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Formaldehyde mutagenesis of the eT1 balanced region in *Caenorhabditis elegans*: Dose–response curve and the analysis of mutational events

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Summary

In this study we have generated a dose–response curve for the formaldehyde induction of recessive lethal mutations in the *eT1(III; V)*-balanced region of *C. elegans*. We have mapped 96 out of 112 formaldehyde-induced lesions to either LGIII or LGV and genetically analyzed 31 lesions that mapped to LGV. Our findings showed that a 4-h treatment with 0.1% formaldehyde gave the best mutation induction frequency with the least side effects. We found that formaldehyde induced putative point mutations, deficiencies and more complex lesions in *C. elegans*. We isolated 11 putative point mutations, 3 of which defined new genes and 8 were alleles of known genes. One of the new genes, *let-450*, is currently the left-most known gene on LGV. We also isolated 5 deficiencies. Our formaldehyde-induced lesions increased the number of zones in the *eT1*-balanced region of LGV from 22 to 34.

Our long-term goal is to map all of the essential genes on 20 map units (m.u.) of the left half of linkage group (LG) V in the nematode *Caenorhabditis elegans*. This region is balanced by the reciprocal translocation *eT1(III,V) (eT1)* (Rosenbluth and Baillie, 1981). A set of deficiencies is essential for this work. Formaldehyde was demonstrated to produce deficiencies in *Drosophila melanogaster* (Slizynska, 1957; O'Donnell et al., 1977) and small deficiencies in *C. elegans* (Moerman and Baillie 1981). For this paper we have developed a formaldehyde dose–response curve and isolated and characterized a number of formaldehyde-induced mutations including some deficiencies.

Many features of *C. elegans* combine to expedite large-scale genetic analysis (see Brenner, 1974). One feature is that *C. elegans* can be maintained frozen in liquid nitrogen. This not only precludes the necessity for stock maintenance but, even more importantly, it slows the accumulation of spontaneous mutations in balanced regions. Mutations may arise through replicational error, chemical mutagenesis or background radiation. Freezing the nematodes in liquid nitrogen effectively eliminates the first two mechanisms and thus protects the balanced region from further insult.

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For the mapping of the essential genes, a set of large γ-irradiation-induced deficiencies is already available. These deficiencies divide (LGV(left) into 22 zones (Rosenbluth et al., 1987). A further subdivision of these zones would facilitate the mapping of newly acquired ethyl methanesulfonate (EMS)-induced lethal point mutations. We have therefore attempted to induce a new set of small deletions in LGV(left). Since Moerman and Baillie (1981) have shown that formaldehyde induces small deletions, we decided to use it as our mutagen and, at the same time, analyze formaldehyde-induced mutagenesis in *C. elegans*. To do so, we used the *eT1* system (Rosenbluth et al., 1983, 1985).

The *eT1* system screens for recessive lethal mutations induced in the region balanced by *eT1*. The latter is a reciprocal translocation that balances at least 20 m.u. of LGIII(right) and at least 20 m.u. of LGV(left). The break on LGIII maps to a location very close to *unc-36* and the homozygous *eT1* individual has an Unc-36 phenotype. *eT1* is stable, it has been intensively studied for several years and has never been shown to break down. It is an excellent system for testing mutagens. *eT1* has been used to develop dose–response curves for the mutagens EMS and γ-irradiation (Rosenbluth et al., 1983). *eT1* is also being used to study the ultraviolet (UV) irradiation induction of recessive lethal mutations (H. Stewart, personal communication).

For this study we used various concentrations of formaldehyde to develop a dose–response curve for the formaldehyde induction of lethals. We isolated 112 strains containing lesions in or very close to the *eT1*-balanced region and genetically analyzed a subset of those mutations. We compared these lethals to lethals induced by either 0.012 M EMS or 1500 R γ-irradiation in a number of respects. We compared the number of lethals that mapped to each of the two linkage groups. We tested for the loss of expression of the markers in strains containing lethal mutations that did not recombine away from a marker during 2-factor mapping. We compared the distribution of outcrossed brood sizes. We also complementation tested a number of the putatively simple formaldehyde-induced lethals on LGV against the set of γ-irradiation-induced rearrangement breakpoints that define 22 zones (Rosenbluth et al., 1987). Once the mutations were mapped to zones, we complementation tested them against all the known genes in the same zones and thus discerned if they were deficiencies, putative point mutations that define new genes or putative point mutations in known genes.

(1) Materials and methods

(i) General

The nomenclature follows the uniform system adopted for *Caenorhabditis elegans* (Horvitz et al., 1979). The nematodes were cultured in petri dishes on a simple agar nematode growth medium streaked with *Escherichia coli* OP50. For details of this as well as procedures for observing and handling the worms see Brenner (1974). The following homozygous *C. elegans* (var. Bristol) strains were obtained from the MRC, Cambridge, England or from the Caenorhabditis Genetics Center at the University of Missouri, Columbia, MO: N2(wild type), *dpy-18(e364), unc-46(e177), eT1(III,V), unc-34(e315* and *e566), unc-60(e677), emb-29(g52), unc-62(e644), dpy-11(e224), unc-68(e540), rol-3(e754), unc-23(e25)*. The mutation *lin-40(e2173)* isolated by S.W. Emmons, was supplied by J. Hodgkin (MRC, Cambridge). The deficiencies *mDf1* and *mDf3* (Brown, 1984) were from D.L. Riddle's laboratory (Columbia, MO). *nDf32* (Park and Horvitz, 1986), *nDf18* and *nDf31* originated in R.H. Horvitz's laboratory (MIT). All mutations denoted with the *s* prefix arose in this laboratory.

(ii) The test strain

Hermaphrodites from a homozygous *dpy-18; unc-46* strain were mated to *dpy-18/eT1; unc-46/eT1* males. Wild-type male offspring were mated to homozygous *eT1* hermaphrodites. One individual wild-type hermaphrodite from the progeny of the latter cross was used to establish the strain BC2200, which was used in all our mutagenesis experiments. The characteristics of *dpy-18/eT1; unc-46/eT1* and of *eT1* have been described previously by Rosenbluth and Baillie (1981).

(iii) Mutagenesis with formaldehyde

We used the method of Moerman and Baillie (1981). Formaldehyde was prepared by warming 5

g of paraformaldehyde (Fisher NO. T353) in 50 ml of 65 °C distilled water and then adding NaOH to clear the solution. The solution was adjusted to pH 7.2 with HCl and then diluted to 500 ml by adding M9 buffer (Brenner, 1974) giving a 1% solution. This solution was further diluted with M9 buffer to give the appropriate formaldehyde concentration. We chose our concentrations based on Moerman and Baillie's (1981) findings. They had shown that a 1% concentration of formaldehyde is lethal to *C. elegans*, but concentrations of 0.1% and 0.07% induced both point mutations and deficiencies in the *unc-22(IV)* region. For each mutagenesis run, we made up a fresh 1% formaldehyde solution.

BC2200 nematodes were washed off the stock plates with M9 buffer. The worms were then collected by centrifugation. They were suspended in either a 0.0%, 0.07%, 0.11%, 0.14% or 0.18% solution of formaldehyde and left at room temperature (20–23 °C) for 4 h. The worms were then removed from the solution with a Pasteur pipette and spotted onto a petri plate. After 2 h, young adult worms were placed on petri plates (either 1 per plate or 3 per plate) with lawns of OP50. These worms were left for 3 days and their F_1 progeny were screened as described below for mutations in the *eT1*-balanced region.

(iv) Screening for mutations

Single F_1 heterozygotes, from the P_0 plates, were placed on individual petri plates (10 mm × 60 mm). From any one P_0 hermaphrodite plate, all F_1 heterozygotes were picked. The progeny of these F_1s were screened for the presence of normal-appearing gravid Dpy-Unc worms. All such F_1 lines were discarded. The absence of mature Dpy-Uncs indicated the presence of a lethal within the screening region. From every plate having no fertile adult Dpy-Uncs, a putative lethal-bearing wild-type heterozygote was picked and used to establish a stock.

(v) Mapping the mutations

Crossing the lethal-bearing strain allows one to map the lethal to a chromosome and to calculate its distance from the appropriate marker. We crossed our lethal-bearing strains to N2 males and then picked several L4 F_1 hermaphrodites from each strain. Any F_1s that gave Unc-36 progeny were discarded. In every case, for one or two of the F_1s, we scored all the F_2s. The lethal was assigned to LGIII(right) if the number of fertile F_2 Dpy-18s was significantly less than one third the number of wild types; if the Unc-46 : wild-type ratio was significantly less than one third, the lethal was assigned to LGV(left). Strains with mutations on both chromosomes were not analyzed further.

(vi) Outcrossed brood sizes

dpy-18(III)/+ ; unc-46(V)/+ hermaphrodites give an average of 332 progeny when not mated (see Results). 10 out of 16 progeny from an *eT1* heterozygous hermaphrodite are aneuploid and do not survive, therefore the total brood size of a *dpy-18/eT1; unc-46/eT1* line is approximately 125. Our lethals are maintained as heterozygotes over *eT1*. When they were mapped, the translocated *eT1* chromosomes were replaced with 'normal' chromosomes thus increasing the brood sizes. The resulting brood sizes are called the outcrossed brood sizes. We call an outbrood size small if it is less than 151, medium if it is greater than 150 but less than 291 and large if it is greater than 290 (only 1 or 2 lines were scored per strain). A recessive lethal with no dominant effect should give an outcrossed brood size of 250. We postulate that medium outcrossed brood sizes are indicative of point mutations or small deficiencies and that small outcrossed brood sizes are associated with more complex mutational events or very large deficiencies. The assumptions about the small and medium outcrossed brood sizes are supported by the results of Rosenbluth et al. (1985). Their smallest outcrossed brood size for a point mutation or a deficiency was 145 [*sDf29(V)*] with most outcrossed brood sizes being greater than 200. Their largest outcrossed brood size for a translocation or duplication was 166 [*sT3(III)*].

(vii) Test for the presence of markers

Several of our strains had mutations that did not separate from their linked marker when crossed to wild-type males. In order to ascertain whether the marker was still expressed in strains where the mutations did not separate from their markers, we crossed these strains to BC1958 (*dpy-18/*

eT1; unc-46/eT1) males. Because aneuploid progeny from heterozygote *eT1* worms die either as eggs or at an early larval stage (L.M. Addison, personal communication), the only F_1 worms that survive to adulthood have these phenotypes: wild type, Unc-36 or Dpy-Unc. The presence of F_1 Dpy-Unc progeny from the above cross indicated that both markers were still expressed. F_1 Dpy-18s indicated that the *unc-46* marker was not expressed, F_1 Unc-46s indicated that the *dpy-18* marker was not expressed, the presence of Dpy-Uncs, Unc-46s and Dpy-18s indicated that at least one of the markers was involved in a more complicated mutational event than a point mutation or a deficiency.

(viii) Mapping to zone on LGV(left)

LGV(left) has been divided into zones by a set of rearrangement breakpoints (Rosenbluth et al., 1987). A subset of these deficiencies (*sDf26, sDf30, sDf33, sDf34* and *sDf35*) uncover all of the region except for the zone between *sDf30* and *sDf35* (see Fig. 2). All of the above deficiencies are balanced over *eT1* and are in strains that also carry *dpy-18*, and all but the strain containing *sDf35* carry *unc-46*. We crossed males from our sample of formaldehyde-induced lethals on LGV(left) to the above set of deficiences. The absence of fertile Dpy-Unc (Dpy-18 for *sDf35* due to pseudolinkage) progeny indicated failure to complement. We thus roughly mapped our lethals. We then used appropriate deficiencies, where necessary (see Figs. 2 and 3), to identify the zone(s) into which each mutation falls. Once a lethal had been mapped to a zone, we complementation tested it against all known genes in that zone. The results of the complementation tests allowed us to assign the mutations to the appropriate genes.

(2) Results

In our formaldehyde treatments of *eT1*, we observed a high incidence of death of the P_0s. Therefore we treated a relatively large number of worms. The number of F_1s from the 0.07%, 0.11% and 0.14% formaldehyde-treated P_0s did not vary significantly from the number of the F_1s from non-mutagenized P_0s. The number of F_1 progeny from the P_0 worms treated with 0.18% formaldehyde was approximately 85% of the untreated P_0 brood size. This indicates that physiological damage plays a major role at higher doses.

The percentage of sterile F_1 individuals increased linearly with formaldehyde dose and in all cases was less than the 2.5% sterility rate observed for 0.012 M EMS-treated worms (unpublished results). Therefore F_1 sterility does not pose a significant problem.

The dose–response data are given in Table 1. The 'dose' refers to the concentration of formaldehyde that the worms were exposed to for 4 h at room temperature. The 'response' is the percentage of fertile F_1s that carried at least 1 lethal mutation within the 40 m.u. balanced by *eT1*. The lethal induction rate for 0.11% formaldehyde is approximately 1.6% (see Table 1). This is similar to the rate for 0.004 M EMS (26/1747 or 1.5%) and greater than the rate for 500 R γ-irradiation (46/4789 or 1.0%) (Rosenbluth et al., 1983).

To establish a value for the outcrossed brood size of *dpy-18/+; unc-46/+* hermaphrodites, we scored all the adult progeny of 7 individuals. The average brood size was 332. We also noted a wild-type : Dpy-18 : Unc-46 : Dpy-Unc ratio that was well within the 95% confidence limits for a

TABLE 1

INDUCTION OF RECESSIVE LETHALS AT VARIOUS FORMALDEHYDE DOSES

Dose	Date of Expt.	Number of F_1s tested	Number of lethals	Lethals (%)
Control	21/06/81 [a]	1812	2	0.11
	27/09/82 [a]	1386	0	0.00
	16/03/87	641	0	0.00
		3839	2	0.05
Formaldehyde				
0.07%	12/05/86	3670	10	0.27
	13/08/86	1163	4	0.34
		4833	14	0.29
0.11%	22/06/86	3682	58	1.58
0.14%	4/09/86	992	16	1.61
0.18%	4/09/86	530	13	2.45
	11/16/86	956	11	1.15
		1486	24	1.62

[a] Rosenbluth et al. (1983).

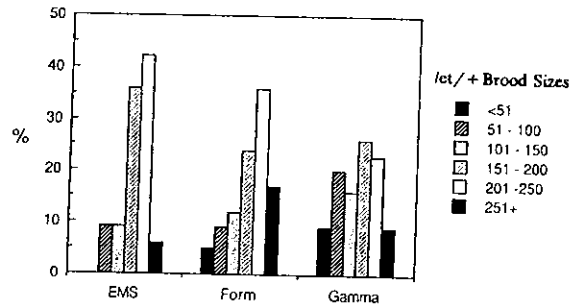

Fig. 1. Outcrossed brood sizes for 34 0.012 M EMS-, 85 0.07%, 0.11%, 0.14% and 0.18% formaldehyde-, and 85 1500 R γ-irradiation-induced lethal mutations in the *eT1*-balanced region.

9 : 3 : 3 : 1 ratio. This shows that if either marker has a detrimental effect, the effect is very small. We also found that there is no significant difference in the percentage of lethals giving small (< 151) outcrossed brood sizes between the low (0.07% and 0.11%) and high (0.14% and 0.18%) formaldehyde doses.

In Fig. 1 we compare the outcrossed brood sizes for lethals induced by 3 different mutagens. The lethals in Fig. 1 do not include those that block development at late larval or adult sterile stages. The mutagens used were: 1500 R γ-irradiation (Rosenbluth et al., 1985; L.M. Addison, personal communication), 0.012 M EMS (unpublished results) and formaldehyde. EMS-generated lethals produce mainly medium outcrossed brood sizes: 82% have between 151 and 290 offspring. γ-Irradiation produced lethal-bearing heterozygotes which varied greatly in outcrossed brood size, with only 56% between 151 and 290. The formaldehyde-induced lethal-bearing heterozygotes gave a distribution of outcrossed brood sizes which fell between that of EMS and γ, with 76% having medium-size broods.

We have mapped to linkage group the formaldehyde-induced lethal mutations of 96 out of a total of 112 strains. We found that 34% lie on LGV(left) and 66% lie on LGIII(right). This includes the strains that have mutations that map to both LGIII and LGV but does not include second site hits on one chromosome. We also found a similar ratio for 0.012 M EMS-induced lethals: 35% on LGV(left) and 65% on LGIII(right) from a sample of 147 lethal-bearing strains (Johnsen et al., 1986). A larger sample of 751 EMS-induced lethals give the same fraction mapping to each chromosome (unpublished results). For 1500 R γ-irradiation, we have the results from the mapping of lethals derived from 2 mutagenesis screens. They produced 67 lethal-bearing strains with lesions restricted to one chromosome (we are not including strains with lesions which map or seem to map to both chromosomes), 40% of the lethals lie on LGIII(right) and 60% lie on LGV(left) (Rosenbluth et al., 1985; L.M. Addison, personal communication).

When we mapped our formaldehyde-induced lethals to either LGIII or LGV, we isolated a number of strains that produced either no Dpy-18 or no Unc-46 progeny. Such strains could arise in 2 possible ways. (1) The lethal is tightly linked to the marker or (2) the marker cannot be expressed in the strain. We tested for the presence of markers (see Materials and methods) 18 strains that failed to show at least one of the markers. 15 strains proved to still express both markers, indicating a lethal tightly linked to a marker. Two strains failed to express one of the markers: BC2772 did not express *dpy-18* and BC2482 did not express *unc-46*. This suggests that the wild-type allele of *dpy-18* in BC2772 and the wild-type allele of *unc-46* in BC2482 may have been translocated from their original locations on the *eT1* chromosomes to a location that is tightly linked to the suppressed marker, or a formaldehyde-induced crossover had occurred between the region containing the marker and the region containing its wild-type allele. One other strain (BC2475) produces Dpy-18, Unc-46 and Dpy-Unc progeny. It is possible that either *dpy-18* or *unc-46* has translocated elsewhere in the genome and has not been lost. These 3 strains demonstrate that formaldehyde can cause complex mutational events.

30 of the 96 strains containing formaldehyde-induced mutations that mapped in or very near the *eT1*-balanced region have lesions that are strictly on LGV(left). 5 strains were lost, 4 of which had low brood sizes, were very sickly and died out and the fifth was outside the balanced region and crossed away. 3 of the remaining strains have markers that behave irregularly (discussed above BC2772, BC2482 and BC2475) and we have stopped working on them. 3 more are sickly and

TABLE 2

DISTRIBUTION AND TYPE OF RECESSIVE LETHAL MUTATION

Dose (%)	Number screened	Number of mutations recovered	Number mapped to V	Deficiencies	Alleles of known genes	Alleles of new genes	Putative complex mutations	Not done
0.07	4833	14	2		1		1	
0.11	3682	58	24	5	5	2	9	3
0.14	992	16	3		1			2
0.18	1486	24	2		1	1		
Total	10993	112	31	5	8	3	10	5

have small brood sizes. We suspect that they contain complex mutational events and therefore we have stopped analyzing them. 1 strain has a medium brood size, is fertile but develops very slowly and we have stopped analyzing it. Initially the latter strain had a lethal mutation, but we think it was just outside the *eT1*-balanced region and crossed away revealing the mutation that causes slow development. 4 strains have lethal mutations that span or are just outside the *eT1* balancer boundary. The analysis of 3 of these is not completed. This leaves 15 strains. 5 map as deficiencies. 10 strains contain 11 putative point mutations (1 strain has 2 putative point mutations), 3 of which define new genes and 8 of which are alleles of known genes (see Table 2).

All 5 of the deficiencies and 7 of the 10 strains containing putative point mutations have medium outcrossed brood sizes. The other 3 strains containing putative point mutations have late larval or adult sterile phenotypes and their outcrossed brood sizes were not scored. The 2 strains that failed to express a marker (BC2772 and BC2482) both have small outcrossed brood sizes whereas BC2475 has a medium outcrossed brood size.

Our map of LGV(left) (Figs. 2 and 3) is based on the map of Rosenbluth et al. (1987). The chromosome, with genes positioned on it, is at the top of the map. The chromosomal rearrangements that break the chromosome into zones are in the middle and the zone designations are on the bottom of the map. The map distances of the genes in zone 1 (*let-450, let-447* and *let-336*) from each other and from *unc-34* are not known. The distances shown for these genes are arbitrary, since there is a recombination suppression phenomenon related to the region to the left of *unc-34* (R.E. Rosenbluth, personal communiction). This recombination suppression phenomenon is currently under investigation in our laboratory. Our formaldehyde-induced deficiency names are shown on the map in single brackets e.g. (*sDf42*). Lesions that previously were putative point mutations and have now been shown to be deficiencies by formaldehyde-induced mutations are on the map with their names in double brackets e.g. ((*sDf53*)). Formaldehyde lesions that are putative point mutations are listed at the bottom of the map in the appropriate zone with their allele names (in brackets) following their gene name. Formaldehyde lesions that define new genes have the letter 'a' in brackets following the gene name e.g. *Let-450*(a) *(s2160)*. We are not using one of the deficiencies (*sDf31*) shown on the Rosenbluth et al. (1987) map. *sDf31* was isolated from a strain with an N2/BO background (Donati, 1985; Rosenbluth et al., 1987). It may still have active Tc1 transposons because it occasionally gives progeny with unexpected phenotypes. We want stable and predictable strains containing deficiencies to define the zones of LGV, therefore we are not using *sDf31*.

The 5 formaldehyde-induced deficiencies are designated *sDf42, sDf44, sDf46, sDf47* and *sDf50*. *sDf46* uncovers the left-most known gene on LGV (*let-450*) suggesting that *sDf46* deletes the end of the chromosome. *sDf46* breaks between *let-347* and *sDf27* thus breaking zone 6B into 6B and 6C (see Fig. 2). There are no known genes in zone 6C.

sDf42 breaks between *unc-60* and *emb-29* splitting zone 4 into 4A and 4B. The right end of *sDf42* breaks between *lin-40* and *let-338* creating zones 8A1 and 8A2 (see Fig. 2).

LINKAGE GROUP V (LEFT)

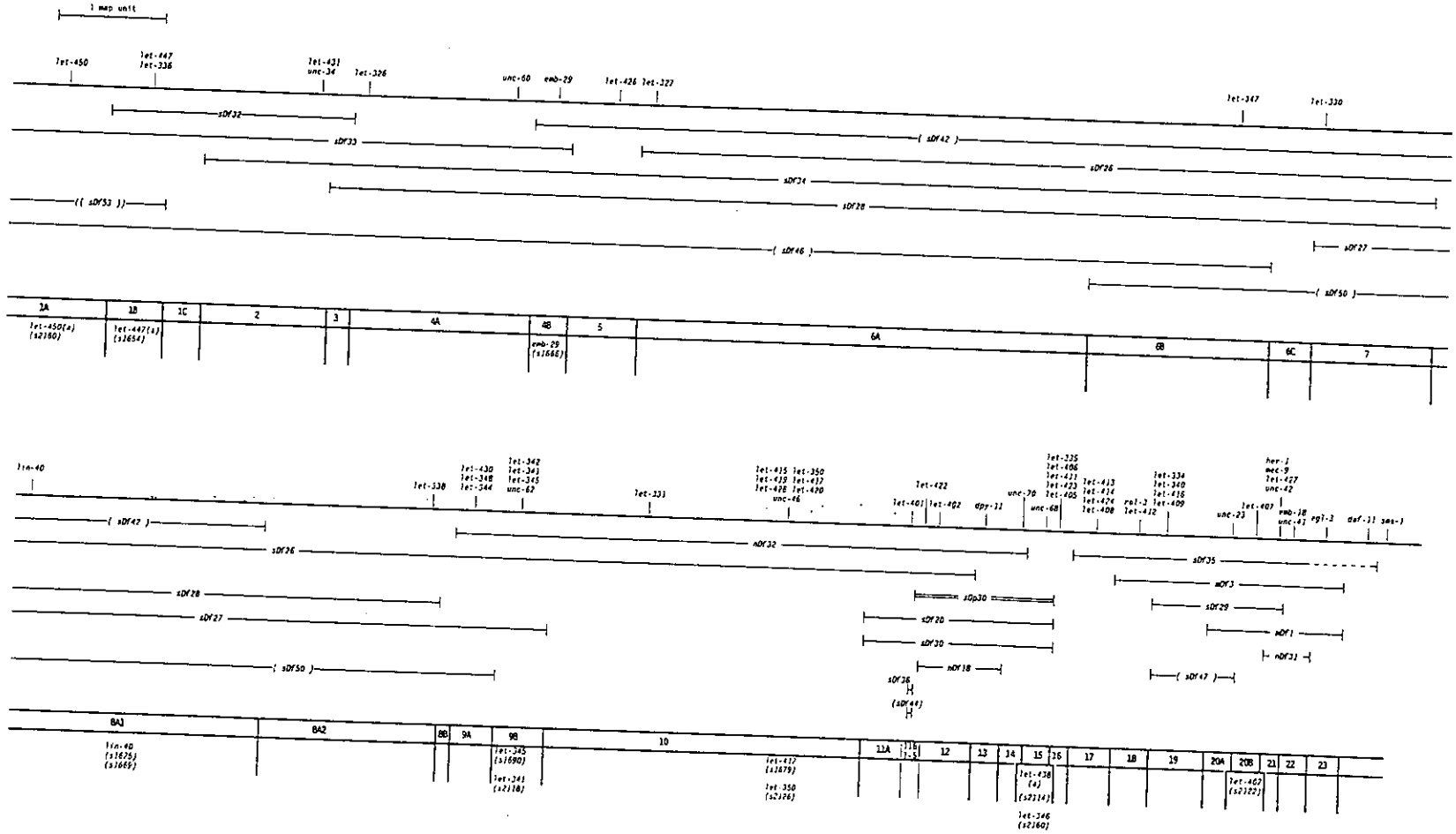

Fig. 2. Genetic map of the *eT1*-balanced region of LGV(left).

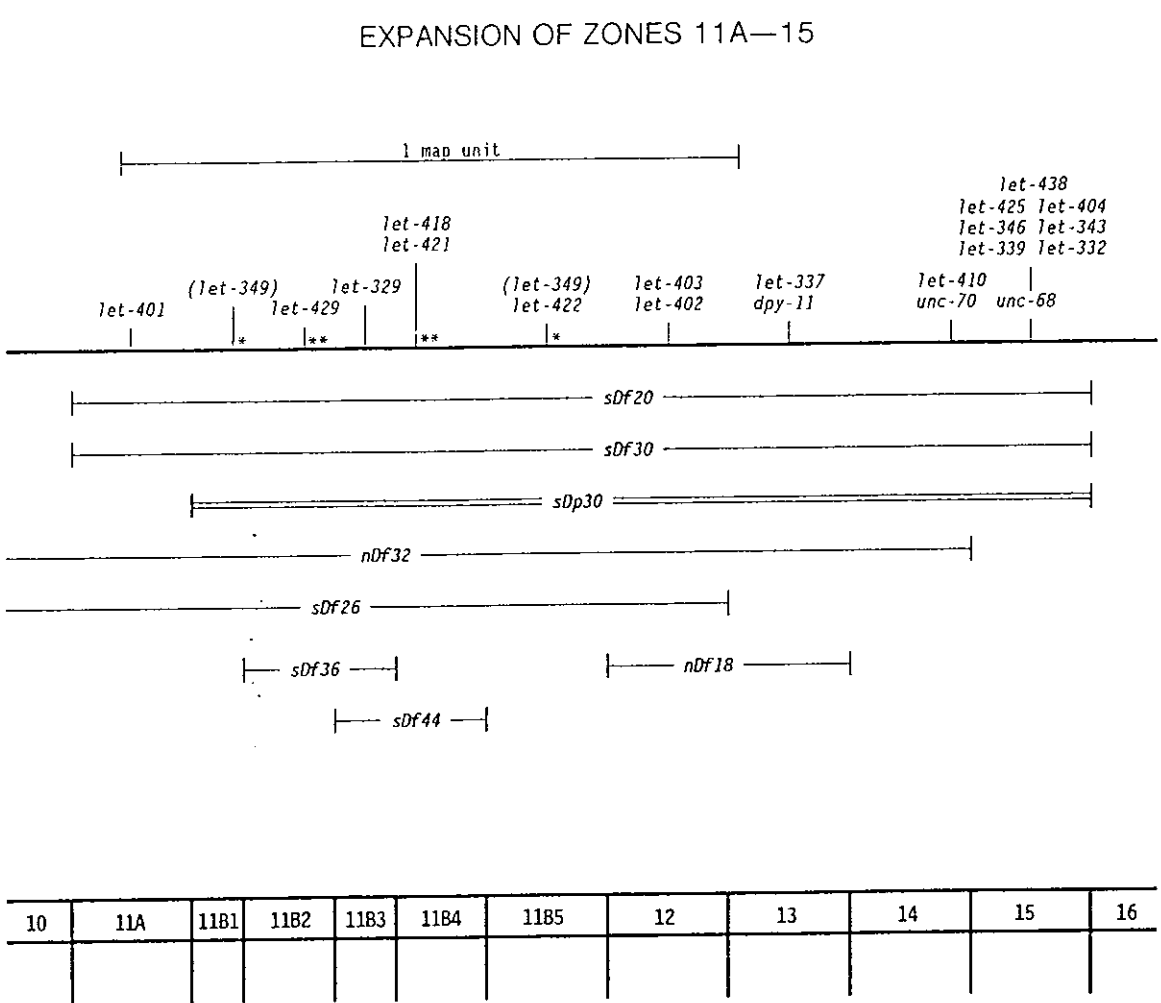

Fig. 3. Expansion of zones 11A through 15. *sDf36* and *sDf44* are floating and have not been right/left positioned relative to each other. *let-349* could be in either 11B1 or 11B5; *let-422* has been placed in 11B5 by 2-factor mapping (see text); *let-429* could be in 11B5 and if so then *let-418* and *let-421* would be in zone 11B2.

sDf50 breaks between *let-327* and *let-347* dividing zone 6 into 6A and 6B. The right break point of *sDf50* divides zone 9 into 9A and 9B. *let-344, let-348* and *let-430* are in 9A and therefore to the left of *let-344, let-342, let-345* and *unc-62* which are in 9B.

The relative positions of genes in zone 11B are not yet clear. *sDf44* is a short deficiency that lies entirely in zone 11B. It partially overlaps *sDf36* which also lies entirely in 11B creating zone 11B3. *let-329* lies in the overlap region (11B3), *let-418* and *let-421* are uncovered by *sDf44* but not by *sDf36*. We do not yet know if *sDf44* overlaps with the right end of *sDf36* as shown on the map (Fig. 3) or the left end of *sDf36*. Therefore, both *let-418* and *let-421* may lie to the left of *sDf36* and *let-420* may lie to the right of *sDf44*. We placed *let-422* in zone 11B5 because 2-factor mapping from *dpy-11* gave a distance of 0.3 m.u. (0.1–0.9) (Rosenbluth et al., 1987) but *let-422* could be in zone 11B1. *let-349* could be in either zone 11B1 or 11B5 and we placed it in both zones on the map (Fig. 3).

The left end of *sDf47* has not been separated from the left end of *sDf29*. However, on the right end, *sDf47* complements *nDf31*, splitting zone 20

into zones 20A and 20B. Zone 20A contains *unc-23* and 20B contains *let-407* (see Fig. 2).

Our formaldehyde screens produced 10 strains containing 11 putative point mutations on LGV. One strain, BC2821, carried a late larval lethal, *s2160*. Upon complementation testing BC2821 against our set of deficiencies we found it to contain 2 mutations, one of which is allelic to *let-346* in zone 15 and has been assigned the allele name *s2165*. The other lesion retains the *s2160* designation. It fails to complement *sDf33* and *sDf53* but complements *sDf34* and *sDf32*, thus showing that *sDf33* extends to the left of *sDf32*. *s2160* defines a new essential gene named *let-450* which lies in zone 1A. *let-450* and zone 1A are the left-most known gene and zone respectively on LGV. *s2160* complements one allele of *let-336* but fails to complement another (*s957*). *s957* is now considered to be a deficiency (*sDf53* that splits zone 1B into 1B and 1C.

s1654 defines a new gene, *let-447*, in zone 1B. *s1654* behaves like a hypermorph. When it is homozygous it usually causes sterility although sometimes a few eggs that do not hatch are laid, but over a deficiency, *s1654*-containing strains consistently give a lot more eggs although none of them hatch. This effect was observed with *sDf32, sDf33, sDf46, sDf53* and several other unpublished deficiencies that uncover the same region.

s1666 is an allele of *emb-29* in zone 4B. Two alleles of *lin-40* (zone 8A1) were isolated, they are *s1669* and *s1675*. *s1669* blocks as a late larval lethal and *s1675* blocks as an adult sterile but occasionally a few nonviable eggs are laid. We mated *s1675* to wild-type males and found that *s1675* is not male rescuable.

s1690 is allelic to *let-345*. *s2118* is allelic to *let-341*. Both *let-345* and *let-341* are in zone 9B. *s1679* is allelic to *let-417*. *s2126* is allelic to *let-350*. Both *let-417* and *let-350* are in zone 10. *s2114* maps to zone 15 but complements all known genes in that zone, we therefore assigned *s2114* the gene name *let-438*.

(3) Discussion

Formaldehyde was shown to be a mutagen in the 1940s (Rapoport, 1946; Kaplan, 1948) but its mechanisms of action were not understood and are still not completely clear (Auerbach et al., 1977). We know that formaldehyde reacts with amino groups in denatured regions of DNA. There is another slower reaction that results in the formation of methylene cross-links between amino groups in DNA (Feldman, 1975). Formaldehyde can also form reactive hydroxyalkyl peroxides and/or free radicals in the presence of oxidizing molecules. Auerbach et al. (1977) proposed that formaldehyde may produce its effects by various mechanisms depending on the organism and mode of application.

Auerbach et al. (1977) argued that the most likely reaction with formaldehyde in biological systems is a condensation reaction with amino groups. This suggests that proteins act as a sink for formaldehyde but the organism can only absorb a certain amount of formaldehyde before physiological damage affects it and its germ cells. Our observation that the F_1 brood size decreased at the higher formaldehyde dose (0.18%) supports this proposal.

Treatment with formaldehyde has been reported to produce several types of mutations in *Drosophila melanogaster* (Slizynska, 1957). She cytologically analyzed nuclei from the salivary glands of female larvae carrying chromosomes from formaldehyde-treated parents. She identified translocations, inversions, deficiencies, repeats, duplications and cases of chromosome loss. She found that the majority of her formaldehyde-induced rearrangements were intrachromosomal (82%). Of 114 mutations 25% were deficiencies and 39% were duplications or repeats. Slizynska also noted that formaldehyde induced approximately 15 times more deficiencies than X-rays at doses that induced similar proportions of sex-linked lethals. O'Donnell et al. (1977) confirmed Slizynska's results. She reported 67% (12 out of 18) formaldehyde mutations in the *D. melanogaster Adh* region were deficiencies. Moerman and Baillie (1981) used formaldehyde to induce *unc-22(IV)* mutations in *C. elegans*. They found that at least 35% (14 out of 40) of those mutations were deficiencies, the remaining 26 were classified as point mutations. 3 of those putative point mutations have since been shown to be internal duplications in *unc-22* (T. Starr, personal communication). Formaldehyde deficiencies delete on average about half as many

genes as do 1500 R γ-irradiation-induced deficiencies (R.E. Rosenbluth and R.C. Johnsen, unpublished results). Therefore formaldehyde has less drastic mutagenic effects than 1500 R γ-irradiation and coupled with the fact that formaldehyde is much cheaper to use than γ-irradiation demonstrates that formaldehyde is an excellent alternative mutagen for the induction of deficiencies.

It is desirable to use the optimum dose of a given mutagen in any mutagenesis experiment. Too high a dose leads to multiple mutational events that will complicate genetic analysis. Too low a dose wastes time, materials and effort. A dose–response curve for a mutagen allows one to derive the optimum dose. From the formaldehyde dose–response curve reported in this paper it appears that a formaldehyde dose of 0.1% is the best dose to use. Table 1 shows the lethal mutation rate at each of the tested concentrations of formaldehyde. These results are similar to those of Kaplan's (1948), in which he transferred Drosophila larvae to a medium containing formaldehyde. In 4 experiments in which the concentration varied 2.5-fold there was no dependence on concentration of the lethal induction rate and the rate was 28 times higher than in the control. Unfortunately these experiments depend on Drosophila eating formaldehyde-containing food. With *C. elegans*, the worms are placed directly into a buffer solution containing formaldehyde and therefore we feel that the conditions of treatment are more easily reproduced.

All the formaldehyde-induced deficiencies and all of the formaldehyde-induced point mutations counted (7 out of 10) gave medium outcrossed brood sizes, with the putative point mutations having an average of 45 more progeny than the deficiencies. Whereas 2 of the 3 strains containing putatively complex mutational events had small outcrossed brood sizes. It may be possible to use the outcrossed brood size as a crude indicator for the type of mutational event induced.

Two-thirds of the lethal mutations induced by EMS (Johnsen et al., 1986) and formaldehyde, mapped to LGIII(right) and one-third to LGV(left). This suggests that there are twice as many genes in the LGIII(right) region than in the LGV(left) region even though both regions are approximately the same length recombinationally, each being about 20 m.u. long. With 1500 R γ-irradiation the ratio is reversed: 40% of the mutations map to LGIII and 60% map to LGV (Rosenbluth et al., 1985; L.M. Addison, personal communication). A partial explanation for the discrepancy between the γ-irradiation results and the EMS and formaldehyde results, is that *eT1* has a defect in the *unc-36* gene (Rosenbluth and Baillie, 1981) and any lesion uncovering *unc-36* would give an Unc-36 phenotype in an *eT1* heterozygote and therefore would be ignored when the F_1 wild types are picked during screening.

However, undetected *unc-36* deletions cannot be the whole explanation, for the following reason. From the 67 γ-ray-induced lethals mapped to chromosome, 26 mapped to LGIII and 41 mapped to LGV. If the same ratio that applies to the EMS and formaldehyde induction of lethals also applies to the induction of lethals by γ-irradiation, then we would expect two-thirds of these lethals to map to LGIII and one-third map to LGV. For 41 lesions on LGV we would expect about 82 on LGIII. We only got 26. Therefore there are approximately 56 missing lesions. If all the missing lesions were undetected *unc-36* deletions then 68% (56/82) of all γ-induced lesions on LGIII(right) would delete *unc-36*. This is far too many and therefore we propose the following hypotheses to account for the 'missing' lesions. One hypothesis requires differential sensitivity of chromosomal areas to γ-irradiation, the other two do not. (1) The *unc-36* region is very sensitive to γ-irradiation, there is a disproportionately large number of lesions that effect *unc-36* and these were not picked up in our screen. (2) There is a haplo-lethal area on LGIII and any mutations that remove it are inviable as heterozygotes and would not be picked up in our screen. P. Mains (personal communication) suggests that *ct45(III)* may identify a haploinsufficient gene in the LGIII(right) region. (3) The LGV(left)-balanced region has more DNA than LGIII(right) but has a lower gene density.

We have isolated 5 deficiencies and 11 putative point mutations induced by formaldehyde. Only 3 of the 11 putative point mutations define new genes. We analyzed a set of EMS-induced lethals in parallel with our analysis of the formaldehyde-induced lethals. 60% of the EMS-induced lethals defined new genes (unpublished results) whereas

less than 30% of the formaldehyde-induced lethals defined new genes. Although this difference is not statistically significant it does suggest that some of our formaldehyde-induced putative point mutations may be deficiencies that delete a small number of genes.

Our formaldehyde-induced mutations have increased the number of zones in the *eT1*-balanced region of LGV (1–20 inclusive) from 22 (Rosenbluth et al., 1987) to 34. We feel that 50–60 would be the optimum number of zones for the analysis of this region. We have several more putative deficiencies that are being analyzed. They should provide the required additional zones.

When looking at the recombination map of LGV(left) it is quite noticeable that the deficiencies in the gene cluster are much shorter than the deficiencies to the left of the cluster. This may reflect a much lower number of DNA base pairs per m.u. near the end of the chromosome than in the middle of the chromosome.

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