Copyright © 1990 by the Genetics Society of America

Essential Genes in the *hDf6* Region of Chromosome *I* in *Caenorhabditis elegans*

Ann Marie Howell[1] and Ann M. Rose

Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

Manuscript received March 27, 1990
Accepted for publication July 21, 1990

ABSTRACT

In this paper we describe the analysis of essential genes in the *hDf6* region of chromosome *I* of *Caenorhabditis elegans*. Nineteen complementation groups have been identified which are required for the growth, survival or fertility of the organism (essential genes). Since ten of these genes were represented by more than one allele, a Poisson calculation predicts a minimum estimate of 25 essential genes in *hDf6*. The most mutable gene in this region was *let-354* with seventeen alleles. An average mutation rate of 5×10^{-5} mutations/gene/chromosome screened was calculated for an ethyl methanesulfonate dose of 15 mM. Mutations were recovered by screening for lethal mutations using the duplication *sDp2* for recovery. Our analysis shows that duplications are very effective for maintenance and mapping of large numbers of lethal mutations. Approximately 600 lethal mutations were mapped in order to identify the 54 that are in the deficiency *hDf6*. The *hDf6* region appears to have a lower proportion of early arresting mutations than other comparably sized regions of the genome.

AN approach to understanding eukaryotic genome organization is to identify, in some way, all the genes and describe their functions. A large subset of genes can be identified through mutation. In the two metazoans that have been most extensively mutationally analyzed, the majority of genes code for essential functions, required for either the survival (vitality) or fertility of the organism. In *Drosophila melanogaster*, for example, HOCHMAN (1971) attempted to identify all the vital loci on chromosome *4* using ethyl methanesulfonate (EMS) mutagenesis. Studies on the zeste-white region of the *X* chromosome (JUDD, SHEN and KAUFMAN 1972; YOUNG and JUDD 1978; SHANNON *et al.*, 1972) have identified most, if not all, the essential genes. In a small region (approximately 0.5 map unit) flanking rosy, HILLIKER *et al.* (1980) identified 23 essential genes; and LEICHT and BONNER (1988) isolated mutants in the genes surrounding the small heat shock protein gene cluster on chromosome *3*. Similar studies have been undertaken in *Caenorhabditis elegans* to identify essential genes (HERMAN 1978; MENEELY and HERMAN 1979, 1981; ROSE and BAILLIE 1980; ROGALSKI, MOERMAN and BAILLIE 1982; SIGURDSON, SPANIER and HERMAN 1984; ROGALSKI and BAILLIE 1985; HOWELL *et al.* 1987; ROGALSKI and RIDDLE 1988; CLARK *et al.* 1988; ROSENBLUTH *et al.* 1988; CHAREST *et al.* 1990). In all of the genomic regions studied, the number of essential loci exceeds the number of nonessential loci previously identified by a variety of selective systems. Thus, identification of essential loci for large genomic regions is feasible and contributes mutant phenotypes for the majority of genes in the region of study.

Using a free duplication of the left third of chromosome I, *sDp2* (ROSE, BAILLIE and CURRAN 1984), we have developed a system to isolate and characterize mutations in essential genes in a large autosomal region (HOWELL *et al.* 1987). A translocation which suppresses recombination on the left half of chromosome *I*, *hT1(I;V)* (MCKIM, HOWELL and ROSE 1988), has also been used to recover lethal mutations. In order to carry out a detailed analysis of a small region, we chose to limit the region to that deleted by the deficiency *hDf6*. Recombination distance across the *hDf6* region is estimated to be 1.5 map units. To date, nearly 600 EMS-induced lethal mutations have been recovered using *sDp2* or *hT1*. We describe here the analysis of 54 mutations which map into the *hDf6* region. These mutations define nineteen essential genes. We have investigated the organization of essential genes in this region with regard to map position, stage of arrest, and mutability.

MATERIALS AND METHODS

General: Wild-type and mutant strains were maintained and mated on Petri plates containing nematode growth media (NGM), streaked with *Escherichia coli* (BRENNER 1974). Unless otherwise noted, all experiments were carried out at 20° (ROSE and BAILLIE 1979). The wild-type N2 strain and some mutant strains of *C. elegans* var. Bristol were obtained from D. BAILLIE, Simon Fraser University, Burnaby, Canada, or the Caenorhabditis Genetics Center at the University of Missouri, Columbia. BW962 (+ *let-354(ct42) dpy-5* +/*unc-73* + + *dpy-14*) was a gift from PAUL

[1] Present address: Fred Hutchinson Cancer Research Center 1124 Columbia Street (M421), Seattle, Washington 98104

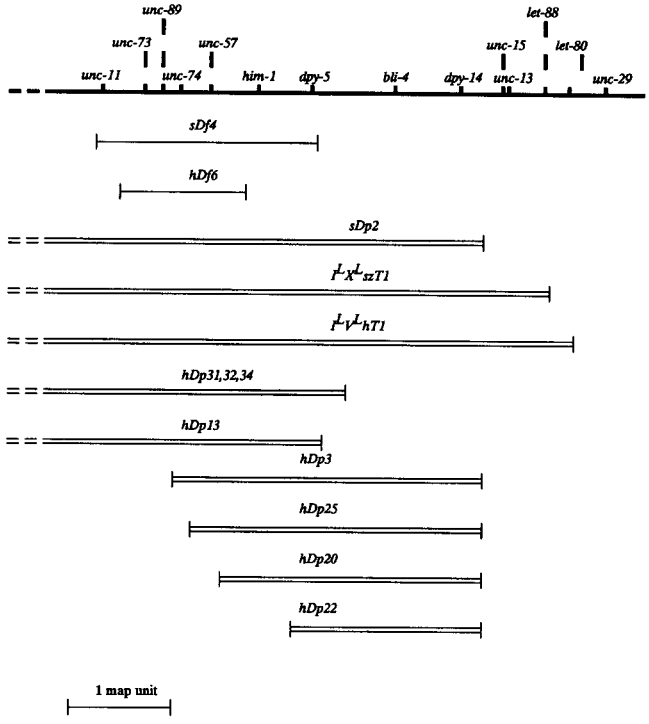

FIGURE 1.—The *unc-11 unc-29* region of chromosome *I* showing rearrangements and markers used. *sDp2*, *hDp13*, *hDp31*, *hDp32* and *hDp34* carry wild-type alleles for *let-362*, near the left end of chromosome *I* (not shown). *hT2(I;III)* covers this entire region and is not shown.

MAINS (MAINS, SULSTON and WOOD 1990) and ZZ1012 (*unc-74 dpy-5*) was a gift from JIM LEWIS (LEWIS *et al.* 1980). The nomenclature in this paper follows the uniform system adopted for *C. elegans* (HORVITZ *et al.* 1979). When describing genotypes of worms carrying duplications or translocations, the genotypes are shown in brackets as in MCKIM, HOWELL and ROSE (1988) for translocation chromosomes, or as in ROGALSKI, BULLERJAHN and RIDDLE (1988) for duplication chromosomes. Confidence intervals (CI) of 95% were calculated using Poisson statistics according to CROW and GARDNER (1959).

Chromosomal rearrangements used: Figure 1 indicates the positions of rearrangements of chromosome *I* used in this study. The duplication, *sDp2*, carries the wild-type alleles of *dpy-5* and *dpy-14* but not of *unc-15* or *unc-13* (ROSE, BAILLIE and CURRAN 1984). Duplications with the h allele designation have been described by MCKIM and ROSE (1990). The duplication, l^Lx^LszT1 was recovered as an aneuploid segregant of *szT1(I;X)* heterozygotes (MCKIM, HOWELL and ROSE 1988).

The translocation *szT1(I;X)* was initially isolated by FODOR and DEAK (1985) as a crossover suppressor for the *X* chromosome. In translocation heterozygotes, recombination is completely suppressed along the left half of chromosome *I* (to *let-88*) (MCKIM, HOWELL and ROSE 1988). In *hT1(I;V)* heterozygotes, recombination is completely suppressed along the left half of chromosome I (to *let-80*) and the left half of LGV (MCKIM, HOWELL and ROSE 1988). *hT2(I;III)* was isolated by K. PETERS in a screen for γ-ray-induced mutations causing pseudolinkage of markers on chromosomes *I* and *III*. Recombination is completely suppressed along the left two thirds of chromosome *I* in translocation heterozygotes (K. PETERS and K. MCKIM, unpublished results).

The deficiency *sDf4* deletes the *unc-11 dpy-5* region (ROSE 1980; HOWELL *et al.* 1987). It has been maintained in strains heterozygous for *bli-4 dpy-14* or the translocation *hT1(I;V)* by selecting phenotypically wild-type heterozygotes each generation. *hDf6* was isolated as a γ-ray-induced lethal mutation balanced over *szT1* (MCKIM, HOWELL and ROSE 1988) and was maintained using either of the duplications, l^Lx^LszT1 or *hDp31*. *hDf7* was isolated previously as a γ-ray-induced lethal mutation recovered using *sDp2* (HOWELL *et al.* 1987). It was found to be a deficiency during the course of this work.

Lethal screening using *sDp2*: In order to screen for lethal mutations of genes in the *hDf6* region of chromosome *I*, the duplication *sDp2* was used as previously described (HOWELL *et al.* 1987). Young adult hermaphrodites were treated with EMS (BRENNER 1974). *dpy-5* + *unc-13*/ *dpy-5 unc-15* +; *sDp2*[+] hermaphrodites were treated with 12, 15 or 17 mM EMS (based on the dosage curves of ROSENBLUTH, CUDDEFORD and BAILLIE 1983). Progeny of individually cultured F_1s were screened for the absence of fertile Dpy-5 Unc-13 individuals. Unc-13 individuals were recovered and lethal mutations were maintained in strains with the genotype (*let-x dpy-5*) *unc-13*/(*let-x dpy-5*) *unc-13*; *sDp2*[+ +]. The parentheses indicate that the *let-* gene could map either to the left or the right of *dpy-5*.

Lethal screening using *hT1*: Young adult *dpy-5 unc-13* +; +/*hT1(I;V)*[+ + *unc-29*;+] hermaphrodites were treated with 17 mM EMS. Individually plated F_1s were screened for the absence of fertile Dpy-5 Unc-13 progeny. Strains were discarded if any fertile Dpy-5 Unc-13 individuals were seen. If no fertile Dpy-5 Unc-13s were seen, the strain was assumed to be carrying a new lethal mutation in the balanced regions of chromosome *I* or *V* and was maintained for further analysis.

Mapping *hT1*-recovered lethals to chromosome *I* and balancing over *szT1*: The lethal bearing strains recovered using *hT1* could have had a lethal mutation on chromosome *I* or *V*. In order to assign each lethal mutation to the correct linkage group, the *hT1* chromosomes were replaced with a normal chromosome *V* and the translocated *I* and *X* chromosomes from *szT1(I;X)*. This eliminated the pseudolinkage of chromosome *I* and *V* in the lethal bearing strains. Lethal strains (*let-x dpy-5 unc-13* +; +/*hT1(I;V)*[+ + + *unc-29*; +] or *dpy-5 unc-13* +; *let-x*/ *hT1(I;V)*[+ + *unc-29*; +]) were mated to Lon-2 males of the genotype *hT2(I;III)* [*dpy-5* +; +; 0]/ *szT1(I;X)*[+ *unc-29*;+; *lon-2*]. Both *hT1* of the hermaphrodites and *szT1* of the males were marked with *unc-29*. This was done so that the *hT1*/*szT1* heterozygotes would be Unc-29 and therefore easily distinguishable from the desired wild-type *let-x dpy-5 unc-13*/*szT1* heterozygotes. The strain used to balance the lethals over *szT1* also carried the translocation *hT2(I;III)* marked with a *dpy-5* allele (EMS-induced on the chromosome by J. BABITY). Since *hT2* reduces recombination in the *unc-29* region, it was used to prevent the *unc-29* mutation from crossing off the *szT1* chromosome. This was not completely effective, however, because in two strains the *unc-29* marker crossed off the *hT1* chromosome creating an unmarked *hT1* chromosome in *trans* to *szT1*. Wild-type out-cross hermaphrodites of genotype *let-x dpy-5 unc-13* +; +/*szT1(I;X)*[+ *unc-29; lon-2*] were allowed to self. The segregation of Lon-2 males indicated the presence of *szT1*. The segregation of many fertile Dpy-5 Unc-13s indicated that the lethal mutation was on chromosome *V*. The presence of one or a few fertile Dpy-5 Unc-13s indicated that the lethal was on chromosome *I* but outside the boundary of crossover suppression. If no fertile Dpy-5 Unc-13 individuals were observed, the lethal mutation was assumed to be in the crossover suppressed region

on chromosome *I*. The *szT1*-balanced strains were used for further analyses.

Complementation tests: The following complementation tests were employed.

Deficiencies: Wild-type males were crossed to lethal-bearing strains (balanced by *sDp2* or *szT1*) and the resulting *let-x dpy-5 unc-13*/+ + + males were mated to deficiency strains. The *sDp2*-carrying males which are also produced by this cross are slower developing and less effective at mating than males lacking the duplication (ROSE, BAILLIE and CURRAN 1984). The absence of fertile *Dpy-5s* (for *sDf4*) or Dpy-5 Unc-13s (for *hDf6 dpy-5 unc-13*) among the outcross progeny indicated failure to complement. A minimum of 30 wild-type outcross males were scored to ensure that the absence of the *let-x*/Df heterozygote was not due to reduced viability.

Duplications: Mapping using duplications was done as described by MCKIM and ROSE (1990). *hDpz* refers to *hDp3*, *hDp13*, *hDp20*, *hDp22* or *hDp25*. Lethal bearing strains (*i.e.*, *let-x dpy-5 unc-13*; *sDp2(I;f)*) were crossed to *unc-11 dpy-14*; *0/szT1(I;X)*[+;*lon-2*] males. The non *sDp2*-bearing males (+ *let-x dpy-5* + *unc-13/unc-11* + + *dpy-14* +) were crossed to either a) *dpy-5 dpy-14*; *hDpz(I;f)* (for *hDp13*) or b) *unc-11 dpy-5*; *hDpz(I;f)* hermaphrodites. Phenotypically wild-type hermaphrodites from cross a) were *let-x dpy-5* + *unc-13*/+ *dpy-5 dpy-14* +; *hDpz* or +*dpy-5 dpy-14 /unc-11* + *dpy-14*; *hDpz* if *hDpz* was *dpy-14*(+). The lethal-bearing hermaphrodites were identified by the fact that they produced many more Dpy-5 progeny than the non lethal-bearing hermaphrodites. Wild-type hermaphrodite progeny from cross b) were + *let-x dpy-5 unc-13/ unc-11* + *dpy-5* +; *hDpz*. From both crosses, fertile Unc-13 progeny were observed in the next generation if the duplication carried *let-x*(+). In some cases where the duplication did not actually carry *let-x*(+), a few fertile Unc-13s were observed. These were recombinants which had lost the lethal mutation on one homolog. They were easily distinguished because they segregated fertile Dpy-5 Unc-13s.

Lethal mutations: Heterozygous males (*let-x dpy-5 unc-13*/ + + +) generated by crossing *sDp2* or *szT1* lethal strains to wild-type males were used for complementation testing. These males were mated to Unc-13 hermaphrodites from lethal-bearing strains. The out-cross progeny were scored. The absence of Dpy-5 Unc-13 males and fertile Dpy-5 Unc-13 hermaphrodites indicated failure to complement.

Nonlethal mutations: Lethal mutations were tested for complementation to nonlethal (visible) mutations. Visible mutations (m) linked to *dpy-5* were crossed to wild-type males. *m dpy-5*/+ + progeny males were mated to Unc-13 hermaphrodites from different lethal-bearing strains. If non-M Dpy-5 progeny were observed, the visible mutation complemented the lethal mutation. If either M Dpy-5 or no Dpy-5 progeny were observed, after a successful mating, the visible mutation failed to complement the lethal mutation.

In order to test if any of the known genes with visible mutant phenotypes had lethal alleles, the visible mutations were first tested for complementation with deficiencies. They were then tested for complementation with lethal mutations in the same region. *hDf6* fails to complement *unc-38* (MAINS, SULSTON and WOOD 1990), *unc-73*, *unc-89*, *unc-74*, and *unc-57*. Lethal mutations were not tested for complementation with *unc-38* or *unc-74* (LEWIS *et al.* 1980) and *unc-89* because known null alleles are not lethal, and are not synthetic lethals with *unc-13*. Mutations in the other unc genes complemented mutations in all of the essential genes in *hDf6*. Thus, no lethal alleles of genes producing morphological mutants were found in *hDf6*.

Stage of arrest of lethal mutations in *hDf6*: The stage of lethal arrest was examined for each of the EMS-induced *sDp2*-rescued lethal mutations in *hDf6*. The animals scored were homozygous for the lethal mutation. They were picked in three ways: (1) as segregants from balanced strains, (2) as progeny of heterozygous hermaphrodites or (3) as arrested Unc-13s resulting from a cross with a duplication which failed to complement the lethal mutation. For strains where arrested Dpy-5 Unc-13s were not easily visible, heterozygous hermaphrodites (*let-x dpy-5 unc-13*/+ + +) were allowed to lay eggs for about eight hours and then removed. Plates were checked the next day to see if all the eggs hatched. If all had hatched the plates were checked later to see when the Dpy-5 Unc-13s arrested development. For strains where arrested Dpy-5 Unc-13 individuals were easily visible, some were picked and allowed to sit for several days to ensure they developed as far as possible. Their lengths were measured and compared to a growth curve of both Unc-13s and Dpy-5 Unc-13s from KR236 (*dpy-5 unc-13*; *sDp2*) prepared by G. DUNCAN (this laboratory). The arresting stages were classified as early larval, mid-larval, late larval or sterile adult. By comparing the sizes to the growth curve of Unc-13 from ROSE (1980), we estimate that early larval corresponded approximately to L1, mid-larval to late L2 or L3, and late larval to late L3 or L4.

RESULTS

Recovery of lethal mutations: Two rearrangements were used to recover mutations in essential genes on chromosome *I*, the duplication, *sDp2* (HOWELL *et al.* 1987), and the translocation, *hT1(I;V)* (MCKIM, HOWELL and ROSE 1988). Using *sDp2*, a total of 561 lethal mutations have been recovered from 31,606 EMS-treated chromosomes (1.8%). Mutations which had been positioned to the right of *dpy-5* by recombination mapping (HOWELL *et al.* 1987; HARRIS 1986; J. BABITY, J. MCDOWALL, A. M. HOWELL, J. S. KIM, R. KISUN, K. MCKIM, K. MCNEIL, D. PILGRIM, K. PETERS, B. RATTRAY and T. STARR, unpublished results) or duplication mapping (A. ROSE and J. MCDOWALL, unpublished results) were not analyzed. In this study, the remaining 444 *sDp2* lethal-bearing strains were analyzed.

The screen using *hT1* recovered 111 lethal mutations (from approximately 750 chromosomes screened). In 12 strains the lethal mutation was lost due to recombination (*i.e.*, they were outside of the boundaries of crossover suppression of *hT1*). Ninety-nine strains were tested to determine whether the lethal mutation in each strain was on chromosome *I* or chromosome *V*. Thirty-eight strains carrying lethal mutations on chromosome *V* were discarded. Two strains appeared to be chromosome *I* lethals when balanced over *szT1*, but were found to be heterozygous for *szT1* and an *hT1* chromosome which had lost the *unc-29* marker. In 13 strains the lethal mutation was on chromosome *I*, but outside of the crossover suppressed region of *szT1* on chromosome *I*. Forty-six strains were recovered which carried chromosome *I* lethal mutations in the crossover suppressed region of *szT1*. Lon-2 males from two of the 46 strains (*h984* and *h1003*) failed to mate after several attempts and

were not positioned, resulting in 44 chromosome *I* lethals that were subsequently examined.

***sDp2* has a lethal mutation in the *hDf6* region:** *hDf6* cannot be maintained in a homozygous state with *sDp2* in the same manner as other *sDp2*-rescued lethal mutations. Two different strains were constructed in attempts to recover an *hDf6 dpy-5 unc-13*; *sDp2* strain. Both *hDf6 dpy-5* + *unc-13*/+ *dpy-5* + *unc-15*; *sDp2* and *hDf6 dpy-5* + *unc-13*/+ *dpy-5 dpy-14* +; *sDp2* hermaphrodites segregated sterile adult Unc-13 hermaphrodites. They did not lay oocytes and were not rescued by male sperm. Any fertile Unc-13s recovered were shown to be recombinants by progeny testing. A possible explanation of this result was that *hDf6* could not be maintained in a genetic background of two deficiency chromosomes to one wild type. However, this was shown not to be the case by constructing two *hDf6/hDf6*; $I^LX^L szT1$ strains. In these strains one of the translocated chromosomes of *szT1* ($I^LX^L szT1$) is being used as a duplication of the left half of chromosome *I*. *hDf6 dpy-5 unc-13*; *unc-3*; $I^L\ X^L$ *szT1* was isolated as an aneuploid segregant from *hDf6 dpy-5 unc-13*; *unc-3*/ *szT1* [+ + +; + *lon-2*]. *hDf6 dpy-5 unc-13*; $I^L\ X^L$ *szT1* was constructed by crossing *hDf6 dpy-5* + *unc-13*/ + + *dpy-14* + males to *dpy-5 dpy-14*; *I X szT1* hermaphrodites and picking the appropriate segregant in the next generation. The viability of the two *hDf6/hDf6*; $I^LX^L szT1$ strains showed that null alleles of all *hDf6* genes can be recovered in a 2:1 ratio of the deficiency chromosome to wild type. Since $I^LX^L szT1$ covers more of chromosome *I* than does *sDp2*, we tested whether the difference in ability to rescue *hDf6* was a result of a difference in size. *hDf6* was tested for complementation by three other duplications, *hDp31*, *hDp32* and *hDp34* (see Figure 1). These three duplications could also rescue *hDf6* in Unc-13 strains (*i.e.*, *hDf6 dpy-5 unc-13; hDpx*). Since these three duplications are shorter than *sDp2*, the inability of *sDp2* to rescue *hDf6* was not a function of its size. Thus, it appears that *sDp2* carries a lethal mutation in the *hDf6* region. The mutation can not be in the *hDf7* region (see later) since *hDf7* is rescuable by *sDp2*. In this study, no mutations were identified in the *hT1* screen which failed to complement the lethal mutation on *sDp2*. It is not known whether the lesion on *sDp2* affects only one gene, or is a small deletion.

Essential genes in *hDf6*: In order to find which lethal mutations from *sDp2* screens might be in the *hDf6* region, 175 of the 444 strains were tested for complementation by *sDf4* (Howell *et al.* 1987; Howell 1989). Since both *hDf6* breakpoints are inside of *sDf4*, it was not necessary to test any lethal mutations outside of *sDf4* for complementation with *hDf6*. The remaining 269 lethal mutations were tested for complementation with *hDf6*. A total of 54 *sDp2*-recovered lethal mutations failed to complement *hDf6*. These define 19 complementation groups (Table 1). Genes were assigned on the basis of EMS-induced mutations only, since γ-ray-induced mutations (described in Howell *et al.* 1987; McKim, Howell and Rose 1988) could be small deficiencies. It was reported by Howell *et al.* (1987) that *h234* was an allele of *let-372*, a gene to the left of *hDf6*. It was later shown that *h234* fails to complement *hDf6*. *h234* now defines the essential gene, *let-373*. The 44 *hT1*-recovered lethals were also mapped (see later) and four of these failed to complement *hDf6*. All four of them were allelic to complementation groups defined by the *sDp2*-recovered lethals (Table 1).

Previously isolated γ-ray-induced mutations were also tested with *hDf6*. One of the γ-ray-induced lethal mutations recovered with *szT1* by McKim, Howell and Rose (1988) mapped to the left of *dpy-5* and failed to complement *hDf6*. This mutation, *h549*, is an allele of *let-354*. Four mutations recovered using *sDp2*

TABLE 1

Stage of arrest of *sDp2*-recovered lethals in *hDf6*

Gene	Allele	Stage of arrest
let-351	*h43*	Mid-larval
let-353	*h46*	Mid-larval
let-354	*h79, h90, h201, h267*	Mid-larval
	h370, h390, h441, h482	
	h504, h508, h693, h803	
	h809, h819, h841, h863	
	h866, h72[a], *h549*[b], *h934*[c]	
let-356	*h83, h501, h679, h871*	Mid-larval
let-366	*h112, h265, h441*	Mid-larval
	h422, h505, h852	
	h890[c]	
let-373	*h234, h573, h70*[a]	Early larval
let-374	*h251*	Mid-larval
let-375	*h259, h391*	Sterile adult (leaky)
let-501	*h714*	Early larval
	h498	Mid-larval
let-502	*h392, h732, h835*	Mid-larval
	h783	Late larval
	h509	Sterile adult
let-503	*h313, h418*	Sterile adult
let-504	*h448*	Sterile adult
	h844	Late larval
	h327[a], *h888*[c]	
let-505	*h426*	Late larval
let-506	*h300*	Late larval
let-507	*h439*	Sterile adult (leaky)
let-508	*h452, h995*[c]	Late larval
let-509	*h521, h522*	Sterile adult (leaky)
	h867	Late larval or adult (leaky)
let-510	*h740*	Late larval
let-511	*h755*	Early larval

Alleles listed are EMS-induced recovered using *sDp2* unless otherwise noted as follows:

[a] γ-Ray-induced *sDp2*-rescued lethal (in Howell *et al.* 1987).

[b] γ-Ray-induced *szT1*-rescued lethal (McKim, Howell and Rose 1988).

[c] EMS-induced *hT1*-rescued lethal.

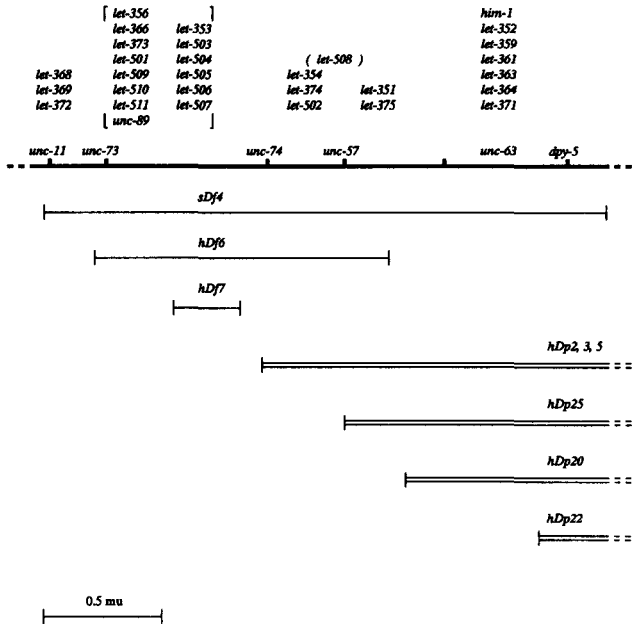

FIGURE 2.—Genetic map of the *hDf6* region including flanking markers, based on data from HOWELL (1989), EDGLEY and RIDDLE (1987) and K. S. MCKIM (unpublished data). The endpoints of *sDf4* are from ROSE (1980) and MCDOWALL (1990). *hDp3* was used to divide *hDf6* into two intervals. *hDp25* separates into two groups those genes which are found both in *hDf6* and in *hDp3*. *hDp25* includes *let-351* but not *let-354* or *let-374*. *let-508* was not tested with *hDp25*. *hDp22* includes *dpy-5* but has not been found to complement any genes to the left of *dpy-5*. Genes in brackets not right-left positioned.

mapped to the left of *dpy-5* (HOWELL *et al.* 1987) and failed to complement *hDf6* (*h54*, *h70*, *h72* and *h327*). Three lie in single complementation groups: *h70* is an allele of *let-373*, *h72* is an allele of *let-354* and *h327* is an allele of *let-504*. *h54* failed to complement alleles of six of the complementation groups in *hDf6* and was renamed *hDf7* (Figure 2). *hDf7* complements lethal mutations outside of *hDf6* and all of the visible mutations in *hDf6* (see below).

Duplication mapping: A number of duplications of chromosome *I* were available (MCKIM and ROSE 1990) and were used to position essential genes. *hDp13* breaks close to the right of *dpy-5* and has been used to position the lethals. Ninety-seven of 173 tested *sDp2*-recovered mutations were complemented by *hDp13*. Thus, *hDp13* conveniently divides the *sDp2* region into two approximately equal regions with regard to lethal mutations. Of 42 *hT1*-recovered mutations that were tested (two were not tested as they mapped outside *hDf6*), 23 were complemented by *hDp13*.

hDp3, which breaks in the *hDf6* region between *unc-74* and *unc-89* (Figures 1 and 2), was used to position the genes in *hDf6* (Table 1). Approximately one third (6/19) of the essential genes in *hDf6* are covered by *hDp3*. Mutations in five of these six genes were tested with *hDp25* (Figure 2).

Stage of arrest of lethal mutants: The lethal mutations recovered with *sDp2* were characterized with regard to their stage of arrest (Table 1). We determined whether multiple alleles of genes arrested at the same developmental stage. The widest range of arrest stages was with mutations of *let-502*. For this gene, three alleles arrest at a mid-larval stage and two at late larval and sterile adult. In two other cases (*let-501* and *let-504*), the arrest stage for the different mutants was similar. All alleles of seven genes (of the ten which have more than one allele) arrested at the same stage. For example, all 17 alleles of *let-354* picked up in our screens arrested development at the same mid-larval stage. Thus, in most cases all alleles of a gene arrest at a similar point in development.

In contrast to these results, a quite different class of *let-354* mutation has been described. The recessive lethal mutation, *ct42*, recovered by P. MAINS (MAINS, SULSTON and WOOD 1990), was found to be an allele of *let-354*. Because *ct42* also had a temperature-sensitive, dominant, maternal-effect lethal phenotype (MAINS, SULSTON and WOOD 1990), ten *let-354* alleles were tested for temperature-sensitive sterility. Late larval Unc-13 hermaphrodites from lethal strains (*i.e.*, *let-354 dpy-5 unc-13*; *sDp2*) were incubated at 25°. Only one allele, *h482*, was sterile at 25°. Many eggs did not hatch and no larvae grew past hatching. None of the other nine alleles tested was sterile at this temperature. *hDf6 dpy-5 unc-13; hDp31* was also tested and found to be fertile at the restrictive temperature. Since *hDf6* must represent the null phenotype of *let-354*, the amorphic phenotype is recessive lethality, not a dominant, maternal-effect lethality.

The *ct42* allele of *let-354* can not be rescued by *sDp2*. Lon males with the genotype *let-354(ct42) dpy-5; 0/szT1*[+ +;*lon-2*] were mated to *dpy-14 unc-29* hermaphrodites. The resulting wild-type males (*let-354(ct42) dpy-5* + +/+ + *dpy-14 unc-29*) were mated to *dpy-5 unc-29; sDp2* (Unc-29) hermaphrodites. Except for recombinants, all wild-type hermaphrodite progeny from that cross were *let-354(ct42) dpy-5* + +/ + + *dpy-14 unc-29; sDp2*. They were kept at 16° because of the dominant temperature-sensitive sterility caused by *let-354(ct42)*. Thirty of the wild-type hermaphrodite progeny were picked and left at 16°. These were expected be of the parental genotype or *let-354(ct42) dpy-5/ let-354(ct42) dpy-5; sDp2* in a 2:1 ratio. Twenty-one were found to be of the parental genotype by progeny testing. The other nine laid fertilized eggs which did not hatch. *hDf6 dpy-5 unc-13; hDp31* were fertile at 16°, indicating this maternal-effect can not be the result of insufficient wild-type product but that the *ct42* allele is as an antimorph (MULLER 1932).

Across the *hDf6* interval, there was a wide range of arrest stages (Table 1). None of the EMS-induced

lethal mutations resulted in embryonic lethality. Some of the sterile adult mutations were leaky, meaning that some of the homozygous lethal Dpy-5 Unc-13 hermaphrodites produced a few progeny. Both alleles of *let-375* and all three alleles of *let-509* were leaky. The escaping progeny from *let-509* never grow past hatching, indicating that these mutations also have a maternal affect. Dpy-5 Unc-13 hermaphrodites from three of these leaky mutant strains, *let-375(h259)*, *let-375(h391)* and *let-507(h439)*, also produced a few inviable progeny after crossing to males. Since the number of out-cross progeny was not much more than self progeny, they are probably not sperm-defective mutations. They also did not exhibit the usual phenotype of sperm-defective mutants, that is they did not lay many unfertilized eggs. These three leaky mutations were not leaky over *hDf6* (and *hDf7* for *let-507*); *i.e.*, over a deficiency their phenotypes were more severe. This result is in agreement with MULLER's (1932) definition of a hypomorphic mutation. None of the other sterile adult mutants gave any progeny after attempted mating to wild-type males.

An egg count was done for *hDf6 dpy-5 unc-13/+ + +* hermaphrodites. One quarter of the eggs should have been homozygotes for *hDf6*. From a total of 70 eggs laid, 17 did not hatch. It thus appears that *hDf6* homozygotes arrest in the embryonic stage. Homozygotes of a second smaller deficiency of this region, *hDf7*, arrest at an early larval stage. All eggs from *hDf7 dpy-5 unc-13/+ + +* hermaphrodites hatched but the Dpy-5 Unc-13s did not develop to the L2 stage. Thus, *hDf7* homozygotes seem incapable of any post-embryonic growth. The earliest blocking mutation identified in *hDf7* was mid-larval. Most of the lethals blocked development at a late larval stage or were sterile adults. The stage of arrest for homozygotes of either deficiency is earlier than that for homozygotes of any single mutation uncovered by them. It is possible that earlier blocking mutations are yet to be identified, or that the earlier block is the result of a cumulative affect of the loss of several gene products.

DISCUSSION

In the *hDf6* region of *C. elegans* the majority of mutable genes code for essential functions. Of the 24 genes identified by mutation, five have morphological mutant phenotypes and 19 have lethal phenotypes (80%). The finding that 80% or more of loci, which produce mutant phenotypes, are essential for survival or fertility has been shown for many regions in *D. melanogaster* (HOCHMAN 1971; JUDD, SHEN and KAUFMAN 1972; HILLIKER *et al.* 1980; LEICHT and BONNER 1988) and *C. elegans* (MEENELY and HERMAN 1979; ROSE and BAILLIE 1980; ROGALSKI and BAILLIE 1985; CLARK *et al.* 1988; ROSENBLUTH *et al.* 1988). For some genes both visible (rare) and lethal (frequent) mutant phenotypes have been described, *unc-70* (PARK and HORVITZ 1986; ROSENBLUTH *et al.* 1988), *him-1* (HOWELL *et al.* 1987), *rol-3* (ROSENBLUTH *et al.* 1988) and *bli-4* (PETERS and ROSE 1988), for example. However, none of the visible genes tested in the *hDf6* region had lethal alleles. Of course there will be genes which would not be identified in mutant screens, those with subtle null phenotypes or redundant members of gene families for example. Thus, the true fraction of genes that can be detected by mutant phenotype will only be known when the number of coding regions (identified by molecular analysis) is known and can be compared to the number of loci identified by mutational analysis. Nevertheless, there is a large number of essential genes in the *C. elegans* genome amenable to the type of analysis described here.

Most of the lethal mutations analyzed in this study were recovered using a free duplication of the left third of chromosome *I*, *sDp2* (as described in HOWELL *et al.* 1987). It is now known that *sDp2* carries a lethal mutation in the *hDf6* region and therefore could not be used to recover alleles of every essential gene in *hDf6*. Others have made use of translocations that suppress recombination to recover lethals (ROSENBLUTH, CUDDEFORD and BAILLIE 1983; CLARK *et al.* 1988; ROSENBLUTH *et al.* 1988). Thus, in order to compare the recovery of lethals using a duplication to that using a translocation, two translocations involving chromosome I were used to screen for and maintain lethal mutations. *szT1(I;X)*, was shown by MCKIM, HOWELL and ROSE (1988) to be inefficient for lethal screening because it spontaneously produces aneuploid strains which appear to be carrying lethal mutations. In the present study, *hT1(I;V)* was used to recover EMS-induced lethal mutations. If we compare the number of lethals mapped to the *hDp13* region, fewer were recovered per chromosome screened using *sDp2* (97/8190 [CI = 79–117] [0.9–1.4%]) than with *hT1* (23/750 [CI = 15–34] [2–4.5%]). This suggests that *hT1* may be more efficient for the recovery of lethal mutations in the left half of chromosome *I*. However, we found no evidence for a large class of genes in which mutations could not be recovered by *sDp2*. For example, the four *hT1*-recovered lethals were all alleles of genes identified by *sDp2*-recovered mutants. Only when a large number of *hT1*-recovered lethals have been analyzed will we know if there are mutations which can not be rescued by a duplication.

Estimate of the number of essential genes in *hDf6*: In this analysis 19 essential genes in *hDf6* were identified. Ten of these genes were represented by more than one EMS-induced *sDp2*-rescued allele. A statistical analysis of the allele frequencies (BEYER 1976; MEENELY and HERMAN 1979) predicted an estimate of a total of 25 essential genes in *hDf6*. The Poisson distribution is applicable if the probability of produc-

ing and detecting a mutant is the same for all genes (LEFEVRE and WATKINS 1986). The latter assumption regarding detection is possibly true for lethal phenotypes. However, the former (regarding mutability) is far from being true (MENEELY and HERMAN 1979; HILLIKER, CHOVNICK and CLARK 1981; LEFEVRE and WATKINS 1986; and this paper). *let-354*, for example, has seventeen alleles while others have none. The high mutation frequency of *let-354* indicated either that the gene is large or very sensitive to mutation (for reasons other than size). Two other highly mutable targets, *unc-54* and *unc-22*, have been cloned and shown to be large genes (MACLEOD, KARN and BRENNER 1981 for *unc-54*; MOERMAN, BENIAN and WATERSTON 1986 for *unc-22*). On the other hand, some mutable targets are known to be very small, *sup-5* (WATERSTON and BRENNER 1978) and *sup-7* (WATERSTON 1981) are examples. In *D. melanogaster*, BARRETT (1980) analyzed published data on lethal analysis and showed that it did not fit the Poisson distribution very well except when the sample sizes were small. One explanation is that there is more than one population of genes (with regard to mutability) in the genome. Initial studies may be detecting a fairly homogeneous population of readily mutated genes. As the sample sizes become larger, a second population is observed consisting of the majority of genes. Thus Poisson estimates for the number of undetected genes increases as more genes are detected. Poisson statistics were never intended to be applied to targets of varying sizes yet to date it is the most appropriate statistical method for estimating gene numbers. The number of very small targets remains an unknown quantity. The estimate of 25 essential genes is a minimum estimate.

The breakpoints of *hDf6* have not yet been identified on the physical map constructed by COULSON and SULSTON (COULSON *et al.* 1986; 1988), however an estimate of the physical size of *hDf6* can been made. *hDf6* extends from 0.5 to 2.0 map units left of *dpy-5* (about 1.5 map units). The amount of recombinational suppression, relative to the genomic average, in this region was estimated to be 1.5 from data of KIM and ROSE (1987) and STARR *et al.* (1989). This would make *hDf6* approximately 750 kb (1.5/300 map units (genome size from EDGLEY and RIDDLE 1987) × 1.5 for suppression of 85 Mb, ignoring the repetitive fraction (genome size from J. SULSTON, personal communication; SULSTON and BRENNER 1974). Estimates of the average number of kilobase pairs (kbp) per gene range from 15 (PRASAD and BAILLIE 1989; STARR *et al.* 1989) to 20 (HEINE and BLUMENTHAL 1986). Thus, by these estimates, there could be 35 to 50 genes in *hDf6*. To date, five nonessential genes and nineteen essential genes have been identified in the *hDf6* region.

Estimate of the number of essential genes in the genome: The recombination distance between genes in the *C. elegans* genome is not uniform (BRENNER 1974; GREENWALD *et al.* 1987; KIM and ROSE 1987; PRASAD and BAILLIE 1989; STARR *et al.* 1989). Thus, we would expect the essential genes to show the same clustering on the genetic map as do the nonessential genes. This is clearly true for chromosome *I* left of *dpy-5* (HOWELL 1989) as well as other regions that have been examined (SIGURDSON, SPANIER and HERMAN 1984; HOWELL *et al.* 1987; CLARK *et al.* 1988; ROSENBLUTH *et al.* 1988). No single small region can be considered representative of the genome as a whole. If the essential gene density in the *hDf6* region were representative of the entire genome, a minimum estimate of approximately 5,000 (25 × 200) total essential genes would be obtained since *hDf6* is 1/200 of the size of the total genome (1.5/300 map units). However, the *hDf6* region is on the left edge of the gene cluster on chromosome *I*, so the gene/map unit density predicted is higher than the genomic average. Adjusting the estimate for a gene density 1.5 times the genome average [based on data from KIM and ROSE (1987) and STARR *et al.* (1989)], we would get a minimum estimate of approximately 3300 essential genes. This is higher than BRENNER's (1974) original estimate of 2000 essential genes. It is similar to the estimates of D. L. BAILLIE (4000; personal communication as cited in MOERMAN and BAILLIE 1979) and CLARK *et al.* (1988) (3500) but lower than ROGALSKI (1983) (5700). These estimates agree fairly well with those based on molecular data. Estimates made by extrapolating from the number of kb per coding region and 85×10^6 bp of nonrepetitive DNA (SULSTON and BRENNER 1974) would predict 4200 to 5700.

Stage of developmental arrest: The stage of lethal arrest was determined for the nineteen essential genes represented by 54 EMS-induced *sDp2*-rescued mutations in *hDf6*. Most alleles of a gene, for which more than one allele has been identified, arrest development at approximately the same developmental stage. This finding has also been described by others in *D. melanogaster* (SHANNON *et al.* 1972) and *C. elegans* (ROSE and BAILLIE 1980; MENEELY and HERMAN 1981; ROGALSKI, MOERMAN and BAILLIE 1982; ROSENBLUTH *et al.* 1988). SHANNON *et al.* (1972) proposed that most variability between alleles is due to leakiness of later arresting mutations. Data from *C. elegans* would support this suggestion. CLARK *et al.* (1988) showed that some later arresting mutations blocked development at an earlier stage when heterozygous with a deficiency, indicating that they are hypomorphs as defined by MULLER (1932). LEICHT and BONNER (1988) proposed that some of the apparent variability between alleles of the essential genes they analyzed in *D. melanogaster* was due to linked second site mutations. Since the lethal mutations de-

scribed in this paper were generated using a relatively low dose of EMS, variability between alleles is unlikely to be due to linked second site mutations. The most variability in stage of arrest was found for alleles of *let-502*, which ranged from mid-larval to sterile adult. The dissimilarities in phenotype could represent varying severities of mutant alleles, or differing mutated functions which are required at different times in development.

Since the stage of lethal arrest was determined in strains which also carried mutations in *dpy-5* and *unc-13*, it is possible that the presence of these mutations affected the phenotypes of the lethal mutations. For example, viable mutant alleles of *unc-11* are lethal in combination with *unc-13* mutations. Some of the other mutations may be synthetically lethal with the marker mutations.

The proportion of arrest stage varies depending on the region of the *C. elegans* genome studied. The *hDf6* region is similar to some other genomic regions for the proportions of mid-larval and adult sterile mutations recovered (MENEELY and HERMAN 1981; CLARK *et al.* 1988), but differs in the scarcity of early larval mutants recovered. For example, only 3 of the 19 genes in *hDf6* appear to be required for development past the first larval stage; whereas, one-third of the essential genes listed in CLARK *et al.* (1988) from the *unc-22(IV)* region were required past an early larval stage. ROSE and BAILLIE (1980) reported that most (13/16) of the essential genes in the region around *unc-15(I)* arrested as early larvae. The *hDf6* region has the smallest proportion (0.16) of early larval lethals. MENEELY and HERMAN (1981), SIGURDSON, SPANIER and HERMAN (1984), ROSENBLUTH *et al.* (1988) and MCDOWALL (1990) studied larger regions of the genome. If the data from these regions are divided into small intervals, regional differences in stages of lethal arrest become evident. Examination of regional differences in developmental arrest stages is suggestive of two conclusions. First of all, the straightforward prediction that most mutants block at L2, a time of maximal growth, for example, does not apply. Second, the distribution of arrest stages is not random throughout the genome, but shows regional patterning. As the number of genes with multiple alleles grows and with the standardization of descriptions of developmental arrest stages, these possibilities can be more rigorously tested.

Lethals as null phenotypes: One third of all recessive lethal mutations in *hDf6* described in this paper are alleles of *let-354*. This gene is a very large mutational target for both recessive and dominant phenotypes. Three of eight dominant temperature-sensitive maternal-effect embryonic lethal mutations recovered in a screen of the whole genome were alleles of *let-354* (MAINS, SULSTON and WOOD 1990; this paper). These alleles also had a nonconditional recessive mid-larval lethal phenotype. *hDf6* represents the null phenotype for *let-354* since the entire gene must be within *hDf6*. *hDf6* does not have a dominant temperature-sensitive maternal-effect lethal phenotype, so this could not be the null phenotype of any gene *hDf6* deletes. Furthermore, most temperature-sensitive mutations are the result of alterations in protein structure, not loss of function. The recessive mid-larval lethal phenotype is most likely the null phenotype of *let-354*.

One of the *sDp2*-recovered EMS-induced alleles of *let-354*, *h482*, showed a dominant temperature-sensitive maternal-effect embryonic lethal phenotype. This phenotype was not as extreme as *ct42*; some of the eggs hatched at 25° but none of the larvae grew after that. With *ct42*, most eggs do not hatch at 20° and none hatch at 25° (MAINS, SULSTON and WOOD 1990). *h482* was rescuable by the duplication and the strain was quite fertile at 20° in contrast to *ct42*. The mutation is similar to *ct42* but its antimorphic behavior is not as strong. PERRIMON *et al.* (1986) showed that in *D. melanogaster* many maternal-effect lethal mutations are rare hypomorphic or antimorphic alleles of ordinary zygotic essential genes. The alleles of *let-354* which show maternal effects are probably of this type; the *let-354* gene product may not normally play a role in embryogenesis. Similar results have been reported by KEMPHUES, KUSCH and WOLF (1988).

Forward mutation rates in *C. elegans*: The data presented here can be used to derive an estimate of the average forward mutation rate per gene in *C. elegans*. The data for *let-354* has been omitted from this calculation because its mutation rate is at least ten times higher than that for the average gene in this region. If we use the dose response curve for EMS mutagenesis published by ROSENBLUTH, CUDDEFORD and BAILLIE (1983) to adjust our estimate of forward mutation rates for different doses, we get 6.8×10^{-5} at 25 mM and 10.1×10^{-5} at 50 mM. Others have estimated 50×10^{-5} at 50 mM EMS (BRENNER 1974) and 8.7×10^{-5} at 25 mM (CLARK 1990). Despite the very different approaches used by us and by CLARK (1990), these two estimates are quite similar. Caution should be exercised in estimating the degree of saturation based on forward mutation rates since until now (this paper; CLARK 1990) no large data sets collected at low EMS doses have been available. Falsely low estimates of the number of genes in any desired category could be predicted by underestimating the average forward mutation rate. We still do not have good estimates on the influence of the genes with very low mutability, however comparisons of mutational targets with physical coding regions suggest that this population is not the major category of genes in the genome.

We estimate a maximum of 75% saturation for essential genes in *hDf6*. The frequency of recovery of mutations should be about the same across the entire *sDp2* region (assuming no regional bias in mutability), since the screen places no bias on the genetic location of the lethal mutations within *sDp2*. Thus, the degree of saturation attained for the entire *sDp2* region should be the same as that for the *hDf6* interval.

The average forward mutation rate for genes in the *hDf6* interval, using an average 15 mM EMS, was 5×10^{-5} mutations/gene/chromosome screened. The forward mutation rate can be used to estimate the number of chromosomes needed to identify all of the genes in a region, or all of the genes of a given type in the genome. In order to have 95% confidence that any gene has been identified, the number of chromosomes screened should be at least three times that required to recover one mutant allele. For the average gene in C. *elegans*, this would require screening 40,000 to 60,000 chromosomes using a dose between 15 and 25 mM EMS.

We wish to thank DAVID BAILLIE, KIM MCKIM, DENISE CLARK and DONALD RIDDLE for valuable discussion about this work; PAUL MAINS for sharing unpublished results and strains; JONATHAN HODGKIN for comments on A.M.H.'s Ph.D. thesis upon which this paper is based. Some of the strains used were provided by the Caenorhabditis Genetics Center, which was supported by contract NO1-9-2113 between the National Institutes of Health and the Curators of the University of Missouri. A.M.H. was supported by a Medical Research Council (MRC) (Canada) Studentship. This work was supported by a grant from the MRC (Canada) to A.M.R.

LITERATURE CITED

BARRETT, J. A., 1980 The estimation of the number of mutationally silent loci in saturation-mapping experiments. Genet. Res. **35:** 33–44.

BEYER, W. H. (Editor), 1976 *CRC Handbook of Tables for Probability and Statistics*. CRC Press, Cleveland, Ohio.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

CHAREST, D. L., D. V. CLARK, M. E. GREEN and D. L. BAILLIE, 1990 Genetic and fine structure analysis of *unc-26(IV)* and adjacent regions in *Caenorhabditis elegans*. Mol. Gen. Genet. (in press).

CLARK, D. V., 1990 The *unc-22* region in *Caenorhabditis elegans*: genetic analysis and molecular mapping. Ph.D. thesis, Simon Fraser University, Burnaby, Canada.

CLARK, D. V., T. M. ROGALSKI, L. M. DONATI and D. L. BAILLIE, 1988 The *unc-22*(IV) region of *Caenorhabditis elegans*: genetic analysis of lethal mutations. Genetics **129:** 345–353.

COULSON, A., J. SULSTON, S. BRENNER and J. KARN, 1986 Towards a physical map of the genome of the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **83:** 7821–7825.

COULSON, A., R. WATERSTON, J. KIFF, J. SULSTON and Y. KOHARA, 1988 Genome linking with yeast artifical chromosomes. Nature **335:** 184–186.

CROW, E. L., and R. S. GARDNER, 1959 Confidence intervals for the expectation of a poisson variable. Biometrika **46:** 441–453.

EDGLEY, M. L., and D. L. RIDDLE, 1987 *Caenorhabditis elegans*. In *Genetic Maps 1987*, Vol. 4, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

FODOR, A., and P. DEAK, 1985 The isolation and genetic analysis of a *C. elegans* translocation (*szT1*) strain bearing an X-chromosome balancer. J. Genet. **64:** 143–157.

GREENWALD, I., A. COULSON, J. SULSTON and J. PRIESS, 1987 Correlation of the physical and genetic maps in the *lin-12* region of *Caenorhabditis elegans*. Nucleic Acids Res. **15:** 2295–2307.

HARRIS, L. J., 1986 Transposable elements in *C. elegans* var. Bristol and *C. briggsae*. Ph.D. thesis, University of British Columbia, Vancouver, B.C.

HEINE, U., and T. BLUMENTHAL, 1986 Characterization of regions of the *Caenorhabditis elegans* X chromosome containing vitellogenin genes. J. Mol. Biol. **188:** 301–312.

HERMAN, R. K., 1978 Crossover suppressors and balanced recessive lethals in *Caenorhabditis elegans*. Genetics **88:** 49–65.

HILLIKER, A. J., A. CHOVNICK and S. H. CLARK, 1981 The relative mutabilities of vital genes in *D. melanogaster*. Drosophila Inform. Serv. **56:** 64–65.

HILLIKER, A. J., S. H. CLARK, A. CHOVNICK and W. M. GELBART, 1980 Cytogenetic analysis of the chromosomal region immediately adjacent to the *rosy* locus in *Drosophila melanogaster*. Genetics **95:** 95–110.

HOCHMAN, B., 1971 Analysis of chromosome 4 in *Drosophila melanogaster*. II. Ethyl methanesulfonate induced lethals. Genetics **67:** 235–252.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

HOWELL, A. M., 1989 Essential genes in a region of chromosome I in *Caenorhabditis elegans*. Ph.D. thesis, University of British Columbia, Vancouver, Canada.

HOWELL, A. M., S. G. GILMOUR, R. A. MANCEBO and A. M. ROSE, 1987 Genetic analysis of a large autosomal region in *Caenorhabditis elegans* by the use of a free duplication. Genet. Res. **49:** 207–213.

JUDD, B. H., M. W. SHEN and T. C. KAUFMAN, 1972 The anatomy and function of a segment of the X chromosome of *Drosophila melanogaster*. Genetics **71:** 139–156.

KEMPHUES, K. J., M. KUSCH and N. WOLF, 1988 Maternal-effect lethal mutations on linkage group II of *Caenorhabditis elegans*. Genetics **120:** 977–986.

KIM, J. S., and A. M. ROSE, 1987 The effect of gamma radiation on recombination frequency in *Caenorhabditis elegans*. Genome **29:** 457–462.

LEFEVRE, G., and W. WATKINS, 1986 The question of the total gene number in *Drosophila melanogaster*. Genetics **113:** 869–895.

LEICHT, B. G., and J. J. BONNER, 1988 Genetic analysis of chromosomal region 67A-D of *Drosophila melanogaster*. Genetics **119:** 579–593.

LEWIS, J. A., C. H. WU, H. BERG and J. H. Levine, 1980 The genetics of levamisole resistance in the nematode *C. elegans*. Genetics **95:** 905–928.

MACLEOD, A. R., J. KARN and S. BRENNER, 1981 Molecular analysis of the *unc-54* myosin heavy-chain of *Caenorhabditis elegans*. Nature **291:** 386–390.

MAINS, P. E., I. A. SULSTON and W. B. WOOD, 1990 Dominant maternal-effect mutations causing embryonic lethality in *Caenorhabditis elegans*. Genetics **124:** 351–369.

MCDOWALL, J. S., 1990 Genetic analysis around the *hP5* polymorphic site in *Caenorhabditis elegans*. M.Sc. thesis, University of British Columbia, Vancouver, Canada.

MCKIM, K. S., A. M. HOWELL and A. M. ROSE, 1988 The effects of translocations on recombination frequency in *Caenorhabditis elegans*. Genetics **120:** 987–1001.

MCKIM, K. S., and A. M. ROSE, 1990 Chromosome I duplications in *Caenorhabditis elegans*. Genetics **124:** 115–132.

MENEELY, P. M., and R. K. HERMAN, 1979 Lethals, steriles, and

deficiencies in a region of the X chromosome of *Caenorhabditis elegans*. Genetics **92:** 99–115.

MENEELY, P. M., and R. K. HERMAN, 1981 Suppression and function of X-linked lethal and sterile mutations in *Caenorhabditis elegans*. Genetics **97:** 65–84.

MOERMAN, D. G., G. M. BENIAN and R. H. WATERSTON, 1986 Molecular cloning of the muscle gene *unc-22* in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **83:** 2579–2583.

MOERMAN, D. G., and D. L. BAILLIE, 1979 Genetic organization in *Caenorhabditis elegans*: fine structure analysis of the *unc-22* gene. Genetics **91:** 95–103.

MULLER, H. J., 1932 Further studies on the nature and causes of gene mutations, pp 213–255 in *Proceedings of the Sixth International Congress of Genetics*, edited by D. JONES. Brooklyn Botanic Gardens, Menasha, Wisc.

PARK, E.-C., and H. R. HORVITZ, 1986 *C. elegans unc-105* mutations affect muscle and are suppressed by other mutations that affect muscle. Genetics **113:** 853–867.

PERRIMON, N., D. MOHLER, L. ENGSTROM and A. P. MAHOWALD, 1986 X-linked female-sterile loci in *Drosophila melanogaster*. Genetics **113:** 695–712.

PETERS, K., and A. M. ROSE, 1988 Complementing alleles of the *bli-4* locus of *Caenorhabditis elegans*. Genome **30:** s294.

PRASAD, S., and D. L. BAILLIE, 1989 Evolutionarily conserved coding sequences in the *dpy-20 unc-22* region of *Caenorhabditis elegans*. Genomics **5:** 185–198.

ROGALSKI, T. M., 1983 A genetic characterization of the region around the *unc-22* gene in *Caenorhabditis elegans*. Ph.D. thesis, Simon Fraser University, Burnaby, B.C.

ROGALSKI, T. M., and D. L. BAILLIE, 1985 Genetic organization of the *unc-22* IV region of *Caenorhabditis elegans*. Mol. Gen. Genet. **201:** 409–414.

ROGALSKI, T. M., A. M.E. BULLERJAHN and D. L. RIDDLE, 1988 Lethal and amanitin-resistance mutations in the *Caenorhabditis elegans ama-1* and *ama-2* genes. Genetics **120:** 409–422.

ROGALSKI, T. M., D. G. MOERMAN and D. L. BAILLIE, 1982 Essential genes and deficiencies in the *unc-22* region of *Caenorhabditis elegans*. Genetics **102:** 725–736.

ROGALSKI, T. M., AND D. L. RIDDLE, 1988 A *Caenorhabditis elegans* polymerase II gene, *ama-1* IV, and nearby essential genes. Genetics **118:** 61–74.

ROSE, A. M., 1980 Genetic studies on the gene coding for paramyosin in Caenorhabditis elegans: *unc-15* and the adjacent region. Ph.D. thesis, Simon Fraser University, Burnaby, B.C., Canada.

ROSE, A. M., and D. L. BAILLIE, 1979 Effect of temperature and parental age on recombination and nondisjunction in *Caenorhabditis elegans*. Genetics **92:** 409–418.

ROSE, A. M., and D. L. BAILLIE, 1980 Genetic organization of the region around *unc-15* (I), a gene affecting paramyosin in *Caenorhabditis elegans*. Genetics **96:** 639–648.

ROSE, A. M., D. L. BAILLIE and J. CURRAN, 1984 Meiotic pairing behavior of two free duplications of linkage group I in *Caenorhabditis elegans*. Mol. Gen. Genet. **195:** 52–56.

ROSENBLUTH, R. E., C. CUDDEFORD and D. L. BAILLIE, 1983 Mutagenesis in *Caenorhabditis elegans*. I. A rapid eukaryotic mutagen test system using the reciprocal translocation *eT1(III;V)*. Genetics **109:** 493–511.

ROSENBLUTH, R. E., T. M. ROGALSKI, R. C. JOHNSEN, L. M. ADDISON and D. L. BAILLIE, 1988 Genomic organization in *Caenorhabditis elegans*: deficiency mapping on linkage group V(left). Genet. Res. **52:** 105–118.

SHANNON, M. P., T. C. KAUFMAN, M. W. SHEN and B. H. JUDD, 1972 Lethality patterns and morphology of selected lethal and semi-lethal mutations in the zeste-white region of *Drosophila melanogaster*. Genetics **72:** 615–638.

SIGURDSON, D. C., G. J. SPANIER and R. K. HERMAN, 1984 *C. elegans* deficiency mapping. Genetics **108:** 331–345.

STARR, T., A. M. HOWELL, J. MCDOWALL, K. PETERS and A. M. ROSE, 1989 Isolation and mapping of DNA probes within the linkage group I gene cluster of *Caenorhabditis elegans*. Genome **32:** 365–372.

SULSTON, J. E., and S. BRENNER, 1974 The DNA of *Caenorhabditis elegans*. Genetics **77:** 95–104.

WATERSTON, R. H., 1981 A second informational suppressor, *sup-7(X)* in *Caenorhabditis elegans*. Genetics **97:** 307–325.

WATERSTON, R. H., and S. BRENNER, 1978 A suppressor in the nematode *Caenorhabditis elegans* acting on specific alleles of many genes. Nature **275:** 715–719.

M. W. YOUNG and B. H. JUDD, 1978 Nonessential sequences, genes, and the polytene chromosome bands of *Drosophila melanogaster*. Genetics **88:** 723–742.

Communicating editor: A. CHOVNICK