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FORMALDEHYDE MUTAGENESIS IN THE NEMATODE *Caenorhabditis elegans*

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Summary

We have found that formaldehyde is capable of inducing mutations in the nematode *Caenorhabditis elegans*. 4 concentrations of formaldehyde were tested. At a concentration of 1%, formaldehyde is lethal to the nematode, and 0.01% formaldehyde did not induce any mutations in approx. 60 000 tested chromosomes. 2 concentrations of formaldehyde, 0.1% and 0.07%, were found to be mutagenic, inducing both point mutations and deficiencies in the *unc-22* region of linkage group IV. 4 of the point mutations have been demonstrated to be alleles of the *unc-22* gene and have been mapped within the locus. 2 of the putative deficiencies have been confirmed. Each spans the *unc-22* gene and at least 2 other genes in the region. A rough estimate of the forward mutation frequency using 0.1% formaldehyde in this region is 3×10^{-5}, while for 0.07% the frequency is 2×10^{-4}.

Our laboratory is presently investigating the genetic organization and control of the *unc-22* gene in *Caenorhabditis elegans* (Moerman and Baillie, 1979). As part of this program we are interested in examining the region around the *unc-22* gene to identify and characterize neighbouring genes. An essential part of this study is to generate a series of deficiencies in this region. Although X-rays can cause deficiencies in the nematode (Meneely and Herman, 1979), note was taken of the early results of Slizynska (1957) and the more recent findings of O'Donnell et al. (1977) demonstrating formaldehyde-induced deficiencies in *Drosophila melanogaster*. These observations led us to examine its mutagenic effects on the nematode. We report here that formaldehyde appears to be capable of inducing both point mutations and deficiencies in the nematode.

Materials and methods

Nematode strains, media and culture conditions. NG agar as described by Brenner (1974) was used throughout this study. *E. coli* (OP-50) in nutrient broth (Difco) at $2-3 \times 10^8$ cells/ml were used for streaking plates. 100-mm Petri plates were used for the maintenance of the strains (at 16°C), and for the isolation of mutations. The wild-type hermaphrodite stock, N2S, used in this study was derived from a single N2 from the Cambridge stock collection. The various strains used in this study are shown in Table 1. The general techniques for nematode genetics have been described (Brenner, 1974).

Mutagenesis with formaldehyde. Formaldehyde was prepared by warming 5 g of paraformaldehyde (Fisher) in 50 ml of 65°C distilled water and adding NaOH to clear the solution. After clearing, the solution was adjusted to pH 7.2, diluted to 150 ml with distilled water, further diluted to 500 ml by adding M9 buffer (Brenner, 1974) and this 1% solution was then kept as a stock solution. All further dilutions from this were done by adding M9 buffer.

N2S worms were mutagenized by washing worms off stock plates with M9 buffer and collecting after centrifugation ($200 \times g$). These worms were then suspended in either a 1%, 0.1% 0.07% or 0.01% solution of formaldehyde. After 4 h at 20°C, they were removed from the formaldehyde with a Pasteur

TABLE 1

MUTANT STRAINS

Linkage group	Stock	Phenotype
IV	*dpy-4 (e1166)* [a]	dumpy
	unc-5 (e152), unc-22 (s8), dpy-4 (e1166)	small, slightly dumpy and twitches
	unc-5 (e152), unc-22 (s12), dpy-4 (e1166)	
	unc-22 (s7) [b,c]	uncoordinated (twitches)
	unc-22 (s34)	uncoordinated (twitches)
	unc-22 (s35)	uncoordinated (twitches)
	unc-22 (s36)	uncoordinated (twitches)
	unc-22 (s55)	uncoordinated (twitches)
	let-51 (s41), unc-22 (s7)/++	segragates twitcher progeny that block in egg
	let-53 (s42), unc-22 (s7)/++	segregates twitcher progeny that block in a late larval stage
	let-56 (s46), unc-22 (s7)/++	segregates twitcher progeny that block in a late larval stage
	let-58 (s48), unc-22 (s7)/++	does not give twitchers block unknown
	let-59 (s49), unc-22 (s7)/++	segregates twitcher progeny that block in a mid-larval stage
	let-60 (s59), unc-22 (s7)/++	segregates twitcher progeny that block in a mid-larval stage

[a] *e*, isolated at Cambridge, England.

[b] *s*, isolated at Simon Fraser University, Canada.

[c] *unc-22* alleles except *s7*, *s8* and *s12* were isolated using 0.1% formaldehyde. *s7* was isolated using 0.025 M ethyl methanesulfonate (EMS). *s8* was isolated using 0.0125 M EMS. *s12* was isolated using 0.05 M EMS.

pipette and spotted onto a Petri plate. After approx. 2 h, 4th-stage larvae and young adult worms were placed on Petri plates (either 10 per plate, 25 per plate or en masse) with lawns of OP-50 (at 26°C). These worms were left for 3 days and the F1 progeny were screened for mutations affecting the *unc-22* gene.

The screen for mutations in the *unc-22* region takes advantage of the fact that mutations in the *unc-22* gene, although mostly recessive under standard conditions, are also conditional dominants. That is, worms when either homozygous or heterozygous for an allele of the *unc-22* gene twitch in a 1% solution of nicotine alkaloid (Sigma), whereas wild-type worms in the solution become rigid. The protocol was to screen in the F1 for worms that twitched in nicotine and then to see if these worms segregated individuals with a twitcher phenotype. Individuals that did not yield homozygous twitcher progeny were candidates for deficiencies and were retained as stocks. These were characterized as described in the Results and discussion. If an F1 worm did segregate individuals that were homozygous for the twitching phenotype, these "twitchers" were also stocked. The procedures for mapping and characterizing mutations that are alleles of the *unc-22* gene have been described in detail elsewhere (Moerman and Baillie, 1979).

Results and discussion

4 concentrations of formaldehyde were tested: 1%, 0.1% 0.07% and 0.01%. We obtained mutations with 0.1% and 0.07% formaldehyde. The high dose of 1% killed the worms. On examining worms plated from this concentration only a few larvae were seen to be alive after 2 days. These could possibly have been protected as either dauer larvae or eggs. With 0.01% we did not obtain any mutations in approx. 60 000 tested F1 chromosomes, nor did we obtain any visible mutations of any type in the F2 after setting up 625 F1 worms.

Using 0.1% formaldehyde we examined approx. 270 000 chromosomes in the F1. In this number we found 7 worms of interest (Table 2). These could be separated into 2 groups; one group segregated homozygous twitchers, the other group gave wild-type progeny and progeny that twitched in 1% nicotine. With 0.1% formaldehyde we also set up 225 of the F1 worms and looked for any type of visible mutation in the F2. We found uncoordinated worms on 3 separate plates. There were progeny-tested and gave only uncoordinated worms.

TABLE 2

FORMALDEHYDE MUTAGENESIS IN THE *unc-22* REGION

Type of disorder	Formaldehyde concentration (%)	Chromosomes tested (approx.)	Number of isolates	Frequency
unc-22 point mutations	0.1	270 000	4	2×10^{-5}
	0.07	200 000	22	1×10^{-4}
unc-22 deficiencies	0.1	270 000	3 [a]	1×10^{-5}
	0.07	200 000	11	5×10^{-5}

[a] One of these may be some other type of chromosomal rearrangement.

Approx. 200 000 chromosomes were examined in the F1 after mutagenesis with 0.07% formaldehyde. We found 33 (Table 2) worms of interest, and again, these could be separated into 2 groups, putative *unc-22* point mutations and *unc-22* deletions. From worms treated with this concentration of formaldehyde we set up 101 F1 worms and examined their progeny for visible mutations. 3 separate mutations were found. One of these was of the uncoordinated (Unc) variety, another was a dumpy (Dpy) and the third was a transformer (Tra − transforms hermaphrodites into males). All 3 mutants were progeny-tested and found to be the result of true germinal mutations. Neither these nor any of the 3 0.1% formaldehyde-induced visible uncoordinated mutations were mapped.

Of the formaldehyde-induced twitchers only 4 were examined closely. The 4, *s34*, *S35*, *s36* and *s55* were phenotypically indistinguishable from EMS- and γ-ray-induced twitchers previously isolated. All 4 were found to be alleles of *unc-22* and mapped within the gene (Table 3). They were mapped relative to *s12*, the rightmost point in the gene, and *s8* which is close to the leftmost border of the gene. Their position within the intragenic map is shown in Fig. 1. As far as we know these are the first formaldehyde-induced mutations that have been mapped within a gene. We had hoped that these mutations might prove to be intragenic deletions because this would give us a means of isolating the *unc-22* gene product and also allow us to determine the direction of transcription. This seems not to be the case. All 4 mutations map as points within the gene. We must exercise caution on this conclusion since the resolution of this system will not allow us to distinguish a point mutation from a small intragenic deletion.

Individuals that did not yield homozygous twitcher progeny were candidates for deficiencies in the *unc-22* region. These were crossed to a series of lethal and visible mutations known to map in this area (Moerman and Baillie, unpublished). Matings were done on 40-mm plates with the lethal or visible mutation in the male. An example cross is: male *let-56(s46) unc-22(s7)*/++ × putative deficiency. In the F1 we looked for males to confirm that the putative deficiency outcrossed, and also for twitcher adults or twitcher larvae depending on whether the putative deficiency only exposed the twitcher gene or also the

TABLE 3

RECOMBINATION DATA BETWEEN THE VARIOUS ALLELES OF THE *unc-22* GENE

Allele pairs tested [a]	Frequency of *unc-22*$^+$	Map distance (in map units) [b]	Left/right
s8/s34	4/126 000	6.4×10^{-3}	*s8-s34*
s12/s34	7/178 000	7.9×10^{-3}	*s34-s12*
s8/s35	6/168 000	7.1×10^{-3}	*s8-s35*
s12/s35	7/184 000	7.6×10^{-3}	*s35-s12*
s8/s36	2/190 000	2.1×10^{-3}	*s8-s36*
s12/s36	8/224 000	7.1×10^{-3}	*s36-s12*
s8/s55	2/132 000	3.0×10^{-3}	*s8-s55*
s12/s55	6/160 000	7.5×10^{-3}	*s55-s12*

[a] Mutation at left was in triple, *unc-5*, *unc-22*, *dpy-4*.

[b] Map distance (d) = $\frac{2(unc\text{-}22^+ \text{ recombinants}) \times 100}{\text{total offspring}}$.

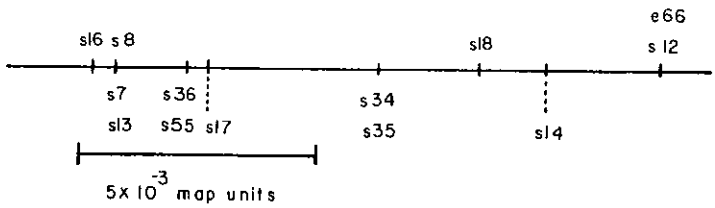

Fig. 1. Fine structure map of the *unc-22* locus showing the relative positions of the formaldehyde-induced alleles. Alleles shown above the line have been left/right positioned relative to one another. The alleles below the line, including the new formaldehyde-induced alleles still must be positioned relative to *s18* and each other.

lethal gene (in this instance a lethal that blocks in the 4th larval stage).

The characterization of the putative deficiencies has not been completed. Of several putative deficiencies only 2 have been examined in any detail. There are *sDf1* and *sDf2*. Both appear to be 1–2 map units in length and uncover *unc-22* but do not expose *dpy-4(e1166)*, the end gene on the linkage group. The 2 deficiencies overlap by at least 0.5 map units and reciprocal crosses give eggs that do not hatch. *sDf1* appears to be a larger deficiency than *sDf2*. It also grows slower and gives fewer progeny. The complementation tests that have been done with these 2 deficiencies are shown in Table 4. All uncovered genes are lethal sites except *unc-22*. A map of the region showing the extent of the deficiencies is shown in Fig. 2. As can be seen the exact rightmost boundary for either deletion has not been determined.

The frequency of obtaining mutations of the *unc-22* gene using 0.1% and 0.07% formaldehyde is about 3×10^{-5} and 2×10^{-4}, resp. The forward mutation frequency of the *unc-22* locus using 0.025 M ethyl methanesulfonate (EMS) is about 10^{-3} (unpublished results). EMS then is more effective as a mutagen in this region of the nematode genome than formaldehyde. Since EMS causes primarily GC to AT transitions (Coulondre and Miller, 1977) the muta-

TABLE 4
COMPLEMENTATION TESTS OF DEFICIENCIES, sDf1 AND sDf2, WITH VARIOUS GENES IN THE *unc-22* REGION [a]

Gene in *unc-22* [b] region tested	SDf1	SDf2
unc-22 (s7)	—	—
let-51 (s41) [c]	+	+
let-53 (s43)	+	+
let-56 (s46)	+	—
let-58 (s48)	—	+
let-59 (s49)	+	—
let-60 (s59)	—	—
dpy-4 (e1166)	+	n.d. [d]

[a] +, complement; —, fail to complement.
[b] All genes tested were the male stock.
[c] All lethal genes are kept as stock linked to *unc-22 (s7)*. Therefore we are testing the *unc-22* gene and a lethal gene in each cross. We score for twitchers in the F1 progeny. If we do not see any adult twitchers then it is scored as a "fail to complement".
[d] n.d., this cross was not done since sDf2 did not extend beyond *let-58*.

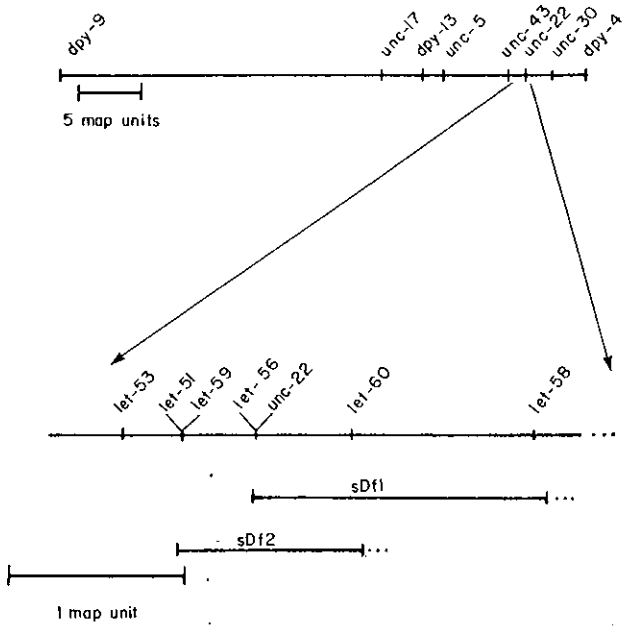

Fig. 2. Map of the *unc-22* region showing the extent of the deficiencies.

tions obtained are, in the main, point mutations. Similar to O'Donnell et al. (1977) we find that a significant proportion of our formaldehyde-induced mutations are deficiencies (14 out of 40 mutations, 35%). Using the same F1 selection protocol for twitchers, we have isolated more than 20 EMS-induced alleles of the *unc-22* gene without finding any deficiencies (unpublished results).

Since the forward mutation rate in this region is quite low using 0.1% formaldehyde there is the possibility that these mutations arose spontaneously. This becomes a more concrete possibility when one considers the data obtained by Schalet (1958) for 13 loci on the X-chromosome of *D. melanogaster* which showed spontaneous mutation frequencies that were between 10^{-6} and 10^{-5}. We have taken the precaution of screening 64 000 N2S chromosomes in an effort to find worms that twitch in 1% nicotine. We did not find any. We also did not obtain any mutants using 0.01% formaldehyde in 60 000 chromosomes. Finally, in over 3 years of keeping the N2S stock plate we have not seen any twitchers. Since this nematode is a self-fertilizing hermaphrodite, any spontaneous twitchers on the plate would give rise to a small clone of twitchers. These would be easily seen in a wild-type background. We conclude then, that the spontaneous mutation frequency is significantly lower than the forward mutation frequency induced by 0.1% formaldehyde.

At any rate, it is clear that the lower concentration of 0.07% formaldehyde does induce mutations in the *unc-22* region of the nematode genome. The demonstration that formaldehyde yields a large percentage of deficiencies makes it a useful tool for the genetic analysis of *Caenorhabditis elegans*. Further improvement on the frequency of mutagenesis may be obtainable by manipulating the protocol described in Materials and methods. Worms incubated

with 0.1% formaldehyde were in poor condition, and an appreciable amount of sterility of the treated worms was detected even at the lower dose of 0.07%. In future we will be experimenting with 0.07% and possibly lower concentrations of formaldehyde.

Auerbach et al. (1977) have pointed out that the widespread use of aldehydes in research, industry and medicine calls for their assessment as potential mutagens. The demonstration of formaldehyde as a mutagen in *Caenorhabditis elegans* suggests that this organism may be useful for this purpose, and for examining the mutagenicity of other chemicals as well.

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