Genet. Res., Camb. (1988), **52**, *pp* 105–118 *With* 1 *text-figure Printed in Great Britain*

Genomic organization in *Caenorhabditis elegans*: deficiency mapping on linkage group V(left)

RAJA E. ROSENBLUTH†, TERESA M. ROGALSKI*, ROBERT C. JOHNSEN, LINDA M. ADDISON AND DAVID L. BAILLIE

Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., Canada V5A 1S6

(*26 November 1987 and in revised form 27 January 1988*)

Summary

In this study we genetically analyse a large autosomal region (23 map units) in *Caenorhabditis elegans*. The region comprises the left half of linkage group V [LGV(left)] and is recombinationally balanced by the translocation *eT1(III;V)*. We have used rearrangement breakpoints to subdivide the region from the left end of LGV to *daf-11* into a set of 23 major zones. Twenty of these zones are balanced by *eT1*. To establish the zones we examined a total of 110 recessive lethal mutations derived from a variety of screening protocols. The mutations identified 12 deficiencies, 1 duplication, as well as 98 mutations that fell into 59 complementation groups, significantly increasing the number of available genetic sites on LGV. Twenty-six of the latter had more than 1 mutant allele. Significant differences were observed among the alleles of only 6 genes, 3 of which have at least one 'visible' allele. Several deficiencies and 3 alleles of *let-336* were demonstrated to affect recombination. The duplication identified in this study is *sDp30(V;X)*. Lethal mutations covered by *sDp30* were not suppressed uniformly in hermaphrodites. The basis for this non-uniformity may be related to the mechanism of *X* chromosome dosage compensation in *C. elegans*.

1. Introduction

The 6 chromosomes constituting the *Caenorhabditis elegans* genome are under intensive analysis at both the genetic and molecular levels. Almost 800 loci have been mapped genetically (Edgley & Riddle, 1987), over half the genome is represented in overlapping cosmid clones (Coulson & Sulston, 1986) and the correlation between the genetic and physical (DNA) maps is being carried out by the co-operative effort of many laboratories. However, the function of at least 90% of the genome still remains to be elucidated. To identify new functional sites in a large fraction of the genome, we have undertaken the genetic analysis of the left half of linkage group V [LGV(left)], a region of approximately 23 map units (m.u.). A region this size should include representatives of most types of chromosomal loci, be they different classes of genes, regulatory sites, or sites affecting chromosome behaviour. One of our long-term goals is to saturate LGV(left) for mutations in essential genes (Johnsen, Rosenbluth & Baillie, 1986). This goal is feasible in *C. elegans* since strains can be maintained indefinitely in liquid nitrogen. To our knowledge the only other studies concerned with saturating a large chromosomal region for mutations in a given class of genes are those by Lefevre & Watkins (Lefevre, 1981; Lefevre & Watkins, 1986) and by the Nüsslein-Volhard group (see Nüsslein-Volhard, Wieschaus & Kluding, 1984) in *Drosophila* and those by Meneely & Herman (1979; 1981) and by Howell *et al.* (1987) in *C. elegans*.

The region we refer to as LGV(left) is recombinationally balanced by the translocation *eT1(III;V)* (Rosenbluth & Baillie, 1981). Genes detected by morphological and behavioural mutants ('visibles') are unevenly distributed along its genetic map (Edgley & Riddle, 1987). Most genes appear 'clustered' near the center of the chromosome, around *dpy-11*, while the region to the left of *unc-46* appears sparsely populated. Whether this uneven distribution reflects an uneven distribution of genes along the DNA, or is due to a non-uniform rate of recombination per length of DNA, will be determined by the correlation of the genetic and physical maps.

In order to identify and position genetic loci along the chromosome we used deficiency, duplication and recombination mapping of recessive lethal mutations and divided LGV(left) into an initial set of zones delineated by rearrangement breakpoints. This strategy has been applied in *C. elegans* to the small regions

* Present address: Division of Biological Sciences, University of Missouri, Columbia, MO 65211, USA.
† Corresponding author.

around *unc-22* IV (Moerman & Baillie, 1979; Rogalski, Moerman & Baillie, 1982; Rogalski & Baillie, 1985; D. V. Clark, unpublished results); *unc-15* I (Rose & Baillie, 1980); and *unc-60* V (McKim *et al.* 1988). The strategy has also been applied to the following larger regions. The 7·5 m.u. on LGX balanced by the duplication *mnDp1(X;V)* (Meneely & Herman, 1979, 1981); the 5 m.u. region between *dpy-10* and *rol-5*, balanced by *mn*C1, on LGII (Sigurdson, Spanier & Herman, 1984); a 9 m.u. region near *unc-54* I (Anderson & Brenner, 1984); and the 15 m.u. region on LGI balanced by *sDp2(I;f)* (Howell *et al.* 1987). We have analysed a total of 110 LGV recessive lethal mutations, isolated in our laboratory. The lethals were isolated to obtain alleles of essential genes as well as deficiencies. This method of isolating deficiencies differs from ones commonly used in *C. elegans* in that it does not require the deletion of a specific genetic site in the screening process. It is, therefore, particularly suited to the recovery of deficiencies in a large chromosomal region. Most of the lethal mutations were isolated by screening over the whole of LGV(left). Thus deficiencies could be expected for sites all across the balanced region. Analysis of the mutations showed that they represent 12 deficiencies, 1 duplication and alleles of 59 essential genes. Thirty of the mutations (representing 10 deficiencies and alleles of 18 genes) were partially analysed in the course of previous studies (Rosenbluth, Cuddeford & Baillie, 1985; McKim *et al.* 1988). The 59 essential genes have been mapped relative to 23 major zones established by the breakpoints of 17 deficiencies (5 coming from other sources, see Materials and Methods). Twenty of the zones are balanced by *eT1(III;V)*.

While the major outcome of this study was the identification of new genes and their placement into chromosomal zones, several additional points of interest emerged. First, and of particular interest, were a number of mutations at the left end of LGV that strongly inhibited recombination. Second were results, obtained with a newly identified (V;X) duplication, that may be relevant to the phenomenon of *X*-dosage compensation in *C. elegans*. Finally, our data indicated that the uneven distribution of visible genes, on LGV, is paralleled by that of the essential genes. The same phenomenon has been demonstrated on the right half of LGII (Herman, 1978; Sigurdson, Spanier & Herman, 1984); on LGI (Rose & Baillie, 1980; Howell *et al.* 1987) and on LGIV (Rogalski & Baillie, 1985). Our lethal mutations, identifying genes or deficiencies, are landmarks for correlating the genetic and physical maps of LGV.

2. Materials and Methods

(i) *General*

The nomenclature follows the uniform system adopted for *C. elegans* (Horvitz *et al.* 1979). Nematodes were cultured on Petri plates containing nematode growth medium streaked with *Escherichia coli* OP50 (Brenner, 1974).

(ii) *Strains*

Unless otherwise indicated, all strains were derived from the wild-type *C. elegans* strain, N2 (var. Bristol). The N2 strain and strains carrying the following mutations were obtained from the Medical Research Council stock collection in Cambridge, England, or from the Caenorhabditis Genetics Center at the University of Missouri, Columbia. LGIII: *dpy-18 (e364)*; LGV: *unc-34(e315* and *e566)*, *unc-60(e677)*, *emb-29(g52)*, *unc-62(e644)*, *unc-46(e177)*, *dpy-11 (e224)*, *unc-68(e540)*, *unc-70(e524)*, *rol-3(e754)*, *unc-23(e25)*, *unc-42(e270)*, *emb-18(g21)*, *unc-41(e268)*, *emb-22(g32)*, *sma-1(e30)*; and the reciprocal translocation *eT1(III;V)*, which carries the recessive *unc-36(III)* defect, *e873*. The mutation *lin-40(e2173)*, isolated by S. W. Emmons, was kindly supplied by J. Hodgkin (M.R.C., Cambridge). Deficiencies *mDf1* and *mDf3* (Brown, 1984) were from D. L. Riddle's laboratory (Columbia, MO). The deficiency *nDf32* (Park & Horvitz, 1986) as well as *nDf18* and *nDf31* originated in R. H. Horvitz's laboratory (M.I.T.). The origin of '*s*'-numbered mutations derived from our laboratory is described below.

(iii) *Origin of recessive lethal mutations ('lethals') on LGV(left)*

Lethals were isolated either in this or one of three previous studies. Treatment with an external mutagen consisted of exposing an adult P_0 hermaphrodite to either 4 h of a given concentration of ethyl methanesulfonate (EMS), or to a given dose of γ irradiation. Radiation was carried out using a ^{60}Co radiation unit (Gamma Cell 200, Atomic Energy of Canada). The dose varied from 296 R/min to 263 R/min. The mutagen treatment and selection system used for each lethal are given in Table 1 (see Results). Four different selection systems were used.

(*a*) The 'eT1 screen' for *unc-46(V)* linked levels in the *eT1(III;V)* balanced region. The characteristics of *eT1(III;V)* (abbreviated '*eT1*'), and our system for selecting lethals balanced by it, have already been described (Rosenbluth & Baillie, 1981; Rosenbluth, Cuddeford & Baillie, 1983). Briefly, the screen involved mutagenizing adult *dpy-18/eT1;unc-46/eT1* hermaphrodites, picking individual wild-type F_1 heterozygotes and screening the F_2 progenies for Dpy Uncs. Lines from those F_1s that produced no mature Dpy Uncs were retained by picking wild-types. These were presumed to carry at least one recessive lethal on either LGIII(right) or LGV(left). In one experiment lines from all F_1s were retained until adult F_2 Dpy Uncs from each had been tested for fertility. Those lines carrying an adult sterile or maternal effect lethal

https://doi.org/10.1017/S0016672300027476 Published online by Cambridge University Press

mutation were then also retained as 'lethal' lines. Adult sterile Dpy Uncs produced no fertilized eggs. Dpy Uncs carrying a maternal effect lethal mutation produced fertilized eggs whose development was arrested in the immediate or subsequent generation. Mutations selected as maternal effect lethals were not analysed in this study. Mutations with numbers between and including *s217* and *s742* were isolated in the above two studies. The remaining mutations from eT1 screens were isolated in this study. Recombination linkage mapping (see below) selected the mutations that were on LGV.

(*b*) The 'nT1 screen' for lethals in the *nT1(IV;V)* balanced region of a Bergerac LGV chromosome. The isolation of these lethals was carried out and described by L. A. M. Donati (1985). The translocation *nT1(IV;V)* balances LGIV(right) and LGV-(left) and is associated with a recessive vulvaless phenotype (Ferguson & Horvitz, 1985). The Bergerac chromosome originally was derived from the wild-type B0 (var. Bergerac) strain. Briefly, the screen involved crossing homozygous *unc-22(s727)IV* Bergerac hermaphrodites with *unc-22(s7) unc-31(e169)/nT1(IV);+/nT1(V)* Bristol males. Individual wild-types F_1 hermaphrodites [*unc-22(s727)/nT1(IV);+/nT1(V)*] were picked and the F_2 progenies were screened for Unc-22s. Lines failing to produce mature Unc-22s were retained and presumed to carry at least one recessive lethal on either LGIV(B0) or LGV(B0). To determine on which chromosome the lethals were positioned, heterozygotes from each strain were crossed to wild-type N2 males and individual *unc-22(s727)*-bearing F_1 hermaphrodites were selected in 1% nicotine (Moerman & Baillie, 1979). Since *nT1* was no longer present, a lethal on LGV(B0) was detected by producing a normal 3:1 phenotypic ratio for Wild:Unc-22, while lethals *cis*-linked to *unc-22(s727)* gave a greater ratio. One of the lethals on LGV(B0), *s743*, was analysed further in the present study. To do so, *s743* was balanced over *eT1(III;V)* instead of *nT1(IV;V)*.

(*c*) The 'd11-u42 screen' for lethals linked to *dpy-11 unc-42(V)* (mutations with numbers between and including *s113* and *s206*). All except three of these lethals were isolated from EMS treated *dpy-11 unc-42/unc-68* P_0 hermaphrodites. Individual heterozygous F_1 hermaphrodites were picked and their F_2 progenies were screened for mature Dpy Unc-42 adults. Strains were established from those F_1s that gave no, or very few, mature Dpy Uncs. Each of these presumably carried a lethal that was either just to the left of *dpy-11*, between *dpy-11* and *unc-42* or just to the right of *unc-42*. Initially the lethal mutations were maintained as *dpy-11 let-(sx) unc-42/unc-68* strains. In some cases either the *dpy-11* or *unc-42* marker was lost. Three mutations, *s115*, *s116* and *s127*, were isolated from treated homozygous *dpy-11 unc-42* or *unc-42* P_0 hermaphrodites. After treatment, the hermaphrodites were mated to N2 males, individual heterozygous F_1 hermaphrodites were allowed to 'self' and the F_2 progenies were screened for the absence of mature Dpy Unc or Unc-42 adults. These mutations were initially also maintained in *dpy-11 let-(sx) unc-42/unc-68* strains. Subsequently *eT1* replaced *unc-68* as a balancer in all strains.

(*d*) The 'u60&d11 screen' for lethals tightly linked to *unc-60* or *dpy-11* (mutations with numbers between and including *s815* and *s833*). The isolation of these mutations was described and carried out by McKim *et al.* (1988). The screen selected for lethals that were close to either *unc-60* or *dpy-11* but were not in the region deleted by *sDf26*. The deficiency *sDf26* deletes most of the region between *unc-60* and *dpy-11* (see Results). In brief, P_0 *unc-60 dpy-11/sDf26* hermaphrodites were treated with EMS, individual wild-type F_1s were picked and their F_2 progenies screened for the absence of mature Dpy Unc adults. Strains producing no or very few mature Dpy Uncs were retained. The lethals were then balanced over *eT1*.

(iv) *Recombination mapping*

(*a*) Linkage mapping the *eT1* balanced mutations. These were linked to either *dpy-18(III)* or to *unc-46(V)*. To determine on which linkage group a particular lethal mapped, heterozygous hermaphrodites were crossed to wild-type males. The self-progeny were scored from those F_1 hermaphrodites that were *dpy-18/+;unc-46/+*, with a lethal *cis*-linked to at least one of the markers. F_1s carrying no *unc-46* linked lethal were expected to give a normal number of Unc-46s (3:1 ratio for Wild:Unc-46) but few Dpy-18s, while those with *unc-46* linked lethals were expected to give relatively few Unc-46s. Only lethals linked to *unc-46* were examined in this study.

(*b*) Two-factor mapping. Recombination distances were measured between lethals and *unc-46* or *dpy-11* by scoring the progenies from the P_0s shown in Table 2 (see Results). The experiments were carried out under the standard mapping conditions suggested by Rose & Baillie (1979). The temperature was 20 °C and all the viable F_1 progeny were counted.

(*c*) Three factor mapping. To establish whether an *unc-46* linked lethal was to the left or right of *unc-46*, *unc-46 let-x/unc-60 dpy-11* heterozygotes, which no longer carried *dpy-18*, were constructed. From these, viable Unc-46 recombinant F_1s were picked. Examination of the F_2 progeny then determined the recombinant chromosome's genotype and, consequently, the lethal's position.

The positions of *dpy-11 unc-42* linked lethals were established by examining the viable recombinants from the *dpy-11 unc-42 let-x/unc-68* heterozygotes. If both Unc-42 and Dpy Unc-42 recombinants appeared, the lethal was to the left of *dpy-11*; if Dpy and Unc-42 (but no Dpy Unc-42) recombinants appeared, it was between the two markers; Dpy plus Dpy Unc-42 recombinants indicated that the lethal was to the right

Table 1. *Assignment of LGV recessive lethal mutations to genes or rearrangements*

Zone	Gene	Mutation	Source of mutation			Phenotype
			Screen[a]	Mutagen[b]	Dose	
1	*let-336*	*s521*	eT1	γ	500 R	Early larval
		s741[d]	eT1	γ	1500 R	Early larval
		s957	eT1	EMS	0·012 M	Early larval
2	*let-431*	*s1044*	eT1	EMS	0·012 M	Adult sterile
		s1049	eT1	EMS	0·012 M	Adult sterile
4	*let-326*	*s238*[d]	eT1	EMS	0·025 M	Mid larval
	emb-29	*s819*[c]	u60&d11	EMS	0·012 M	Egg lethal
5	*let-426*	*s826*[c]	u60&d11	EMS	0·012 M	Mid larval
6	*let-327*	*s247*[d]	eT1	EMS	0·025 M	Slow development; translucent
	let-347	*s1035*	eT1	EMS	0·012 M	Late larval
7	*let-330*	*s573*	eT1	EMS	0·004 M	Mid larval
		s1702[e]	eT1	Spo		Mid larval
8A	*lin-40*	*s1053*	eT1	EMS	0·012 M	Adult sterile
		s1704[e]	eT1	EMS	0·025 M	Adult sterile–maternal (over *Df*)
	let-338	*s503*	eT1	γ	500 R	Mid larval
		s1020	eT1	EMS	0·012 M	Mid larval
9	*unc-62*	*s472*[f]	eT1	Spo		Putative egg lethal
	let-341	*s1031*	eT1	EMS	0·012 M	Putative egg lethal
	let-342	*s1029*	eT1	EMS	0·012 M	Mid larval
	let-344	*s376*	eT1	EMS	0·012 M	Putative egg lethal
	let-345	*s578*	eT1	EMS	0·004 M	Mid larval
	let-348	*s998*	eT1	γ	1500 R	Mid larval
	let-430	*s1042*	eT1	EMS	0·012 M	Adult sterile
10	*let-331*	*s427*	eT1	EMS	0·004 M	Mid larval (15 °C); slow development (20 °C)
	let-350	*s250*	eT1	EMS	0·025 M	Late larval–adult sterile
	let-415	*s129*	d11-u42	EMS	0·025 M	Late larval
	let-417	*s204*	d11-u42	EMS	0·025 M	Early larval
		s1313[e]	eT1	EMS	0·012 M	
	let-419	*s219*	eT1	EMS	0·025 M	Mid larval
	let-420	*s723*[d]	eT1	γ	1500 R	Late larval–adult sterile
		s1046	eT1	EMS	0·012 M	Adult sterile
		s1058	eT1	EMS	0·012 M	Adult sterile
	let-428	*s1070*	eT1	EMS	0·012 M	Adult sterile
11A	*let-401*	*s193*	d11-u42	EMS	0·025 M	Mid larval
11B	*let-349*	*s217*	eT1	EMS	0·025 M	Early larval
		s502	eT1	γ	500 R	Early larval
		s572	eT1	EMS	0·004 M	Late larval (15 °C); wild type (20 °C)
	let-418	*s1045*	eT1	EMS	0·012 M	Adult sterile–maternal
	let-421	*s288*	eT1	EMS	0·012 M	Late larval–maternal
	let-422	*s194*	d11-u42	EMS	0·025 M	Early larval
		s738[d]	eT1	γ	1500 R	Early larval
		s739[d]	eT1	γ	1500 R	Early larval
		s1321[e]	eT1	EMS	0·012 M	
11B′	*let-329*	*s575*	eT1	EMS	0·004 M	Early larval
	let-429	*s584*	eT1	EMS	0·004 M	Adult sterile
12	*let-402*	*s127*	d11-u42	EMS	0·05 M	Mid larval
		s500	eT1	γ	500 R	Early larval
		s992	eT1	γ	1500 R	Early larval
	let-403	*s120*	d11-u42	EMS	0·025 M	Mid–late larval
		s246[e]	eT1	EMS	0·025 M	
		s498	eT1	γ	500 R	Late larval
13	*let-337*	*s382*[e]	eT1	EMS	0·012 M	
		s825[c]	u60&d11	EMS	0·012 M	Mid larval
		s1018	eT1	EMS	0·012 M	Late larval–maternal
		s1024	eT1	EMS	0·012 M	Late larval–maternal
14	*let-410*	*s815*[c]	u60&d11	EMS	0·012 M	Mid larval
	unc-70	*s115*	d11-u42	EMS	0·05 M	Mid larval

https://doi.org/10.1017/S0016672300027476 Published online by Cambridge University Press

15	*let-332*	*s234*	eT1	EMS	0·025 M	Putative egg lethal
		s369	eT1	EMS	0·012 M	Putative egg lethal
		s1021	eT1	EMS	0·012 M	Early larval (leaky)
	let-339	*s1019*	eT1	EMS	0·012 M	Mid larval (15 °C); leaky (20 °C)
	let-343	*s816*[c]	u60&d11	EMS	0·012 M	Early–mid larval
		s1025	eT1	EMS	0·012 M	Putative egg lethal
	let-346	*s373*	eT1	EMS	0·012 M	Late larval
		s1026	eT1	EMS	0·012 M	Late larval
	let-404	*s119*	d11-u42	EMS	0·025 M	Mid larval
	let-425	*s385*	eT1	EMS	0·012 M	Adult sterile
16	*let-335*	*s232*	eT1	EMS	0·025 M	Mid larval
	let-405	*s116*	d11-u42	EMS	0·05 M	Early larval
		s388	eT1	EMS	0·012 M	Mid larval
		s829[c]	u60&d11	EMS	0·012 M	Mid larval
	let-406	*s514*	eT1	γ	500 R	Mid larval
	let-411	*s223*	eT1	EMS	0·025 M	Late larval
	let-423	*s818*[c]	u60&d11	EMS	0·012 M	Early larval
17	*let-408*	*s195*	d11-u42	EMS	0·025 M	Late larval
		s827[c]	u60&d11	EMS	0·012 M	Putative egg lethal
	let-413	*s128*	d11-u42	EMS	0·025 M	Putative egg lethal
	let-414	*s114*	d11-u42	EMS	0·025 M	Mid larval
		s207	d11-u42	EMS	0·05 M	Mid larval
	let-424	*s248*	eT1	EMS	0·025 M	Adult sterile
		s384	eT1	EMS	0·012 M	Adult sterile
18	*let-412*	*s579*	eT1	EMS	0·004 M	Adult sterile
	rol-3	*s126*	d11-u42	EMS	0·025 M	Early larval
		s422	eT1	EMS	0·004 M	Mid larval
		s501	eT1	γ	500 R	Early larval
		s742[d]	eT1	γ	1500 R	Mid larval
		s833[c]	u60&d11	EMS	0·012 M	Mid larval
		s1030[e]	eT1	EMS	0·012 M	
		s1040	eT1	EMS	0·012 M	Fertile (15 °C); Mid larval (20 °C)
19	*let-334*	*s383*	eT1	EMS	0·012 M	Mid larval
		s908	eT1	EMS	0·012 M	Early larval
	let-340	*s1022*	eT1	EMS	0·012 M	Mid larval
	let-409	*s206*	d11-u42	EMS	0·05 M	Early larval
		s823[c]	u60&d11	EMS	0·012 M	Early larval
	let-416	*s113*	d11-u42	EMS	0·025 M	Late larval
20	*let-407*	*s118*	d11-u42	EMS	0·025 M	Early larval
		s830[c]	u60&d11	EMS	0·012 M	Early larval
21	*let-427*	*s1057*[e]	eT1	EMS	0·012 M	Adult Sterile
11A–15	*sDf20*[d]	*s565*	eT1	γ	1500 R	
6–12	*sDf26*[d]	*s721*	eT1	γ	1500 R	
7–9	*sDf27*[d]	*s556*	eT1	γ	1500 R	
3–8A	*sDf28*[d]	*s722*	eT1	γ	1500 R	
19–21	*sDf29*[d]	*s728*	eT1	γ	1500 R	
11A–15	*sDf30*[d]	*s740*[h]	eT1	γ	1500 R	
1–7	*sDf31*	*s743*[i]	nT1	N2/BO		
1–3	*sDf32*[g]	*s583*	eT1	EMS	0·004 M	
1–4	*sDf33*[g]	*s993*	eT1	γ	1500 R	
2–7	*sDf34*[g]	*s996*	eT1	γ	1500 R	
17–23	*sDf35*	*s821*[c]	u60&d11	EMS	0·012 M	
11B′	*sDf36*[f]	*s473*	eT1	Spo	*s473* fails to complement *let-329*(*s575*) and *let-429*(*s584*)	
11B–15	*sDp30*	*s740*[h]	eT1	γ	1500 R	

[a] LGV regions screened (see Materials and methods): eT1 and nT1 = LGV(left). u60&d11 = Regions that are adjacent to *unc-60* and *dpy-11* but are not deleted by *sDf26*. d11–u42 = Regions adjacent to or within the *dpy-11* to *unc-42* interval. [b] Spo = spontaneous. [c] Isolated and mapped by McKim *et al.* (1988). [d]Partially mapped previously (Rosenbluth *et al.* 1985). [e] Each of the following pairs of mutations were carried on the same chromosome. *s246* and *s1704*; *s382* and *s1312*; *s1030* and *s1313*; *s1057* and *s1702*. [f] Previously (Rosenbluth *et al.* 1985) *s472* was assigned to *let-328*; *s473* was assigned to *let-329*. [g] Partially mapped by McKim *et al.* (1988). [h] *sDf30* and *sDp30* were isolated from the same strain and are assumed to be the result of a single transposition. [i] Isolated by Donati (1985).

https://doi.org/10.1017/S0016672300027476 Published online by Cambridge University Press

Table 2. *Two-factor mapping data for lethal mutations at 20 °C*

Zone[a]	Gene	Mutation	P_0 hermaphrodite	Adult F_1 progeny: *let* to marker recombinants[b]	Total	Marker[c]	Distance[d] (m.u.)
1	*let-336*	*s521*	*dpy-18/+;let-336 unc-46/++*	0	488	u46	0
		s741	*dpy-18/+;let-336 unc-46/++*	132	2172	u46	13·0 (11·0–15·0)
		s957	*dpy-18/+;let-336 unc-46/++*	134	2149	u46	13·4 (11·3–15·5)
3/4	*sDf32*		*dpy-18/+;sDf32 unc-46++*	0	1692	u46	0
4	*let-326*	*s238*	*dpy-18/+;let-326 unc-46/++*	126	1592	u46	17·3 (14·6–20·0)
4/5	*sDf33*		*dpy-18/+;sDf33 unc-46/++*	120	2344	u46	10·8 (9·0–12·6)
6	*let-327*	*s247*	*dpy-18/+;let-327 unc-46/++*	184	2683	u46	14·8 (12·8–16·8)
	let-347	*s1035*	*dpy-18/+;let-347 unc-46/++*	132	3028	u46	9·1 (7·6–10·6)
7	*let-330*	*s573*	*dpy-18/+;let-330 unc-46/++*	37	943	u46	8·3 (5·7–10·9)
7/8	*sDf34*		*dpy-18/+;sDf34 unc-46/++*	0	1180	u46	0
8A	*lin-40*	*e2173*	*lin-40 dpy-11/++*	97	2391[e]	dll	8·5 (6·9–10·1)[f]
	let-338	*s1020*	*dpy-18/+;let-338 unc-46/++*	74	4351	u46	3·5 (2·7–4·3)
8A/B	*sDf28*		*dpy-18/+;sDf28 unc-46/++*	0	807	u46	0
9	*let-344*	*s376*	*dpy-18/+;let-344 unc-46/++*	18	1276	u46	2·9 (1·6–4·1)
	unc-62	*s472*	*dpy-18/+;unc-62 unc-46/++*	26	1925	u46	2·7 (1·6–3·8)
	let-342	*s1029*	*dpy-18/+;let-342 unc-46/++*	27	2136	u46	2·6 (1·6–3·6)
	let-345	*s578*	*dpy-18/+;let-345 unc-46/++*	10	866	u46	2·3 (1·1–4·2)
10	*let-331*	*s427*	*+let-427 unc-46+/unc-60++dpy-11*	7	894	u46	1·2 (0·5–4·1)[g]
10/11	*sDf20*		*dpy-18/+;unc-46 sDf20/++*	7	1935	u46	0·7 (0·3–1·8)
	sDf30		*dpy-18/+;unc-46 sDf30/++*	6	1442	u46	0·8 (0·8–1·8)
11A	*let-401*	*s193*	*let-401 dpy-11+unc-42/++unc-68+*	6	1473	d11	0·6 (0·2–1·3)
11B	*let-422*	*s194*	*let-422 dpy-11+unc-42/++unc-68+*	3	1407	d11	0·3 (0·1–0·9)
12	*let-402*	*s127*	*let-402 dpy-11+unc-42/++unc-68+*	1	1229	d11	0·1 (0·0–0·5)
	let-403	*s120*	*let-403 dpy-11+unc-42/++unc-68+*	1	2583	d11	0·1 (0·0–0·2)
14	*unc-70*	*s115*	*dpy-11 unc-70+unc-42/++unc-68+*	4	659	d11	0·9 (0·2–2·4)
15	*let-404*	*s119*	*dpy-11+let-404 unc-42/+unc-68++*	2	1377	d11	0·2 (0·0–0·8)
16	*let-405*	*s116*	*dpy-11+let-405 unc-42/+unc-68++*	9	1817	d11	0·7 (0·3–1·4)
17	*let-408*	*s195*	*dpy-11+let-408 unc-42/+unc-68++*	8	1268	d11	1·0 (0·4–1·9)
	let-413	*s128*	*dpy-11+let-413 unc-42/+unc-68++*	21	1298	d11	2·5 (1·5–3·5)
	let-414	*s114*	*dpy-11+let-414 unc-42/+unc-68++*	12	1298	d11	1·4 (0·7–2·5)
		s207	*dpy-11+let-414 unc-42/+unc-68++*	10	1558	d11	1·0 (0·5–1·8)
18	*rol-3*	*s126*	*dpy-11+rol-3 unc-42/+unc-68++*	11	1068	d11	1·6 (0·8–2·8)
19	*let-409*	*s206*	*dpy-11+let-409 unc-42/+unc-68++*	6	1152	d11	0·8 (0·3–1·7)
	let-416	*s113*	*dpy-11+let-416 unc-42/+unc-68++*	19	1604	d11	1·8 (1·0–2·6)
20	*let-407*	*s118*	*dpy-11+let-407 unc-42/+unc-68++*	34	1534	d11	3·3 (2·2–4·4)

[a] In the case of a deficiency, the zones indicated are the ones separated by the breakpoint nearest to *unc-46*. [b] When *unc-46* was the marker, only Unc-46 (i.e. not Dpy Unc) phenotypes were scored as recombinants. [c] u46 = *unc-46*. d11 = *dpy-11*. [d] Distance (with 95% confidence limits) from *let* mutation to *marker*. For the *unc-46* marker [except from *let-331*(*s427*)], distance = $100[1-\sqrt{(1-4U)}]$, where U = frequency of Unc-46 recombinants. For the *dpy-11* marker [except from *lin-40*(*e2173*)], distance = $100[1-\sqrt{(1-3D)}]$, where D = frequency of (Dpy + Dpy Unc) recombinants. For more than 14 recombinants the confidence limits are based on $1{\cdot}96\sqrt{npq}$ recombinants, where n = total number of progeny; p = frequency of recombinants scored and q = frequency of the remaining progeny. For 14 or less recombinants the limits were based on a table by Stevens (1942). [e] Includes larval F_1s. [f] From *lin-40*(*e2173*), distance = $100[1-\sqrt{(1-4D)}]$, where D = frequency of the Dpy recombinants. [g] From *let-331*(*s427*), distance = $100[1-\sqrt{(1-3U)}]$, where U = frequency of the Unc-46 recombinants.

of *unc-42*. In this study we examined only lethals that were to the left of *unc-42*. The positions of lethals from the 'u60&d11' screen had been determined in a similar manner by McKim *et al.* (1988).

(v) *Complementation tests*

(*a*) Lethal *vs.* lethal. These complementation tests required that the two lethal mutations be tightly *cis*-linked to a common visible marker. No such marker was common to all the lethal mutations; e.g. some were linked to *unc-46*, others to *dpy-11*, etc. Advantage was, therefore, taken of the characteristics of *eT1*. In crosses between two *eT1* heterozygotes only euploid progeny survive. Thus, surviving F_1s that are homozygous for normal LGIII must also be homozygous for normal LGV. Therefore, all LGV lethals were balanced over *eT1* and 'pseudolinked' to a common LGIII marker, *dpy-18*(*e364*). Two *dpy-18/eT1*-(*III*);*let-x/eT1*(*V*) individuals, each with a different lethal mutation or deficiency, were crossed. Comple-

https://doi.org/10.1017/S0016672300027476 Published online by Cambridge University Press

mentation was indicated if mature Dpy (or Dpy Unc) progeny appeared among the F_1s.

(*b*) Lethal *vs.* visible marker (*m*). In one type of test *dpy-18/eT1;let-x/eT1* males or hermaphrodites were crossed to either *m/m* or *m/+* individuals. Appearance of the marker phenotype among the F_1s indicated a failure to complement. If no F_1 marker was observed, several wild-type F_1s were picked to see if any segregated both Dpy and marker phenotypes. The appearance of both indicated that the wild-type F_1 had been *dpy-18/+;let-x +/+m* and that the lethal complemented the marker mutation. As an alternative test, F_1 phenotypes were examined from *dpy-18/eT1;let-x/eT1* hermaphrodites crossed to *dpy-18/eT1;m/eT1* males. Dpy non-marker progeny indicated complementation; Dpy marker progeny indicated failure to complement.

3. Results

A total of 110 recessive lethal mutations ('lethals') on LGV(left), obtained from a variety of selection systems, has been analysed. Data derived in the course of other studies have been indicated as such in the appropriate places.

(i) *Sources of the lethal mutations*

Table 1 (columns 3–6) compiles the mutations and their sources. The mutations were recovered from four types of screening protocols (see Materials and methods) on the following bases: 12 EMS-induced ones as tightly linked to *unc-60* or *dpy-11* ('u60&d11' screen); 17 EMS-induced ones as tightly linked to the *dpy-11-unc-42* interval ('d11-u42' screen); one, on a Bergerac chromosome, as balanced by the translocation *nT1(IV;V)* ('nT1' screen); and 81, linked to *unc-46*, as balanced by the translocation *eT1(III;V)* ('eT1' screen). The mutation from the nT1 screen arose in an N2/B0 heterozygote. Of the 81 eT1 mutations, 55 were EMS-induced, 23 were γ-ray induced and three arose spontaneously.

(ii) *Mapping the lethal mutations*

Appropriate complementation tests were carried out between (*a*) the lethals *inter se*, (*b*) the lethals and deficiencies from other sources (*mDf1, mDf3, nDf18, nDf31, nDf32*) and (*c*) the lethals and mutations of established genes. On the basis of these tests each mutation was classified as either being a deficiency or as belonging to a single complementation group. A mutation was classified as a deficiency if it failed to complement at least 3 (in the case of EMS mutations) or at least 2 (in the case of γ and spontaneous mutations) other complementing mutations. Of the 110 lethals examined, 12 were identified as deficiencies, one of which was found to be associated with a duplication, *sDp30* (described below). Eight deficiencies were recovered after γ-ray treatment, 2 after EMS treatment, 1 was the mutation recovered from the N2/B0 heterozygote, and 1 was a spontaneous mutation. The latter, *s473*, had originally been classified as an allele of *let-329* (Rosenbluth, Cuddeford & Baillie, 1985). Since it failed to complement both *let-329(s575)* and *let-429(s584)* it was reassigned as *sDf36*. The remaining 98 mutations fell into 59 complementation groups.

Together with recombination data, the complementation tests led to the construction of the map in Fig. 1. The breakpoints of deficiencies and *sDp30* divided LGV(left) into a number of 'zones'. As the study was progressing, and as the number of zones was increasing, we decided to assign major zone numbers to the first 20 identified zones that were balanced by *eT1* (i.e. those to the left of *unc-42*), and to assign sub-zone numbers to those later identified in the region. As can be seen, zones 8 and 11 have already been divided into two sub-zones each, and the zones to the right of the *eT1* region have been numbered 21–23. At least one gene was identified in every major zone except zone 3. However, we believe an essential gene must exist in zone 3 to account for the fact that *sDf32* and *sDf28* failed to complement. The positions of *let-326* and *emb-29* relative to *unc-60* (in zone 4) had been established previously by three-factor mapping (McKim *et al.* 1988). Two essential genes, *emb-18* and *emb-22*, had been positioned near *unc-42* by Cassada *et al.* (1981). Deficiency mapping now placed *emb-18* into zone 22, while *emb-22* was neither deleted by any of the deficiencies, nor balanced by *eT1*. The position of *ges-1* was based on data from J. M. McGhee (personal communication), those of *her-1* and *egl-3* on data from C. Trent (personal communication), that of *mec-9* on data from M. Chalfie (personal communication) and that of *daf-11* on data from S. Brown (1984). The deficiency *sDf36* lies completely in zone 11B. The region deleted by it has been given the temporary name, 11B′.

The zone in which *unc-46* was located could not be established by deficiency mapping. The nearest deficiencies on either side of it were *sDf27* to the left and *sDf20* and *sDf30* to the right. Since all three had been induced on *unc-46(e177)* marked chromosomes, complementation tests could not determine whether or not these deficiencies deleted *unc-46*. However, recombination mapping showed that none of them did so. In the case of *sDf27*, the evidence was based on *let-331(s427)*, which complements all deficiencies except *sDf26*. Three factor mapping placed it to the left of *unc-46* and, therefore, between *sDf27* and *unc-46*: all seven Unc-46 recombinants from *+let-331 unc-46 +/unc-60+ +dpy-11* hermaphrodites carried the *unc-60* marker. In the case of *sDf20* and *sDf30*, it was found that each of these deficiencies could recombine with *unc-46*. Thus, *unc-46*'s position in zone 10 was established.

Based on deficiency mapping, Table 1 orders all the lethal mutations according to their zone positions and

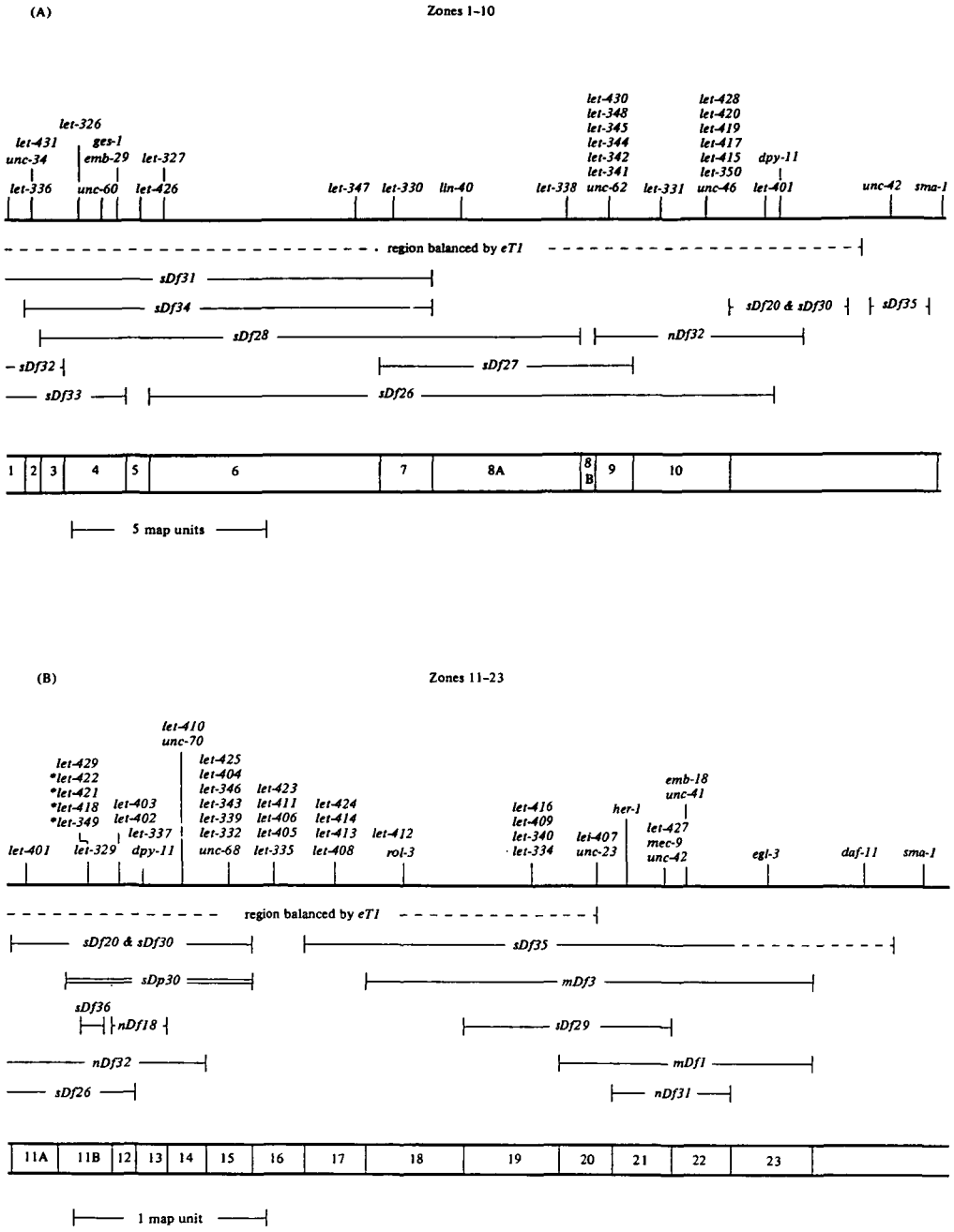

Fig. 1. Partial map of LGV(left). The right-hand breakpoints of *mDf1* and *mDf3* as well as both breakpoints of *nDf31* are based on data from C. Trent (personal communication). The position of *let-336* within zone 1 is arbitrary since *let-336* alleles inhibit recombination (see text). In (B) zones 11–23 are shown with an expanded scale. * Indicates genes in zone 11B that are not deleted by *sDf36*.

shows their respective gene or deficiency assignments. The 17 deficiencies used, including the *mDf* and *nDf* deficiencies (see Materials and methods), provided deletions for the whole region between *let-336* and *sma-1*, except for zone 16. Seven mutations fell into none of the deleted regions. Therefore, they could have been either to the left of *sDf31*, *sDf32* and *sDf33*, if these do not extend to the end of the chromosome, or they could have been in zone 16. Three factor recombination mapping placed all seven to the right of *unc-46* or *dpy-11* and, therefore, into zone 16. In any one zone, all mutations were complementation tested against each other. Gamma-ray and spontaneously induced mutations were considered putative deficiencies which, in certain instances, might stretch into adjacent zones. For example, mutations in zone 9 might be deficiencies stretching into zone 10. Therefore, such mutations were tested also against mutations in the neighbouring zone. The strains bearing *let-407* alleles, in zone 20, produced the occasional fertile Dpy Unc, suggesting that *eT1* balancing ends within zone 20.

https://doi.org/10.1017/S0016672300027476 Published online by Cambridge University Press

Five of the 59 complementation groups belonged to genes identified previously by other than *let* gene names. These were *emb-29* (Cassada *et al.* 1981), *lin-40* (S. W. Emmons & J. Hodgkin, personal communication), *unc-62, unc-70* and *rol-3*. The latter three had been identified by 'visible' alleles (Brenner, 1974). In zone 14, *s115/unc-70(e524)* heterozygotes had an Unc-70 phenotype. Other lethal alleles of *unc-70* had already been isolated by Park & Horvitz (1986). In zone 18, two EMS and two γ-ray induced lethals all produced a few sickly Rol-3 phenotypes as heterozygotes with *rol-3(e754)*. Three other lethals were classified as *rol-3* on the basis of failing to complement these lethal *rol-3* alleles. Finally, in zone 9, the spontaneous mutation, *s472*, which had originally been classified as an allele of *let-328* (Rosenbluth *et al.* 1985), failed to complement *unc-62(e644)* by giving an Unc-62 phenotype. It was therefore reassigned to *unc-62*.

Two-factor mapping results for the distances of some lethals from either *unc-46* or *dpy-11* are shown in Table 2. For *lin-40* we used the allele *e2173* since one of our alleles, *s1053*, gave anomalous results and the other, *s1704*, was carried on the same chromosome as *let-403(s246)*. Since homozygous *e2173* has a slow-moving, slow-developing, sterile phenotype (S. W. Emmons & J. Hodgkin, personal communication) recombinant Dpys were easily identified and all slow-developing larvae were included in the total progeny count. Although generally recombination distances were consistent with the positions of genes and rearrangement breakpoints, as determined by complementation mapping, some discrepancies were noted. Those to the right of *dpy-11* (in zones 12–20) presumably were due to the relative low number of recombinants recovered in the small distances involved. More interesting were the discrepancies to the left of *unc-46*. Here, the allele *let-336(s521)*, in zone 1, and the deficiencies *sDf32, sDf34* and *sDf28* each failed to recombine with *unc-46*. Furthermore, as mentioned previously (McKim *et al.* 1988), the other two *let-336* alleles, *s741* and *s957*, recombined at a lower frequency than was consistent with their position based on deficiency mapping. This was also true for the deficiency *sDf33*, which also lies at the left end of LGV.

(iii) *Distribution of genes on LGV(left)*

The earliest map of *C. elegans* (Brenner, 1974) already showed that genes identified by EMS mutagenesis are not distributed evenly along the recombination map. Our data for the EMS induced lethals recovered over *eT1* demonstrated this phenomenon to be true also for the essential genes on LGV. In Table 3 the region balanced by *eT1* is divided into five intervals varying in size. Column 2 gives their lengths in map units and column 3 gives the number of genes in which EMS induced mutations were recovered while screening the whole *eT1* balanced region. As can be seen from column 4, the genes are predominantly concentrated in zones 9–20 (*unc-62–unc-42*), the region within which visible genes also appear to cluster.

Table 3. *Distribution of genes within which EMS induced mutations were recovered from the 'eT1' screening system*

Zones	Approx. map units	No. of genes	Genes/ map unit
1–4	2	3	1·5
5–8	11	5	0·5
9–10	5	11	2·2
11–17	2	17	8·5
18–20	2	4	2·0

(iv) *Multiple mutations*

Since we were working with chromosomes balanced over a large region, we expected to recover some strains that carried multiple lethal mutations. Those multiple events that occurred in different LGV zones would be detected by the deficiency mapping. Several 'two-hit' events were found, and in four cases both lethals were included in our analysis (see Table 1, footnote *e*). Whether or not these were caused by the mutagen treatment or had occurred spontaneously could not be determined in most cases. Since most of them would be induced by the mutagen, we attempted to minimize this problem by generally keeping the doses relatively low (Table 1). With EMS, the majority of concentrations used were 0·012 M or lower; while with gamma irradiation the doses were 500 or 1500 R.

In a long-term study as is the present one, we did anticipate the occurrence of spontaneous mutations since, for convenience, it was necessary to keep strains in the unfrozen condition for long periods of time. Based on a spontaneous lethal mutation frequency of 1/1600 (Rosenbluth *et al.* 1983), a rough calculation predicts a 1 in 31 chance that after one year at 15 °C, a given lethal carrying heterozygous *eT1* hermaphrodite would carry an additional lethal on LGIII or LGV. An example of one such detected mutation is *let-330(s1702)*. It is believed to have a spontaneous origin since the chromosome bearing it originally produced an adult sterile phenotype and only later a larval lethal one. Complementation tests showed that over *sDf28* the chromosome was a larval lethal (due to *s1702*), but over *nDf31* it was an adult sterile (due to *s1057*). Other examples of apparently spontaneous mutations appearing in the strains were noticed. These included (*a*) two more cases where the homozygous mutant phenotype of a chromosome became more severe with time, (*b*) the appearance of a lethal on an LGV chromosome in a region previously shown to be free of lethals (two cases), (*c*) the appearance of

a lethal on LGIII as noticed in a repeated linkage mapping experiment (one case); and (*d*) the appearance of fertile Dpys segregating in a strain (19 cases). Five of the new Dpys were examined further. Two proved to be alleles of *dpy-5(I)*. The other three were on an *eT1* chromosome and proved to be allelic to the *dpy-18(e364)* marker used for LGIII. Whether the three were spontaneous mutations or due to rare recombination (or conversion) events is not known. Only in the first two above examples would there by secondary mutations on the normal LGV.

To get a better measure of how prevalent spontaneous mutations might be on the normal LGIII or LGV chromosomes in our strains, we assumed that they would occur on the *eT1* balancer at the same frequency. Consequently we also checked the strains for the presence of *eT1*-linked lethals. By the end of the study, out of about 100 strains examined, only three lacked viable *eT1* homozygotes. Thus, for most strains, especially those carrying only putative point mutations, long term maintenance on Petri plates did not cause a serious problem for the analysis. It should be pointed out, however, that the deficiency strains required occasional replacement from the frozen stock, due to increased sterility. This was especially true for the strains carrying *sDf26, sDf27* or *sDf28*.

(v) *Phenotypes*

The phenotypes of the various mutants are listed in Table 1. They generally ranged from egg lethals to adult steriles. Four genes [*lin-40* (zone 8A); *let-418* and *let-421* (zone 11B); and *let-337* (zone 13)] had alleles that produced occasional maternal effect lethal phenotypes. In addition, one mutation, *let-327(s247)*, though originally classified as a lethal, produced slow developing, sickly appearing, fertile Dpy Uncs. Mutations in several genes produced adult sterile hermaphrodites. These were tested for fertility in the presence of N2 males. None appeared to be rescuable by male sperm.

The mutants developing beyond the early larval stage were easily observed as dying Dpy Uncs (or Dpys) in the *eT1*-balanced strains. However, those strains that did not exhibit easily identifiable Dpys presumably carried either egg or early larval lethals. To distinguish between these two lethal stages was not possible in the presence of *eT1*. Due to the translocation itself, the *eT1* strains segregated two classes of dying aneuploids: egg and early larval lethals (L. M. Addison, unpublished results). These, therefore, masked the analysis of the lethal mutations. To circumvent this problem, putative egg or early larval lethals were analysed by examining the progeny of hermaphrodites heterozygous for lethals that were no longer balanced over *eT1*.

(vi) sDf30 *and* sDp30

In a previous study a recessive lethal was assigned to *sDf30* because it failed to complement two complementing lethals, *let-329(s473)* and *s738* (Rosenbluth, Cuddeford & Baillie, 1985). [Note: *s473* is now known as *sDf36*.] In those tests *sDf30* was carried by the male parent and the failure to complement was based on the absence of adult Dpy Unc F_1 males was well as the sterility (in the case of *s473*) or absence (in the case of *s738*) of adult Dpy Unc F_1 hermaphrodites. Since then we found that the reciprocal tests, in which *sDf30* was carried by the hermaphrodite, produced adult Dpy Unc males. Similar results have been obtained with other lethals now known to be in zones 11B–15. In most tests where *sDf30* was carried by the male and no Dpy Unc male progeny survived, sibling Dpy Unc hermaphrodites became adults many of which were fertile. The simplest interpretation of these results was that some of the LGV genes deleted by *sDf30* had been transposed to LGX. These putatively transposed genes we now named *sDp30(V;X)*. Thus, we assumed that the genotype of our *sDf30*-bearing parental hermaphrodites was *dpy-18/eT1(III);unc-46 sDf30/eT1(V);sDp30(V;X)* and that of the males was *dpy-18/eT1(III);unc-46 sDf30/eT1(V);sDp30/0(X)*. These assumptions were confirmed by our ability to extract the two rearrangements into two separate strains: *dpy-18/eT1(III);unc-46 sDf30/eT1(V)*, carrying only *sDf30*, and *unc-60 dpy11(V);sDp30(V;X)*, carrying only *sDp30*. The fact that *sDp30/0* males were viable indicated that the transposition had not inactivated an *X*-linked essential gene.

The extents of *sDf30* and *sDp30* are shown in Fig. 1. Deletion by *sDf30* (zones 11–15) was based on the lethality of Dpy (Unc) male progeny from those complementation tests in which the male parent carried both *sDf30* and *sDp30*; while coverage by *sDp30* in zones 11–15 was based on the viability of Dpy males from tests in which it was the hermaphrodite parent that carried both *sDf30* and *sDp30*. (Note: In later experiments it was possible to test for deletion by *sDf30* using the duplication-free strain as either male or hermaphrodite parent, and scoring for lethal Dpys of both sexes.) The duplication covered all the lethals in these zones except *let-401(s193)*. The left end-point therefore subdivided zone 11 into 11A and 11B. To determine whether *sDp30* extended to the right of zone 15 and covered any of our zone 16 lethals, the following experiment was done. P_0 *dpy18/dpy-18(III);(unc-46)let-x/unc-46* + *(V)*; +/+*(X)* hermaphrodites were crossed to *dpy-18/eT1(III);unc-46 sDf30/eT1(V);sDp30/0(X)* males. Twelve self-fertilizing wild-type F_1 hermaphrodites were picked individually. It was assumed that all received the *sDp30* duplication. Half of the hermaphrodites should have been *dpy18/eT1;(unc-46) let-x/eT1;sDp30/+* and should have produced no mature Dpy(Unc) F_2s if *sDp30* did not cover the

https://doi.org/10.1017/S0016672300027476 Published online by Cambridge University Press

Table 4. *Fertility of* dpy-18(III);let-x(sy)/sDf30(V);sDp30/+(X) *hermaphrodites*

Zone	Gene (*x*)	Allele (*y*)	Ratio of fertile:sterile adult hermaphrodites at different temperatures		
			15 °C	20 °C	24 °C
11B	*let-349*	*s217*	11:0	10:0	4:1
		s502	4:5	4:9	9:0
		*s572*cs	13:0		
	let-418	*s1045*	10:0	12:0	9:0
	let-421	*s288*	12:0	6:0	13:0
	let-422	*s194*	No adults	No adults	No adults
		s738	No adults	No adults	No adults
		s739	No adults	No adults	No adults
11B′	*let-329*	*s575*	0:13	0:52	8:5
	let-429	*s584*	0:10	0:9	7:2
	sDf36	*s473*	No adults	0:14	0:9
12	*let-402*	*s127*	0:10	1:6	4:2
		s500	0:5	1:4	3:3
		s992	0:9	10:12	10:7
	let-403	*s120*	0:17	0:11	3:0
		s246	0:11	0:14	9:1
		s498	0:4	0:14	8:2
13	*let-337*	*s825*	12:0	12:0	17:2
		s1018	12:0	17:0	12:1
		s1024	13:1	19:0	13:0
14	*let-410*	*s815*	10:4	14:3	14:2
	unc-70	*s115*	12:0	24:0	12:0
15	*let-332*	*s234*	14:3	14:0	6:0
		s369	9:1	11:1	7:1
		s1021	10:2	12:1	13:0
	let-339	*s1019*cs	11:0		
	let-343	*s816*	0:12	1:11	2:11
		s1025	0:13	0:13	3:18
	let-346	*s373*	0:7	4:2	5:2
		s1026	0:9	15:4	12:1
	let-404	*s119*	0:11	0:7	2:9
	let-425	*s385*	1:10	12:2	9:1

lethal. All zone 16 mutations, except *s116*, were tested in this manner. Each produced some wild type F_1s that did not segregate mature Dpy(Unc)s, suggesting that none of the identified genes in zone 16 were covered by *sDp30*. However, the possibility exists that among the genes there were some similar to *let-422* (see below). Only as males, but not as hermaphrodites, are homozygous mutants of this gene rescued by *sDp30*.

Suppression of the mutant phenotype by *sDp30* was not always complete in hermaphrodites. Table 4 gives the ratio of fertile:sterile adult hermaphrodites of the genotype *dpy-18(III);let-x(sy)/sDf30(V); sDp30/+(X)*. This genotype had the same mutant:wild gene dosage ratio as did the fertile *let-x(sy)/eT1* stock strains. However, while most hermaphrodites reached adulthood, there were some adults that were sterile at 15 °C, indicating that for some genes the duplication did not produce enough wild type product. (It should be noted that the stock strains were fertile at 15 °C.) The variable response of the hermaphrodites suggested two possibilities. One was that the level of expression of all genes on *sDp30* was below normal but the amount of wild-type product required for a normal phenotype varied among the genes. Alternatively, the genes on *sDp30* were expressed with varying degrees of efficiency. Consistent with both ideas was the fact that the sterility at 15 °C was gene- (rather than allele-) specific; i.e. different alleles of a given gene exhibited similar patterns of fecundity. Interestingly, the genes whose mutations caused hermaphrodite sterility were not necessarily in contiguous zones. Also of interest was the fact that increased temperature apparently increased the efficiency with which mutant phenotypes were suppressed by the duplication.

4. Discussion

The work reported here involves the detailed genetic analysis of the left half of LGV. The 110 recessive lethal mutations identified 12 deficiencies (*sDfs*), one duplication and alleles of 59 essential genes. Deficiency mapping (using also *mDf* and *nDf* deficiencies) divided

https://doi.org/10.1017/S0016672300027476 Published online by Cambridge University Press

the region into 23 zones. Twenty of these zones were balanced by *eT1(III; V)*.

The lethals belonging to the 59 complementation groups added considerably to the number of genes identified on LGV. A total of 74 genes (including visibles) has now been mapped relative to the 23 zones. Sixty-six of the genes lie in the *eT1* balanced region. Of these, 5 lie in an interval (zone 16) that is not yet spanned by a deficiency. The latter interval may contain a gene requiring two wild-type copies for viability. For 3 genes there are both visible and lethal mutations. The fact that for *unc-62* only 1 lethal allele (*s472*) has, as yet, been identified leaves open the possibility that *s472*, which arose spontaneously, is a small deletion that includes a neighbouring essential gene. On the other hand, the lethal alleles of *unc-70* and *rol-3* are probably true severe alleles of the 2 genes. This conclusion is based on the fact that several lethal alleles exist for each of these genes (see Park & Horvitz, 1986 for *unc-70* alleles).

As was expected, the EMS lethals, that were recovered over *eT1*, showed the distribution of essential genes detected by EMS mutagenesis to follow that of the visible genes. To the left of *unc-62* the genes were distributed relatively sparsely compared to the region between *unc-62* and *unc-42*. To determine how well the distribution of genes on the genetic map correlates with their distribution at the DNA level, will require comparing a length of cloned DNA between precisely positioned genes in the sparse region with one between genes in the cluster. For this purpose, the identified genes in zones 5–8 become particularly valuable landmarks.

The deficiency *sDf31*, which was derived from the Bergerac × Bristol cross and is carried on a Bergerac (B0) chromosome, will be valuable for the mapping of those restriction fragment length differences (RFLDs) between Bergerac and Bristol (Emmons *et al.* 1979; Rose *et al.* 1982), that are known to map on LGV(left). Preliminary data indicate that it behaves as a crossover suppressor between itself and *dpy-11* (unpublished results). Therefore, the genotype of a heterozygous strain, consisting of an *sDf31* chromosome over a normal lethal-bearing Bristol LGV chromosome (the lethal being outside the *sDf31* region), should remain stable and produce no viable homozygotes for the region to the left of *dpy-11*. Using genomic DNA from such a strain, RFLDs in the region deleted by *sDf31* will show only the Bristol pattern, while those to the right of *sDf31* will show both patterns.

Important for understanding chromosome behavior is the finding that several other mutant chromosomes affected recombination. The chromosomes were detected by comparing results from deficiency mapping with those from recombination mapping. Thus, three deficiencies, *sDf28, sDf32* and *sDf34*, as well as the γ-ray induced *s521* allele of *let-336* all failed to recombine with *unc-46*. In addition, *sDf33* and two other alleles of *let-336* partially inhibited recombination. To deduce the bases for these effects will require further investigation. The fact that *sDf33* recombined to some extent with *unc-46* rules out a simple interpretation.

While the focus of this study was on the mapping of the lethal mutations, an attempt was also made to observe their arrest phenotypes. These ranged from egg lethality to a few cases of maternal effect lethality. Most of the lethals died as larvae. Only one definite egg lethal, *emb-29(s819)*, was observed, although several other lethals were classified as putative egg lethals. The early phenotypes should be interpreted cautiously. Their severity may be due to mutations in the background genotype – either due to the genetic markers used or due to undetected secondary mutations 'fixed' in the balanced regions. Furthermore, nine of the early larval lethals were not induced by EMS. Eight were recovered after γ-ray mutagenesis and one arose spontaneously. These mutations may, in fact, prove to be small deletions deleting more than one gene.

Phenotypic differences among alleles of the same gene may reflect those genes whose products are required at more than one stage of development. Of the LGV genes with lethal mutations, 26 (including *emb-29, unc-62* and *unc-70*) had multiple alleles. As yet, significant phenotypic differences have been observed among the alleles of only a few of these genes: *let-349, let-337, let-408, unc-62, unc-70*, and *rol-3*. The last three exhibit the most striking variety of phenotypes, each having a visible allele.

The duplication *sDp30* carried a number of wild type LGV genes on the *X* chromosome. Our finding that in hermaphrodites *sDp30* did not completely suppress all lethal mutations suggests that some genes were not fully expressed in the duplication. This raises the following two questions. First, regarding the variability of suppression: were all the transposed wild type genes uniformly under-expressed and did the mutant genes vary in their sensitivity to dosage, or were only a few genes under-expressed? That is to say, did the variability of suppression reside in the sensitivity of the mutant genes on LGV or in the expression of the transposed genes on *sDp30*? Second, was the under-expression of genes in the duplication (whether uniform or partial) related to the fact that the genes were now associated with the *X* chromosome? If so, our findings may be relevant to the problem of *X*-dosage compensation in this organism. *C. elegans* compensates for the difference in dosage of *X*-linked genes between XO males and XX hermaphrodites (Meneely & Wood, 1987). It does so by equalizing *X*-linked mRNA transcripts in the two sexes (Meyer & Casson, 1986). Whether compensation is achieved by elevating expression in the single *X* chromosomes of males or by reducing expression in the two *X* chromosomes of hermaphrodites or by a combination of these two mechanisms is under investigation. Based on data of Meneely & Wood (1987), using *dpy-21* and *dpy-26*, and those of Meyer

& Casson (1986), using *dpy-27* and *dpy-28*, reduction of *X* chromosome expression in hermaphrodites appears to be part of the mechanism. We may, therefore, speculate that in our experiments (1) reduction of *X* chromosome expression occurred in hermaphrodites, (2) this reduction spread to the linked autosomal genes on *sDp30* and (3) either the genes were variably affected by the spreading effect or the mutant phenotypes were variably sensitive to reduced doses of wild type product. The phenomenon of spreading would mimic an aspect of *X*-dosage compensation in mammals. In those organisms compensation is achieved by an almost total inactivation of one *X* chromosome in females (*X*-chromosome inactivation), and this inactivation has been demonstrated to spread to autosomal genes translocated to the *X* (reviewed by Gartler & Riggs, 1983). To test whether in our experiments the apparent underexpression of certain *sDp30* genes was due to the effects of *X*-dosage compensation, we plan to study *sDp30*'s properties in the background of mutant genes known to affect dosage compensation in *C. elegans* (Hodgkin, 1983; Meneely & Wood, 1984, 1987; Wood *et al.* 1985; Meyer & Casson, 1986; Villeneuve & Meyer, 1987). In addition, we hope to acquire new duplications that carry the same genes as *sDp30* but are transposed to autosomes.

In conclusion, this study has laid the groundwork for the characterization of the large LGV region balanced by *eT1*. Deficiencies, which divide LGV(left) into manageable zones, span the whole region, except for the interval defining zone 16, and 54 new genes have been identified, making LGV(left) the best analysed region of its size in *C. elegans*.

We would like to thank Margaret Rogalski and Fay Gin for excellent technical assistance. We also wish to thank Robert Devlin for isolating the *sDf33* and *sDf34* deficiencies as well as the two mutations *s992* and *s998*. We thank Meenal Khosla for the isolation of *s908*, Helen I. Stewart for assistance in maintaining the many unfrozen strains required for this study, and Karen Beckenbach for constructing Fig. 1. Finally, we are grateful to Ann M. Rose and Denise V. Clark for valuable discussions during the course of this study. The work was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Muscular Dystrophy Association of Canada to D.L.B.

References

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94.

Brown, S. J. (1984). Genetic interactions affecting muscle organization in the nematode *C. elegans*. Ph.D. Thesis, University of Missouri, Columbia.

Cassada, R., Isnenghi, E., Culotti, M. & von Ehrenstein, G. (1981). Genetic analysis of temperature-sensitive embryogenesis mutants in *Caenorhabditis elegans*. *Developmental Biology* **84**, 193–205.

Coulson, A., Sulston, J., Brenner, S. & Karn, J. (1986). Toward a physical map of the genome of the nematode *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences USA* **83**, 7821–7825.

Donati, L. A. M. (1985). A genetic analysis of the right arm of linkage group IV of *Caenorhabditis elegans*, with emphasis on the *sDf2* region. M.Sc. Thesis, Simon Fraser University, Burnaby, B.C.

Edgley, M. L. & Riddle, D. L. (1987). *Caenorhabditis elegans*. In: *Genetic Maps 1987*, vol. 4 (ed. S. J. O'Brien). Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Emmons, S. W., Klass, M. R. & Hirsh, D. (1979). Analysis of the constancy of DNA sequences during development and the evolution of the nematode *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences USA* **76**, 1333–1337.

Ferguson, E. L. & Horvitz, H. R. (1985). Identification and characterization of 22 genes that affect vulva cell linages of the nematode *Caenorhabditis elegans*. *Genetics* **110**, 17–72.

Gartler, S. M. & Riggs, A. D. (1983). Mammalian X-chromosome inactivation. *Annual Review of Genetics* **17**, 155–190.

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

Hodgkin, J. (1983). X chromosome dosage and gene expression in *Caenorhabditis elegans*: two unusual dumpy genes. *Molecular and General Genetics* **192**, 452–458.

Horvitz, H. R., Brenner, S., Hodgkin, J. & Herman, R. K. (1979). A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. *Molecular and General Genetics* **175**, 129–133.

Howell, A. M., Gilmour, S. G., Mancebo, R. A. & Rose, A. M. (1987). Genetic analysis of a large autosomal region in *Caenorhabditis elegans* by the use of a free duplication. *Genetical Research* **49**, 207–213.

Johnsen, R. C., Rosenbluth, R. E. & Baillie, D. L. (1986). Genetic analysis of linkage group V(left) in *Caenorhabditis elegans*. *Genetics* **113**, s11.

Lefevre, G. (1981). The distribution of randomly recovered X-ray-induced sex-linked genetic effects in *Drosophila melanogaster*. *Genetics* **99**, 461–480.

Lefevre, G. & Watkins, W. (1986). The question of the total gene number in *Drosophila melanogaster*. *Genetics* **113**, 869–895.

McKim, K. S., Heschl, M. F. P., Rosenbluth, R. E. & Baillie, D. L. (1988). Genetic organization of the *unc-60* region in *Caenorhabditis elegans*. *Genetics* **118**, 49–59.

Meneely, P. M. & Herman, R. K. (1979). Lethals, steriles and deficiencies in a region of the X chromosome of *Caenorhabditis elegans*. *Genetics* **92**, 99–115.

Meneely, P. M. & Herman, R. K. (1981). Suppression and function of X-linked lethal and sterile mutations in *Caenorhabditis elegans*. *Genetics* **97**, 65–84.

Meneely, P. M. & Wood, W. B. (1984). An autosomal gene that effects X-chromosome expression and sex determination in *Caenorhabditis elegans*. *Genetics* **106**, 29–44.

Meneely, P. M. & Wood, W. B. (1987). Genetic analysis of X-chromosome dosage compensation in *Caenorhabditis elegans*. *Genetics* **117**, 25–41.

Meyer, B. J. & Casson, L. C. (1986). *Caenorhabditis elegans* compensates for the difference in X chromosome dosage between the sexes by regulating transcript levels. *Cell* **47**, 871–881.

Moerman, D. M. & Baillie, D. L. (1979). Genetic organization in *Caenorhabditis elegans*: fine structure analysis of the *unc-22* gene. *Genetics* **91**, 95–103.

Nüsslein-Volhard, C., Wieschaus, E. & Kluding, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. *Roux's Archives of Developmental Biology* **193**, 267–282.

Park, E.-C. & Horvitz, H. R. (1986). *C. elegans unc-105*

mutations affect muscle and are suppressed by other mutations that affect muscle. *Genetics* **113**, 853–867.

Rogalski, T. M. & Baillie, D. L. (1985). Genetic organization of the *unc-22 IV* gene and the adjacent region in *Caenorhabditis elegans*. *Molecular and General Genetics* **201**, 409–414.

Rogalski, T. M., Moerman, D. G. & Baillie, D. L. (1982). Essential genes and deficiencies in the *unc-22IV* region of *Caenorhabditis elegans*. *Genetics* **102**, 725–736.

Rose, A. M. & Baillie, D. L. (1979). The effect of temperature and parental age on recombination and nondisjuction in *Caenorhabditis elegans*. *Genetics* **92**, 409–418.

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

Rose, A. M., Baillie, D. L., Candido, E. P. M., Beckenbach, K. A. & Nelson, D. (1982). The linkage mapping of cloned restriction fragment length differences in *Caenorhabditis elegans*. *Molecular and General Genetics* **188**, 286–291.

Rosenbluth, R. E. & Baillie, D. L. (1981). The genetic analysis of a reciprocal translocation, *eT1(III;V)*, in *Caenorhabditis elegans*. *Genetics* **99**, 415–428.

Rosenbluth, R. E., Cuddeford, C. & Baillie, D. L. (1983). Mutagenesis in *Caenorhabditis elegans*. I. A rapid eukaryotic mutagen test system using the reciprocal translocation, *eT1(III;V)*. *Mutation Research* **110**, 39–48.

Rosenbluth, R. E., Cuddeford, C. & Baillie, D. L. (1985). Mutagenesis in *Caenorhabditis elegans*. II. A spectrum of mutational events induced with 1500 R of gamma radiation. *Genetics* **109**, 493–511.

Sigurdson, D. C., Spanier, G. J. & Herman, R. K. (1984). *Caenorhabditis elegans* deficiency mapping. *Genetics* **108**, 331–345.

Stevens, W. L. (1942). Accuracy of mutation rates. *Journal of Genetics* **43**, 301–307.

Villeneuve, A. M. & Meyer, B. J. (1978). *sdc-1*: a link between sex determination and dosage compensation in *C. elegans*. *Cell* **48**, 25–37.

Wood, W. B., Meneely, P., Schedin, P. & Donahue, L. (1985). Aspects of dosage compensation and sex determination in *Caenorhabditis elegans*. *Cold Spring Harbor Symposia on Quantitative Biology* **50**, 575–583.