.5 mM MgCl<sub>2</sub>, 0.45% NP-40, 0.45% Tween 20, 0.01% gelatin, 120 µg/ml Proteinase K). Eggs in lysis buffer were then digested at 65°C for 45 min and the enzyme inactivated at 95° C for 15 min. Thermo-cycling reactions were performed in an Idaho Technology model 1605 Air Thermo-cycler using "Low" (20 mM Mg<sup>2++</sup>) 10× reaction buffer obtained from Idaho Technologies. Taq polymerase was obtained from BioCan Scientific. Nucleotide triphosphates were obtained from Pharmacia. Thermal profiles for all PCRs were: 1 min at 94° C, followed by 30 cycles of 10 s at 94° C, 20 s at 53°-60° C, 40 s at 72° C, followed by a final extension of 2 min at 72°C. Ramp speed was set at 9 (fastest). Primers were designed based on cosmid sequences available from GenBank. All PCRs were run in duplicate with positive control primers. PCR products were run on 1% agarose gels. Experimental primers that produced no PCR product indicate that the sequence from which the primer pairs were designed is deleted by the deficiency.

### Results

Recovery and mapping of lethal mutations

From the UV- or gamma ray-treated  $P_o$  hermaphrodites we retained 36 lethal mutations. These were left-right positioned with respect to *dpy-17*. Only those that mapped tightly to *dpy-17* or to its right were retained for complementation testing with EMS lethals.

The screening of 10401 EMS-treated chromosomes for the presence of *sDp3*-balanced lethals yielded 151 mutations. Complementation tests with the retained UV and gamma-ray lethals identified six deficiencies: *sDfs 125, 127* and *130*, induced by UV irradiation; *sDfs 121, 128 and 135*, from the gamma-ray screen.



Fig. 1 Partial genetic and physical map of the dpy-17 region. Essential genes are clustered by zone. Genes are arbitrarily ordered within each zone by gene name. Cosmids shown are the outmost cosmids known to be deleted by the deficiency shown beneath. Scale is based on the physical positions of the cosmids (Pmap) as listed in the public database ACeDB (available by anonymous FTP from ncbi.nlm.nih.gov, directory repository/acedb). The deletion of daf-4 by sDf121 is based on personal communication from H. Honda and I. Mori. The positions of the other non-*let* genes as well as nDf20 are based on ACeDB

The EMS-induced lethal mutations were positioned with respect to 14 chromosomal zones determined by rearrangement breakpoints and left-right positioning with respect to dpy-17 (Fig. 1). Inter se tests within each zone then established that the 151 mutations represented 112 genes (Table 1). Of these, two had already been identified by others: mup-4 (Gatewood and Bucher 1997), and let-972 (E. A. Bucher, personal communication). Owing to the large number of lethal mutations generated, we did not perform extensive complementation tests against the 75 genetic markers previously identified. Therefore, it is possible that some of these lethal mutations may be allelic with existing lethal or visible mutations in this region. Thirty of the genes were hit multiple times in this study: twenty-two had two hits. seven had three, and one had five hits.

Distribution of essential genes along the chromosome

The left sDp3 breakpoint is between daf-7 and dpy-1, while the right-hand one is between mig-10 and dpy-19 (Fig. 1). The duplication covers about 17 mu to the left of dpy-17 and only 2 mu to its right.

Zone 14 is defined as the region balanced by sDp3and to the left of sDf130. The lethal gene closest to dpy-17 in this region is only approximately 2 mu away from dpy-17. Thus zone 14 comprises about 15 mu or 80% of the balanced recombination distance. However, only 5% of the genes for which we recovered EMS-induced mutations fall into this zone. Furthermore, none of them map more than 5 mu from *dpy-17*. That is, they lie within the right-most 3 mu of zone 14. Two reasons probably account for this. First, as pointed out in Materials and methods, lethals to the left of dpy-17 could have crossed off in the F1 heterozygotes during the screening protocol. Second, the already established genetic map shows a clustering of other genes in the dpy-17 to *unc-32* region, relative to the rest of the left arm of chromosome III (Hodgkin et al. 1997).

Ninety-five percent of the essential genes are distributed among zones 1-13 (Fig. 1). Genes in zones 11 and 12 are deleted by both *sDf121* and *sDf130*, but are

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7Early larval Early larval $te-713$ $2240$ Early larval Late larval $te-373$ $2323$ Steril Late larval $7$ Early larval Early larval $te-771$ $2325$ Early larval $te-337$ $te-337$ $23234$ Mid larval $te-337$ $7$ Early larval Early larval $te-771$ $23256$ Early larval $te-737$ $te-337$ $23234$ Mid larval $te-337$ $7$ Early larval Early larval $te-721$ $23256$ Early larval $te-737$ $te-337$ $23234$ Mid larval $te-337$ $7$ Early larval Early larval $te-721$ $23250$ Mid larval $te-737$ $te-337$ $23234$ Mid larval $te-731$ $8$ Mid larval Early larval $te-747$ $te-737$ $23260$ Mid larval $te-737$ $te-737$ $23261$ Mid larval $te-731$ $8$ Mid larval $te-747$ $te-737$ $23261$ Mid larval $te-737$ $te-737$ $2361$ Mid larval $te-731$ $8$ Mid larval $te-747$ $te-737$ $2360$ Mid larval $te-737$ $te-737$ $2361$ Mid larval $te-731$ $8$ Mid larval $te-747$ $te-737$ $2360$ Mid larval $te-737$ $te-737$ $2360$ Mid larval $te-731$ $8$ Mid larval $te-747$ $te-737$ $2360$ Mid larval $te-737$ $te-737$ $2360$ Mid larval $te-731$ $8$ Mid larval $te-747$ $te-737$ $te-737$ $2360$ Mid larval $te-737$ $te-737$ $2360$ <td>244/20 8244</td> <td>S244</td> <td>0 4</td> <td>Late larval</td> <td>9</td> <td>let-755</td> <td>\$2600 -2575</td> <td>Mid larval</td> <td></td> <td>let-826</td> <td>11828</td> <td>Sterile<sup>c</sup></td>	244/20 8244	S244	0 4	Late larval	9	let-755	\$2600 -2575	Mid larval		let-826	11828	Sterile <sup>c</sup>
$C_{12}$ Sterife         7 $(e_{7}7)$ $(246)$ Late larval $(e_{7}32)$ $(262)$ $(223)$ $(233)$ Late larval $(e_{7}33)$ $(233)$ Late larval $(e_{7}3)$ $(230)$ Mid larval $(e_{7}3)$ $(230)$ $(e_{7}3)$ $(230)$ $(e_{7}3)$ $(230)$ $(110)$ $(100)$ $(100)$ $(100)$ $(100)$ $(100)$ $(100)$ $(100)$ $(100)$ $(100)$ $(100)$ $(100)$ $(100)$ $(100)$ $(1$	$\frac{1}{2}$	975 975	<u> </u>	Early larval Early larval		C0/-121	82570 82630	Early larval		let-827	82820 82827	Sterile <sup>c</sup>
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4.1         Early larval $2332$ Late larval $e_{1-73}$ $2303$ Maternal ffeed           3.6         Early larval $e_{1-73}$ $2303$ Maternal $e_{1-73}$ $2303$ Maternal           3.6         Early larval $e_{1-73}$ $2303$ Maternal $e_{1-73}$ $2303$ Maternal           3.6         Early larval $e_{1-73}$ $2303$ Early larval $e_{1-73}$ $2303$ $e_{1-73}$ $a_{1-73}$	21-844 s25	s25	.67	Early larval <sup>c</sup>		let-721	s2447	Maternal effect <sup>c</sup>	12	let-732	s2611	Early larval <sup>c</sup>
8.3         Early larval $ler.725$ $2.2454$ Sterile $ler.733$ $2.467$ Sterile           8.6         Early larval $ler.733$ $2.467$ Sterile         Sterile           8.6         Early larval $ler.733$ $2.3621$ Mid larval         Sterile           8.6         Early larval $ler.733$ $2.2621$ Mid larval         Late larval           8.6         Early larval $ler.733$ $2.363$ Mid larval $ler.732$ $2.3292$ Early larval           2.7         Mid larval $ler.736$ $2.3806$ Early larval $ler.836$ $2.3033$ Late larval           2.8         Mid larval $ler.736$ $2.3876$ Mid larval $ler.736$ $2.3876$ Mid larval           3.8         Early larval $ler.736$ $2.3876$ Mid larval $ler.736$ $2.3777$ Mid larval           4.7         Early larval $ler.715$ $2.3677$ Mid larval $ler.716$ $2.3777$ Mid larval           3.8         Mid larval $ler.716$ $2.3275$ Mid lar	21-972 s24	s24	41	Early larval			s2812	Late larval <sup>c</sup>			s2808	Maternal effect <sup>c</sup>
4.8         Early larval $2260$ Mid larval $22621$ Mid larval           5.8         Fairly larval $er.728$ $22757$ Mid larval $er.761$ $22621$ Mid larval           6         Early larval $er.728$ $22075$ Mid larval $er.823$ $22797$ Early larval           2.3         Mid larval $er.756$ $22013$ Early larval $er.823$ $22797$ Early larval           3.3         Late larval $er.756$ $22061$ Early larval $er.823$ $22797$ Early larval           3.3         Early larval $er.736$ $22866$ Early larval $er.828$ $22877$ Mid larval           3.4         Early larval $er.817$ $22876$ Mid larval $er.730$ $22777$ Mid larval           3.4         Early larval $er.747$ $22876$ Mid larval $er.730$ $22777$ Mid larval           3.4         Early larval $er.817$ $22873$ Mid larval $er.730$ $22777$ Mid larval           3.4	s25	s25	83	Early larval		let-725	s2454	Sterile		let-733	s2467	Sterile <sup>c</sup>
50         Early larval $ler.728$ $s.2573$ Mid larval $ler.761$ $s.2661$ Mid larval           7.6         Early larval $ler.776$ $s.2733$ Early larval $ler.761$ $s.261$ Early larval           7.7         Mid larval $ler.776$ $s.2613$ Early larval $ler.782$ $s.2793$ Early larval           6.7         Late larval $ler.767$ $s.2603$ Early larval $ler.836$ $s.2903$ Early larval           6.7         Late larval $ler.767$ $s.2613$ Early larval $ler.836$ $s.2793$ Early larval           6.7 $s.2615$ Early larval $ler.700$ $s.2777$ Mid larval           8.1         Early larval $ler.714$ $s.2279$ Early larval $ler.700$ $s.2777$ Mid larval           9.1         Early larval $ler.716$ $s.2373$ Mid larval $ler.738$ $s.2435$ Early larval           9.1         Early larval $ler.718$ $s.2373$ Mid larval $ler.738$ $s.2435$ Early larval	24-722 s24	<i>s</i> 24	48	Early larval			s2800	Mid larval <sup>c</sup>			s2621	Mid larval
88Mid larval $ler-747$ $2.2456$ Mid larval $ler-732$ $2.5791$ Late larval10.6Early larval* $ler-756$ $2.207$ Early larval* $ler-732$ $2.2927$ Early larval*22.2Mid larval $ler-756$ $2.2064$ Late larval* $ler-836$ $2.2927$ Early larval*23.8Late larval* $ler-757$ $s.2809$ Early larval* $ler-836$ $s.2803$ Late larval*23.8Late larval* $ler-876$ $s.2819$ Mid larval $ler-836$ $s.2803$ Late larval*23.8Mid larval $ler-816$ $s.2809$ Early larval* $ler-836$ $s.2803$ Late larval*33.0Early larval* $ler-814$ $s.2829$ Mid larval $ler-836$ $s.2803$ Late larval*33.0Early larval* $ler-774$ $s.2823$ Mid larval* $ler-836$ $s.2803$ Late larval*33.1Early larval* $ler-817$ $s.2823$ Mid larval* $ler-736$ $s.2773$ Mid larval*33.1Early larval* $ler-716$ $s.2372$ Mid larval* $ler-736$ $s.2373$ Mid larval*33.1Mid larval* $ler-716$ $s.2833$ Early larval* $ler-736$ $s.2373$ Mid larval*33.1Mid larval* $ler-716$ $s.2373$ Mid larval* $ler-736$ $s.2373$ Mid larval*33.2Mid larval* $ler-716$ $s.2373$ Sarral* $ler-736$ $s.2373$ Mid larval*33.3Mid larval* $le$	s24	<i>s</i> 24	150	Early larval		let-728	s2573	Mid larval		let-761	s2461	Mid larval
320       Early larval <sup>6</sup> $ce-823$ $s2792$ Early larval <sup>6</sup> $322$ Mid larval <sup>6</sup> $ce-328$ $s2792$ Early larval <sup>6</sup> $63$ Late larval <sup>6</sup> $ce-328$ $s2792$ Early larval <sup>6</sup> $63$ Late larval <sup>6</sup> $ce-366$ $s2809$ Early larval <sup>6</sup> $ce-336$ $s2809$ Early larval <sup>6</sup> $63$ Late larval <sup>6</sup> $ce-756$ $s2809$ Early larval <sup>6</sup> $ce-336$ $s2909$ Early larval <sup>6</sup> $530$ Early larval <sup>6</sup> $ce-836$ $s2879$ Mid larval $ce-710$ $s2377$ Mid larval $530$ Early larval <sup>6</sup> $ce-817$ $s2829$ Mid larval $ce-710$ $s2577$ Mid larval $530$ Early larval <sup>6</sup> $ce-710$ $s2377$ Mid larval $ce-710$ $s2577$ Mid larval $510$ Early larval <sup>6</sup> $ce-710$ $s2379$ Mid larval $ce-730$ $s2677$ Mid larval $510$ Early larval <sup>6</sup> $ce-730$ $s2379$ $carly larval6       ce-730 s2377       Mid larval         510       Early lar$	<u>s</u> 2:	s2:	588	Mid larval		let-747	s2456	Mid larval		let-782	s2591	Late larval
842       Early larval <sup>6</sup> $ler.756$ $s.503$ Early larval <sup>6</sup> $ler.328$ $s.2797$ Early larval <sup>6</sup> 6.22       Mid larval $ler.756$ $s.2809$ Early larval <sup>6</sup> $ler.386$ $s.2803$ Late larval <sup>6</sup> 6.35       Late larval $ler.774$ $s.2819$ Mid larval $ler.346$ $s.2478$ Mid larval         5.81       Early larval $ler.774$ $s.2819$ Mid larval $ler.346$ $s.2371$ Mid larval         5.80       Early larval $ler.774$ $s.2829$ Early larval <sup>6</sup> $ler.710$ $s.2478$ Mid larval         5.80       Early larval <sup>6</sup> $ler.774$ $s.2829$ Mid larval <sup>6</sup> $ler.710$ $s.2577$ Mid larval         5.80       Early larval <sup>6</sup> $ler.714$ $s.2823$ Mid larval <sup>6</sup> $ler.710$ $s.2577$ Mid larval         5.81       Sterile $ler.716$ $s.2327$ $s.2835$ Early larval <sup>6</sup> $ler.7736$ $s.2435$ Early larval <sup>7</sup> 5.81       Sterile $ler.716$ $s.2337$ $s.2435$ Early larval <sup>7</sup> $ler.736$ $s.2332$ Mid larval <sup>7</sup>	s2	$S_{S}$	816	Early larval <sup>c</sup>			s2807	Early larval <sup>c</sup>		let-823	s2792	Early larval <sup>c</sup>
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463       Late larval $ler-767$ $s2464$ Late larval $s2846$ Early larval         558       Early larval $ler-707$ $s2819$ Mid larval $ler-700$ $s2378$ Mid larval         580       Early larval $ler-714$ $s2819$ Mid larval $ler-710$ $s2377$ Mid larval         830       Early larval $ler-714$ $s2829$ Mid larval $ler-710$ $s2577$ Mid larval         830       Early larval $ler-716$ $s2823$ Mid larval $ler-716$ $s2577$ Mid larval         831       Early larval $ler-716$ $s2832$ Mid larval $ler-736$ $s2577$ Mid larval         831       Early larval $ler-716$ $s2373$ Early larval $ler-736$ $s2638$ Early larval         831       Sterile $ler-736$ $s2373$ Early larval $ler-736$ $s2372$ Mid larval         832       Mid larval $ler-736$ $s2373$ Early larval $ler-736$ $s2475$ Mid larval         834       Early larval $ler-736$ $s2332$ Early larval <td>2t-754 S2</td> <td>S.</td> <td>222</td> <td>Mid larval</td> <td></td> <td></td> <td>s2809</td> <td>Early larval<sup>a</sup></td> <td></td> <td>let-836</td> <td>s2803</td> <td>Late larval<sup>c</sup></td>	2t-754 S2	S.	222	Mid larval			s2809	Early larval <sup>a</sup>		let-836	s2803	Late larval <sup>c</sup>
436       Late larval $2373$ Mid larval $13$ $ler770$ $52773$ Mid larval         530       Early larval $ler774$ $52015$ Early larval $s2571$ Mid larval         530       Early larval $ler774$ $52015$ Early larval $ler713$ $5277$ Mid larval         509       Early larval $ler715$ $5273$ Mid larval $s2573$ Mid larval         509       Early larval $ler715$ $5273$ Mid larval $ler715$ $5277$ Mid larval         501       Early larval $ler715$ $52735$ Mid larval $ler775$ $5590$ Late larval         502       Early larval $ler775$ $52335$ Early larval $ler775$ $52590$ Late larval         501       Early larval $ler775$ $52335$ Early larval $ler775$ $52590$ Late larval         502       Early larval $ler773$ $52347$ Early larval $ler737$ $52375$ Mid larval         502       Early larval $ler770$ $52324$ Early larval $ler773$ $52475$	21-766 S2	S,	:463	Late larval		let-767	s2464	Late larval <sup>c</sup>		let-840	s2846	Early larval <sup>c</sup>
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8/4Sterile* $ler-8/8$ $s.2835$ Early larval* $s.2835$ Mid larval* $s.2832$ Mid larval*85/1Early larval*9 $ler-718$ $s.2445$ Early larval* $ler-737$ $s.2332$ Mid larval*433Mid larval* $ler-797$ $s.2745$ Early larval* $ler-738$ $s.2475$ Embryo*574Embryo* $ler-797$ $s.2824$ Early larval* $ler-738$ $s.2475$ Early larval*574Embryo* $ler-797$ $s.28350$ Embryo* $ler-739$ $s.2593$ Early larval*592Late larval* $ler-798$ $s.28350$ Early larval* $ler-763$ $s.2580$ Early larval*592Late larval* $ler-799$ $s.28356$ Early larval* $ler-796$ $s.2580$ Early larval*592Late larval* $ler-719$ $s.28356$ Early larval* $ler-796$ $s.2352$ Mid larval*592Late larval* $ler-719$ $s.28356$ Early larval* $ler-796$ $s.23794$ Late larval*583Early larval* $ler-812$ $s.2475$ Sterile $ler-812$ $s.2794$ Late larval*628Late larval* $ler-719$ $s.23580$ Early larval* $ler-812$ $s.2801$ Late larval*587Early larval* $ler-812$ $s.2805$ Late larval* $ler-812$ $s.2845$ Sterile*628Early larval* $ler-825$ $s.2806$ Late larval* $ler-820$ $s.2845$ Sterile*633Ea	21-821 S2	$S_{2}$	804	Early larval <sup>c</sup>		let-817	s2823	Mid larval <sup>c</sup>			s2590	Late larval
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2440Embryo <sup>c</sup> $let-797$ $s2824$ Early larval $let-739$ $s2593$ Early larval $574$ Embryo <sup>c</sup> $let-798$ $s2870$ Embryo <sup>c</sup> $let-763$ $s2593$ Early larval $574$ Embryo <sup>c</sup> $let-798$ $s2870$ Embryo <sup>c</sup> $let-763$ $s2475$ Early larval $592$ Late larval $let-799$ $s2870$ Early larval $let-763$ $s2579$ Early larval $592$ Late larval $let-799$ $s2836$ Early larval $let-796$ $s2822$ Mid larval $528$ Late larval $let-810$ $s2836$ Early larval $let-796$ $s2822$ Mid larval $528$ Early larval $let-796$ $s2822$ Mid larval $let-812$ $s2801$ Late larval $813$ Early larval $let-727$ $s2455$ Sterile $let-812$ $s2843$ Early larval $813$ Early larval $let-810$ $s2589$ Early larval $let-810$ $s2845$ Sterile $813$ Early larval $let-727$ $s22602$ Late larval $let-822$ $s2806$ Late larval $8747$ Mid larval $let-825$ $s2806$ Late larval $let-820$ $s2845$ Mid larval $5577$ Early larval $let-822$ $s2806$ Late larval $let-822$ $s2845$ Mid larval $5577$ Early larval $let-822$ $s2805$ Late larval $let-822$ $s2845$ Mid larval $5577$ Early larval $let-822$ $s2817$	1up-4 S2	S.	2433	Mid larval		let-780	s2576	Sterile		let-738	s2435	$Embryo^{c}$
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482Sterile $let-799$ $s2852$ Early larvale $s2580$ Early larval592Late larvale $let-809$ $s2844$ Early larvale $s2580$ Early larvale528Late larvale $let-809$ $s2844$ Early larvale $let-796$ $s2322$ Mid larvale628Late larvale $let-810$ $s2836$ Early larvale $let-812$ $s2822$ Mid larvale628Late larvale $let-810$ $s2836$ Early larvale $let-812$ $s2801$ Late larvale433Early larvale $let-810$ $s2836$ Early larvale $let-812$ $s2801$ Late larvale813Early larvale $let-727$ $s2589$ Early larval $let-812$ $s2843$ Early larvale813Early larval $let-812$ $s2806$ Late larval $let-819$ $s2845$ Sterile813Early larval $let-825$ $s2806$ Late larval $let-820$ $s2845$ Sterile877Mid larval $let-822$ $s2806$ Late larval $let-820$ $s2845$ Mid larval877Farly larval $let-823$ $s2806$ Late larval $met-32$ $s2805$ Maternal effect	s2	$S_{S}$	574	Embryo <sup>c</sup>		let-798	s2850	Embryo <sup>°</sup>		let-763	s2475	Early larval
592       Late larval <sup>c</sup> let-809 $s2844$ Early larval <sup>c</sup> let-796 $s2822$ Mid larval <sup>c</sup> 628       Late larval       let-810 $s2836$ Early larval <sup>c</sup> let-812 $s2822$ Mid larval <sup>c</sup> 628       Late larval       let-810 $s2836$ Early larval <sup>c</sup> let-812 $s2801$ Late larval <sup>c</sup> 433       Early larval <sup>c</sup> let-719 $s2455$ Sterile       let-813 $s2801$ Late larval <sup>c</sup> 813       Early larval <sup>c</sup> let-727 $s2589$ Early larval <sup>c</sup> let-813 $s2801$ Late larval <sup>c</sup> 474       Mid larval       let-825 $s2806$ Late larval <sup>c</sup> let-820 $s2843$ Sterile <sup>c</sup> 587       Farlv larval       let-822 $s2806$ Late larval <sup>c</sup> let-822 $s2845$ Mid larval <sup>c</sup> 587       Farlv larval       let-822 $s2805$ Late larval <sup>c</sup> let-822 $s2845$ Mid larval <sup>c</sup> 587       Farlv larval       let-822 $s2805$ larval <sup>c</sup> let-822 $s2845$ Mid larval <sup>c</sup>	21-768 S2	$S_{2}$	482	Sterile		let-799	s2852	Early larval <sup>c</sup>			s2580	Early larval
628       Late larval $let-810$ $s2836$ Early larval <sup>c</sup> $let-812$ $s2794$ Late larval <sup>c</sup> 483       Early larval <sup>c</sup> 11 $let-719$ $s2455$ Sterile $let-813$ $s2801$ Late larval <sup>c</sup> 813       Early larval <sup>c</sup> $let-719$ $s2455$ Sterile $let-813$ $s2801$ Late larval <sup>c</sup> 813       Early larval <sup>c</sup> $let-812$ $s2801$ Late larval <sup>c</sup> $let-819$ $s2843$ Early larval <sup>c</sup> 474       Mid larval $let-825$ $s2806$ Late larval <sup>c</sup> $let-820$ $s2845$ Sterile <sup>c</sup> 587       Farb larval $let-822$ $s2806$ Late larval <sup>c</sup> $let-822$ $s2845$ Mid larval <sup>c</sup> 587       Farb larval $let-822$ $s2817$ Late larval <sup>c</sup> $let-822$ $s2845$ Mid larval <sup>c</sup>	<u>s2</u>	$S_{2}$	592	Late larval <sup>c</sup>		let-809	s2844	Early larval <sup>c</sup>		let-796	s2822	Mid larval <sup>c</sup>
#83Early larval11 $ler-719$ $s2455$ Sterile $ler-813$ $s2801$ Late larval813Early larval $ler-819$ $s2843$ Early larval813Early larval $ler-819$ $s2843$ Early larval813Early larval $ler-825$ $s2602$ Late larval813Early larval $ler-820$ $s2843$ Early larval814Early larval $ler-825$ $s2806$ Late larval815Early larval $ler-825$ $s2806$ Late larval816 $ler-822$ $s2848$ Mid larval827Early larval $ler-822$ $s2848$ 837Early larval $ler-822$ $s2848$ 847Mid larval $ler-822$ $s2848$ 847Mid larval $ler-822$ $s2848$ 848Mid larval $ler-822$ $s2848$ 849Mid larval $ler-822$ $s2848$ 840Iarval $ler-822$ $s2848$ 841Iarval $ler-822$ $s2845$ 842Early larval $ler-822$ $s2845$ 843Early larval $ler-822$ $s2845$ 844Early larval $ler-822$ $s2845$ 845Early larval $ler-822$ $s2845$ 844Early larval $ler-822$ $s2845$ 844Early larval $ler-822$ $s2845$ 844Early larval $ler-822$ $s2845$ 845Early larval $ler-822$ $s2845$ 846Early larva	820	\$20	528	Late larval		let-810	\$2836	Early larval <sup>c</sup>		let-812	s2794	Late larval <sup>c</sup>
813Early larval $lef-727$ $s2589$ Early larval $lef-819$ $s2843$ Early larval73Early larval $lef-825$ $s2602$ Late larval $lef-820$ $s2845$ Sterile <sup>c</sup> 774Mid larval $lef-825$ $s2806$ Late larval <sup>c</sup> $lef-822$ $s2848$ Mid larval <sup>c</sup> 87Farly larval $lef-832$ $s2817$ Late larval <sup>c</sup> $mel-33$ $s2805$ Maternal effect <sup>c</sup>	st-702 \$24	\$24	183	Early larval <sup>c</sup>	11	let-719	s2455	Sterile		let-813	\$2801	Late larval <sup>c</sup>
7.3Early larval $ler-784$ $s2602$ Late larval $ler-820$ $s2845$ Sterile <sup>c</sup> 7.4Mid larval $ler-825$ $s2806$ Late larval <sup>c</sup> $ler-822$ $s2848$ Mid larval <sup>c</sup> 87Farlv larval $ler-832$ $s2817$ Late larval <sup>c</sup> $mel-33$ $s2805$ Maternal effect <sup>c</sup>		s28	13	Early larval <sup>c</sup>		let-727	\$2589	Early larval		let-819	s2843	Early larval <sup>c</sup>
74     Mid larval     ler-825     s2806     Late larval     ler-822     s3848     Mid larval       87     Early larval     ler-832     s2817     Late larval     mel-33     s2805     Maternal effect'	vt-711 \$24	47S	73	Early larval		let-784	\$2602	Late larval		let-820	s2845	Sterile <sup>c</sup>
7 Early larval <i>lef-832 s2817</i> Late larval <i>mel-33 s2805</i> Maternal effect	824	74 C S	74	Mid Jarval		let-825	s2806	Late larval <sup>c</sup>		let-822	s 2848	Mid larval <sup>c</sup>
	23	5	587	Early larval		let-832	\$2817	Late larval <sup>c</sup>		mel-33	s2805	Maternal effect <sup>c</sup>

**Table 1** Ethyl methane sulfonate induced lethal mutations recovered in the *dpv-17 III* region of *Caenorhabditis elegans* 

Early larval Sterile Mid larval Late larval <sup>c</sup> Mid larval Sterile <sup>c</sup> Early larval <sup>c</sup>
s2480 s2607 s2595 s2603 s2603 s2853 s2853
let-706 let-758 let-759 let-776 let-791 let-831
14
Early larval Early larval Early larval Early larval Early larval Early larval Early larval Early larval
\$2462 \$2479 \$2837 \$2876 \$2810 \$2810 \$2810 \$2614 \$2614
11/12 let-707 let-734 let-741 let-753 let-753
Sterile Sterile Early larval Early larval Mid larval Early larval Late larval
\$2465 \$2481 \$2582 \$2471 \$2443 \$2443 \$2443 \$2432
let-714 let-717 let-754 let-769 let-769

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Refer to Fig. 1 for the location of each zone on the map Worms were characterized as described in Materials and methods unless otherwise noted

Characterized by observation under dissecting microscope after 3-4 days Worms are very slow developers

distinguished from each other by virtue of their position with respect to dpy-17. Sixteen genes were not separated from *dpy-17* by recombination mapping (Fig. 1).

None of the genes in this study maps to zones 8 or 10. Physical markers define both zones. In the case of zone 8, the left-hand breakpoints of sDf125 and sDf127 are distinguishable. Three primer pairs were tested from C23G10. sDf127 deletes the two sets of right-hand primers but not the left-hand primer pair, while sDf125 deletes all three primer pairs. Neither *sDf125* nor *sDf127* deletes the primer pair from F42A10. In the case of zone 10, the primer pair from cosmid C05D2 distinguishes the right breakpoints of sDf121 and sDf130. The set of primers is deleted by *sDf121* but not by *sDf130*.

Genes in zones 2 and 9 are to the right of dpy-17, but are not deleted by any of the deficiencies used in this study. Those in zone 2 are covered by sDp8, while those in zone 9 are not. This may imply that there are haploinsufficient loci in these zones. However, nDf17 deletes these regions and is viable as a heterozygote (Ellis et al. 1991). Assuming *nDf17* is not discontinuous, this suggests that there are no haplo-insufficient loci in the two zones.

# PCR deficiency endpoint mapping

The region in which mutations were recovered spans approximately 4.5 Mb of sequence. PCR deficiency mapping was used to anchor the genetically defined zones to the physical sequence map. All 14 zones were assigned physical breakpoints, with the closest cosmids from which primers were designed acting as physical markers. The data are tabulated in Table 2. For each sDf the cosmids at the ends are listed. "Out" refers to the primer sequence being present in the homozygous sDf embryo. "In" means that the sequence is deleted.

# Phenotypes

The developmental arrest stages of the homozygous EMS-induced lethal mutations included embryos, larvae, sterile adults, as well as some cases of Mel (Table 1). The larval lethals are the most common with 76% of the lethals recovered arresting as larvae. Generally, different alleles of the same gene arrest at approximately the same stage. In a few cases different alleles arrest at significantly different stages, e.g., let-722 (zone 1A), let-714 (zone 5) and let-732 (zone 12). The distribution of the earliest arrest stages of the genes is displayed in Fig. 2.

# Discussion

We have recovered a set of 151 EMS-induced recessive lethal mutations under the balancer sDp3. These mutations identify 112 essential genes in a 7 mu, 4.5 Mb region to the left of unc-32III. Of these genes, only two

Deficiency	Left		Right		
	Out <sup>a</sup> cosmid	In <sup>b</sup> cosmid	Out cosmid	In cosmid	
sDf130	C32A3 3416-4060	R13G10 4233-5506	C05D2 30876-31406	ZC155 1227-1792	
sDf121	F10G11 891-1513	F35G12 35188-35567	F54E7 6725-7529	C05D2 30876-31406	
sDf127	C23G10a 1778-2723	C23G10b 2626-3117	R13A5 <sup>c</sup>	T20B12 6500-6838	
sDf125	F42A10 18575-19251	C23G10a 1778-2723	K04C2 3805-4495	R13F6 4747-5611	
sDf135	B0361 10625-11306	T20B12 6500-6838	R13A5 <sup>c</sup>	T20B12 6500-6838	
sDf128	C29E4 11250-12030	C06G4 4540-5157	F44B9 7092-7950	C06G4 4540-5157	

Table 2 Polymerase chain reaction (PCR) deficiency mapping data. Numbers below cosmids correspond to base pair positions on Genbank cosmid entry

<sup>a</sup> "Out": the PCR site is not deleted by the deficiency

<sup>b</sup> "In": the site is deleted

<sup>c</sup> No Genbank entry. Primer sequence R13-F (5'-TTTCGGATAGGGGTCAGA-3'), R13-R(5'-GGTTTTCATTTCGTTCAT-3')

were previously identified. Assuming that our region is representative of the entire C. elegans genome and that all essential genes are equally mutable, it is possible to estimate the total number of essential genes in C. elegans from our data. We predict from a Poisson distribution of our mutations in the region spanning zones 1-13, the zones with distinct physical boundaries, that this region contains a minimum of 234 essential genes. As all genes are not equally mutable this number is at best a rough estimate. The sequence data predict a total of 920 genes in the region. This indicates that at least 25% of these genes are essential under laboratory conditions. Extrapolating this value to the 16400 genes predicted in C. elegans, we estimate that there are approximately 4200 essential genes in the C. elegans genome. This number is not in accordance with the 2850 predicted by Johnsen and Baillie (1991), and is considerably higher than the 3300 estimated by Howell and Rose (1990) and the 3500 estimated by Clark (1990). Several factors may explain why our predicted number is much higher. It is possible that this region is not representative of the rest of the C. elegans genome or is dissimilar to the regions studied in other experiments. Genes (both those predicted



Fig. 2 Histogram of the number of genes whose most severe alleles block development at the indicated developmental stages. The stages are egg lethal, early, mid, late larval lethal, sterile adults and maternal-effect lethal (*Mel*)

by sequencing as well as genetically identified genes) are not distributed evenly across the chromosomes. There is an obvious clustering of genes in the central regions of the chromosomes and fewer genes on the arms. Such positional effects may therefore lead to different gene estimates when based on data from different regions.

The distribution of essential genes across the *dpy-17* region is relatively uniform. Most zones have a comparable number of essential gene hits per unit of physical distance. This is due in part to the chromosomal position of the region studied. Almost all of the 14 zones are within the central cluster of chromosome III as defined by Barnes et al. (1995). The authors denoted pal-1 as the left-most marker within the cluster; *pal-1* falls within zone 12. Therefore all essential genes to the right of pal-1 are in the gene-rich cluster. This explains the scarcity of essential genes in zone 14 in addition to the fact that they may have been lost during the screening process. Zone 13, although not in the cluster, has approximately the same essential gene density as those zones within the cluster. This is most likely due to the fact that the cluster boundary is gradual (Barnes et al. 1995). Interestingly, the essential gene density is greatest in zones 11 and 12, while immediately to the right, no essential genes were identified in zone 10, which is of a comparable size. Whether this is statistically significant has yet to be determined.

All developmental block stages are represented in our study but most of the essential gene mutations block as early larvae. This is in accordance with the essential gene mutation studies performed on chromosome V (Johnsen and Baillie 1990) and on chromosome IV (Clark and Baillie 1992). The low number of Mels is most likely a representation of our screen criteria. The screen was not designed to isolate Mels. The Mels that were isolated were probably picked up as sterile adults. They were not identified as Mels until further phenotypic characterization was performed, since they laid very few eggs and did not have a particularly gravid appearance. Phenotypes of essential gene mutations may be more complex than simply the arrest stage of development. We report here only the block stages but for some we have observed more detailed phenomena, such as, defective cell divisions, improper gonadogenesis, clear, small, and very slowly developing worms. An example of a more specific phenotype is *let-733(s2621)* in which there is abnormal cytokinesis and cell division. Therefore, within this set of lethal mutations specific developmental defects may lead to the overall lethal phenotype.

Different alleles of a gene generally block at approximately the same developmental stage. We suspect that this reflects the zygotic null phenotype for these genes. EMS predominantly induces  $G/C \rightarrow A/T$  transitions and therefore would generate numerous nonsense alleles, resulting in truncated protein products. The few genes with alleles that arrest at significantly different stages are interesting. The differing arrest stages suggest that the gene may have separate functions at different developmental times. It is also possible that we have generated mutations in the same gene with varying degrees of severity. The actual nature of these different arrest stages will be determined when these lethal alleles are further characterized.

In conclusion, we have divided the dpy-17 region of the genome into 14 zones using chromosomal rearrangements – primarily deficiencies isolated in the UV and gamma-ray lethal screens reported in this paper. These zones have subsequently been anchored to the physical map by PCR-based deficiency endpoint mapping. Zones are delineated both genetically and physically. We mapped the 112 genes into these 14 physically defined zones and thus have narrowed the candidate sequences for each. Their mutations provide us with tools from which it will be possible to positionally clone these essential genes. Using systematic rescue experiments (Janke et al. 1997), we have already positioned some genes to cosmids and even to the correct coding element (Vatcher et al. 1998).

Our collection of mutations for 112 essential genes is a small part of the next step in the *C. elegans* genomic sequencing project. These genes constitute between 2.5 and 4.5% of all essential genes in *C. elegans*. As the *C. elegans* genome sequencing project nears the completion of the entire genomic sequence, researchers must focus on post-sequencing strategies to correlate the genomic sequence of predicted genes with biological function. Mutational analysis is an excellent method with which to achieve this goal.

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