NOTES

Recovery of duplications by drug resistance selection in *Caenorhabditis elegans*

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We have devised a scheme that facilitates rapid screening for duplications of essential loci. Our scheme takes advantage of the *lev-1(x22)* mutation, which confers resistance in a recessive fashion to the potent anthelmintic levamisole. We have tested our methodology by recovering two gamma ray induced duplications of *let-56*, the first essential gene to the left of *unc-22*. One of the duplications is attached to the fourth chromosome. The other duplication is attached to the X chromosome. This duplication contains a functional copy of the *unc-22* gene, as well as functional copies of several essential loci adjacent to *unc-22*. Results we have obtained during analysis of this duplication are compatible with the notion that the copy of the *unc-22* gene located on the duplication is subject to X chromosome dosage compensation.

Key words: *C. elegans*, duplication, *let-56*, *unc-22*, X chromosome.

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Les auteurs ont mis au point une méthodologie qui facilite la sélection rapide de duplications de locus essentiels. Cette méthodologie tire avantage de la mutation *levl(x22)*, qui confère de la résistance d'une façon récessive à la puissante levamisole anthelmintique. La méthode a été testée par le recouvrement de duplications induites par deux rayons gamma du gène *let-56*, le premier gène essentiel situé à gauche du gène *unc-22*. L'une des duplications est attachée au quatrième chromosome, l'autre au chromosome X. Cette dernière duplication contient une copie fonctionnelle de l'*unc-22*, ainsi que des copies fonctionnelles de plusieurs locus essentiels qui sont adjacents à l'*unc-22*. Les résultats obtenus au cours d'analyses de cette duplication sont compatibles avec la notion que la copie du gène *unc-22* localisée sur la duplication est sujette à une compensation de dosage du chromosome X.

Mots clés : *Caenorhabditis elegans*, duplication, *let-56*, *unc-22*, chromosome X.

[Traduit par la rédaction]

We have devised a scheme that has allowed us to screen for chromosomal duplications of vital loci that lie within the region of chromosome IV genetically balanced by the translocation *nT1(IV;V)* in *Caenorhabditis elegans* (Ferguson and Horvitz 1985; Clark et al. 1988; see Fig. 1). On chromosome IV, *nT1* has been shown to effectively balance the 21 map unit interval from *lin-1* to *dpy-4* (Clark et al. 1988; Fig. 1). This interval represents about 7% of the *C. elegans* genome and, based on recent estimates of approximately 15 000 genes in the genome (Sulston et al. 1992; Waterston et al. 1992), would contain over 1000 genes.

Materials and methods

Our duplication selection scheme makes use of the recessive *lev-1(x22)(IV)* mutation and selects candidate duplication bearing individuals based on their resistance to 1 mM levamisole (Lewis et al. 1980). A flow diagram illustrating the scheme is shown in Fig. 2. To test our scheme, we screened for duplications of *let-56*, the first essential locus to the left of *unc-22(IV)* (Schein et al. 1993; Fig. 3). Animals homozygous for the *let-56(s2321)* mutation grow extremely slowly compared with individuals heterozygous for the mutation. *let-56(s2321)* homozygous individuals attain adulthood after approximately 2 weeks at 20°C and are sterile (M.A. Marra, unpublished data). We chose for our screen a mutagen that causes chromosome breakage, expecting to recover duplications containing a wild-type copy of *let-56*. We presumed these duplications would be derived from *nT1(IV)* (see Fig. 2).

One thousand and fifty young gravid hermaphrodites of the genotype *let-56(s2321) unc-22(s7) lev-1(x22)/nT1(IV); +/nT1(V)* were treated with 1500 R of gamma radiation (Rosenbluth et al. 1985; see Fig. 2). Fifty treated individuals (the P_0 generation) were placed on each of 21 plates and allowed to lay eggs for two 24-h broods. Three days after the brood had been set, F_1 animals were collected in M9 buffer (Sulston and Hodgkin 1988) and spotted onto NGM plates (Sulston and Hodgkin 1988) containing 1 mM levamisole (Lewis et al. 1980). Twenty-four hours later, these plates were inspected. No recombination was expected between *let-56* and *lev-1* because of the presence of *nT1(IV;V)*, which suppresses recombination in this interval (Ferguson and Horvitz 1985; Clark et al. 1988; see Fig. 1). Therefore, individuals homozygous for *lev-1(x22)* (and thereby resistant to levamisole) would also be homozygous for *let-56(s2321)* and, in the absence of a suppressor, would appear as arrested larvae at the time of inspection. We screened the levamisole-containing plates for exceptional adult mobile hermaphrodites, which were in a background of developmentally arrested larvae or paralyzed hypercontracted individuals. These exceptional adult mobile individuals would be candidate duplication-bearing animals.

Results and discussion

Six fertile animals displaying the characteristic Unc-22 "twitching" phenotype (Moerman and Baillie 1979; Waterston et al. 1980) and exhibiting varying degrees of resistance to levamisole were recovered from a population of approxi-

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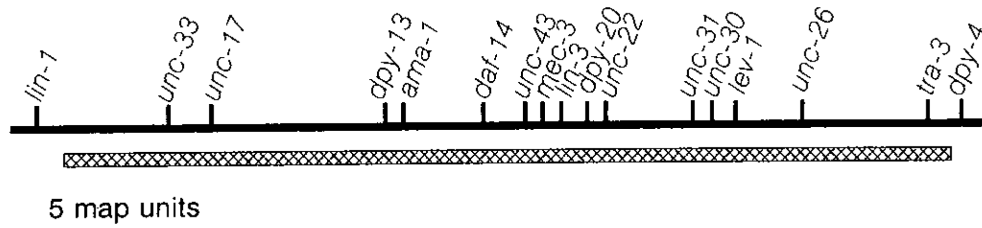

FIG. 1. Partial genetic map of the right-most portion of chromosome IV. The region of chromosome IV recombinationally balanced by the *nT1(IV;V)* translocation is indicated by the cross-hatched line beneath the genetic map (Ferguson and Horvitz 1985; Clark et al. 1988).

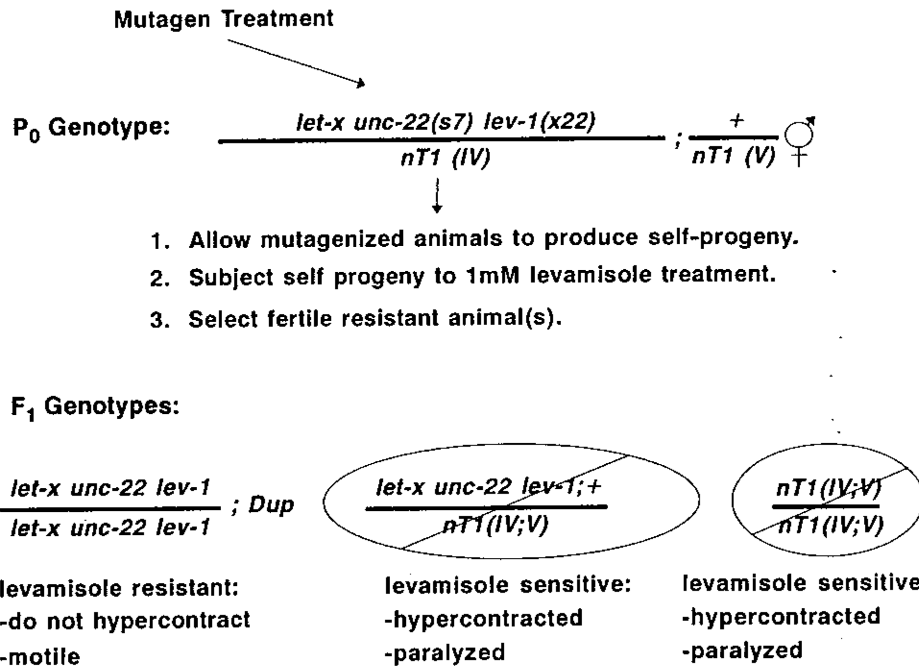

FIG. 2. Generalized flow diagram illustrating the methodology used to recover duplications of *let-56(s2321)*. P_0 hermaphrodites of the indicated genotype are mutagenized, and F_1 animals are subjected to treatment with 1 mM levamisole after the majority of F_1 animals have reached a late larval/early adult stage. In this way we avoid selecting *let-56(s2321) unc-22(s7) lev-1(x22)* arrested homozygotes as candidate duplication-bearing individuals. After levamisole selection, candidate duplication-bearing animals are recognized as individuals who are motile and not hypercontracted. Levamisole-sensitive individuals are severely paralyzed and in many cases die following exposure to 1 mM levamisole.

mately 24 000 F_1s. Four of these individuals appeared only partially resistant to the effects of levamisole. Progeny testing of these four animals revealed that, in each case, the majority of the eggs that were laid failed to hatch. In addition, each of the four animals produced some progeny that lacked vulvae. Both of these characteristics are indicative of the presence of *nT1(IV;V)* (Ferguson and Horvitz 1985). These animals were presumed to be resistant to levamisole because of the induction of an *unc-22* mutation on the *nT1(IV)* chromosome that conferred partial levamisole resistance and were not analysed further. Unc-22 individuals are weakly resistant to the effects of levamisole, probably because of their inability to hypercontract upon exposure to the drug (Brenner 1974; Lewis et al. 1980). The two remaining animals exhibited strong resistance to levamisole. In addition, neither of these individuals produced progeny that indicated the presence of *nT1(IV;V)*. One of these animals displayed a considerably more severe "twitch" than did the other and more closely resembled *unc-22(s7)* homozygous individuals.

The phenotypes of the self progeny produced by this "severe twitcher" were examined. In the F_1, fertile twitchers and sterile twitchers were found. We proposed that the fertile twitchers carried a duplication that covered the *let-56* locus. To determine whether the putative duplication carried in this strain could be present in two copies without detriment to the organism, we picked 20 animals to separate plates and observed their progeny. Several of the animals failed to segregate sterile twitchers, producing only fertile twitcher progeny. This indicated to us that the duplication had become linked to a chromosome, and in these animals was present on both homologues. The duplication was designated *sDp11*. A single animal derived from a parent that had failed to segregate sterile twitcher progeny was selected and used to establish a strain (BC4274). The genotype of this animal was thought to be *let-56(s2321) unc-22(s7) lev-1(x22) sDp11*. However, our results could also be explained if *let-56(s2321)* had recombined away from the *unc-22(s7) lev-1(x22)* marked chromosome during the course of our experiments. If this had occurred, the genotype of BC4274 individuals would be *unc-22(s7) lev-1(x22)*. A recombination event resulting in the loss of the lethal mutation was unlikely for two reasons. First, in the strain that was mutagenized, the mutations *let-56(s2321)*, *unc-22(s7)*, and *lev-1(x22)* were balanced by the *nT1(IV;V)* rearrangement, which eliminates

crossing-over between them (see Figs. 1 and 2). Second, *let-56* is recombinationally close to *unc-22*, separated from it by appromixately 0.5 map units (Schein et al. 1993). We confirmed that the lethal mutation had not recombined away from the other markers on chromosome IV as follows. BC4274 individuals were mated with wild-type (N2) males, and wild-type L4 hermaphrodites resulting from this cross were picked to separate plates and allowed to produce self progeny. One hundred and one young Unc-22 larvae were selected from among the self progeny and were set on separate plates to observe their development. Of these 101 individuals, 3 failed to develop into fertile adults, displaying the sterile adult terminal phenotype characteristic of *let-56(s2321)* homozygous individuals. We concluded that *let-56(s2321)* was present in BC4274 individuals and had not recombined away from the *unc-22(s7)* and *lev-1(x22)* markers. However, because the sterile phenotype segregated only infrequently in this experiment, it appeared that the duplication of *let-56* we had isolated was attached to chromosome IV at a site recombinationally close to the *let-56(s2321)* mutation. In addition, our results showed that this duplication that contained wild-type *let-56* activity did not contain a functional copy of the *unc-22* locus, as individuals that carried either one or two copies displayed an Unc-22 phenotype. Therefore, one of the duplication breakpoints must lie between *let-56* and *unc-22* or possibly within the *unc-22* locus itself.

The other levamisole-resistant animal we recovered following the gamma ray mutagenesis screen displayed a much weaker "twitch" than did BC4274 individuals and exhibited greater motility. The phenotypes of the progeny produced by this "weak twitcher" were examined. In the F_1, three distinct phenotypes were noted. We observed the parental fertile weak twitcher phenotype in addition to sterile severe twitcher animals and fertile nontwitcher animals. The sterile twitcher animals resembled *let-56(s2321) unc-22(s7) lev-1(x22)* homozygous individuals. We selected individual nontwitcher animals for progeny testing and found only nontwitcher offspring among the progeny of these animals, whereas weak twitcher individuals consistently produced the three classes of offspring described above. These observations led us to propose that we had recovered a duplication that contained a wild-type copy of the *let-56* locus and affected the *unc-22* locus in some way. The weak twitching phenotype we had observed was intriguing. Animals homozygous for the *unc-22(e66)* allele and carrying a single copy of the free *unc-22(+)*-bearing duplication *nDp5* (Beitel et al. 1990) did not exhibit a weak twitching phenotype but rather appeared wild type (this study; G. Beitel, personal communication). As a consequence of these observations, we considered two possibilities. Either the duplication we had recovered contained an altered copy of the *unc-22* gene that was incapable of encoding completely wild-type *unc-22* function, or the duplication had become attached to a chromosome where it was subject to some type of position effect down-regulation so that it produced a less than wild-type amount of *unc-22* gene product. We believed the first possibility to be unlikely for the following reason. If we had recovered a duplication that contained an altered copy of the *unc-22* gene, then we should never have observed nontwitcher progeny segregating from the weak twitchers which were, presumably, homozygous for *unc-22(s7)*. None of the self progeny would carry a wild-type copy of *unc-22* and would all therefore twitch. All known mutant alleles of *unc-22* display the twitching phenotype; the *unc-22* gene has been shown to be sensitive to even apparently innocuous lesions, all of which produce the characteristic twitch (Benian et al. 1993). Support for the second hypothesis was provided by the absence of arrested animals among the progeny of the nontwitcher hermaphrodites. If the duplication we had recovered was free, then it seemed likely that an occasional sterile twitcher would be seen among the progeny of the nontwitcher animals. This was not observed. Therefore, the duplication had probably become attached to a chromosome. If so, it seemed possible that it might have become linked to a chromosomal region that was insufficiently transcriptionally active to produce enough wild-type *unc-22* gene product to overcome the twitching phenotype produced by the homozygous *unc-22(s7)* allele. We selected a single nontwitcher hermaphrodite to establish the strain BC4430 and designated the duplication carried by this strain as *sDp10(IV)*.

To ascertain that *unc-22(s7)* was homozygous in BC4430 animals, we mated N2 males with BC4430 hermaphrodites and selected male progeny. Interestingly, none of the male progeny that resulted from the above cross twitched when immersed in a 1% nicotine solution, which causes individuals heterozygous for *unc-22* mutations to twitch in a fashion resembling individuals homozygous for *unc-22* mutations (Moerman and Baillie 1979; 1981). We mated single males from this cross with *dpy-20(e1282) unc-26(e345)* hermaphrodites. Outcross progeny were easily distinguished from self progeny, as self progeny were Dpy–Unc and outcross progeny were wild type. Thirty single male matings were performed, and the progeny resulting from each cross were examined in a 1% nicotine solution. None of the crosses resulted in the production of hermaphrodite progeny that twitched in nicotine, although, in each of the crosses, there were approximately equal proportions of twitching and nontwitching males. These results were consistent with the idea that BC4430 individuals were homozygous for *unc-22(s7)* and carried a duplication of *unc-22* that was attached to the X chromosome and present on both homologues.

To determine whether the *let-56(s2321)* mutation was still present in BC4430 and to test for *sDp10*'s coverage of *dpy-20* and *unc-26*, individual L4 hermaphrodites resulting from the cross described above were picked to individual plates and allowed to produce self progeny. These hermaphrodites were of two possible genotypes, either + +/*dpy-20(e1282) unc-26(e345)*; *sDp10* or *let-56(s2321) unc-22(s7)lev-1(x22)/dpy-20(e1282) unc-26(e345);sDp10*. The genotype of the latter animals was recognized by the appearance of twitcher progeny. The phenotypes of the progeny produced by these hermaphrodites were scored. Sterile twitcher animals resembling *let-56(s2321) unc-22(s7) lev-1(x22)* homozygous individuals were recovered, indicating that *let-56(s2321)* was retained in the BC4430 stock and had not recombined away from the *unc-22(s7) lev-1(x22)* markers. In addition, the frequency of Dpy–Unc animals observed in these experiments indicated that the duplication did not contain functional copies of either the *dpy-20* or *unc-26* genes.

To determine whether *sDp10* covered other genes between *dpy-20* and *unc-26*, we constructed a male strain (BC4475) in which both chromosome IV's were wild type and *sDp10* was present on the X chromosome. + +;*sDp10* males were mated to *unc-22(s7) unc-31(e169)* hermaphrodites. L4 hermaphrodites of the genotype *unc-22(s7) unc-31(e169)*/+ +;

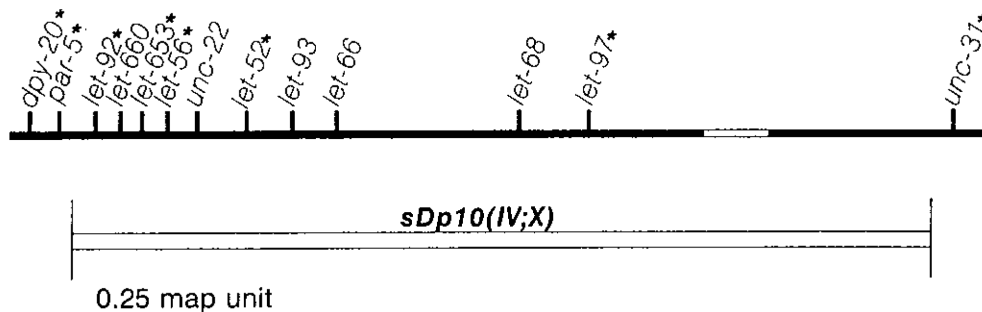

FIG. 3. Partial genetic map of the *dpy-20–unc-31* interval showing the extent of *sDp10*'s coverage of the fourth chromosome. The duplication's genetic breakpoints are indicated by the vertical lines. Markers used in complementation tests with *sDp10* are indicated by asterisks. The open bar between *let-97* and *unc-31* indicates the map in this area has been truncated to fit on the figure.

sDp10 resulting from this cross were picked to individual plates and allowed to produce self progeny in four 24-h broods. The progeny of four hermaphrodites were scored. The frequency of Unc-31 animals observed among the self progeny indicated that the duplication did not contain a functional copy of the *unc-31* gene.

To test whether the duplication carried functional copies of any of the vital loci between *dpy-20* and *unc-31*, ++;*sDp10* males were crossed with hermaphrodites of the genotype *let-(sx) unc-22(s7) [unc-31(e169)]/nT1(IV)*; *+/nT1(V)*, where *let-(sx)* was an allele of one of the essential genes indicated in Fig. 3; some lethal alleles were on chromosomes marked with *unc-31(e169)*. At least 15 F_1 hermaphrodite progeny that failed to twitch in nicotine were picked to individual plates. These F_1 animals were either *let-(sx) unc-22(s7) [unc-31(e169)]/+++;sDp10* or *+++/nT1(IV)*; *+/nT1(V)*; *sDp10*. The progeny of these animals were examined for the presence of twitcher animals. The presence of multiple fertile adult weak twitcher animals [genotype *let-(sx) unc-22(s7) [unc-31(e169)]* and carrying a single copy of *sDp10*] indicated that *sDp10* carried a wild-type copy of the lethal mutation being tested. In cases where lethal mutations had been induced on chromosomes marked with *unc-31(e169)*, the presence of adult Unc-31 animals that twitched weakly indicated that *sDp10* complemented the lethal mutation. When either weak twitchers or weakly twitching Unc-31 animals were seen, several were picked to individual plates for examination and progeny testing. Results obtained during this complementation analysis have allowed us to position the genetic breakpoints of *sDp10* on chromosome IV as shown in Fig. 3.

If *sDp10* had become attached to the X chromosome, as our data indicated, it seemed possible that the copy of *unc-22* contained within *sDp10* might be subject to X chromosome dosage compensation. If so, this could explain the sex difference we noticed for individuals that were homozygous for *let-56(s2321) unc-22(s7) lev-1(x22)* and carrying a single copy of *sDp10*. Hermaphrodites of this genotype exhibited the weak twitching phenotype discussed above, whereas males of this genotype did not exhibit a weak twitch. We propose that in these hermaphrodites, X chromosome dosage compensation has extended into *sDp10*, resulting in insufficient amounts of wild-type *unc-22* transcript to completely mask the Unc-22 phenotype produced by the homozygous *unc-22(s7)* allele. This apparent extension of dosage compensation into X-attached autosomal duplications in *C. elegans* has been suggested in the case of *sDp30(V;X)* (Rosenbluth et al. 1988).

The isolation of *sDp10* and *sDp11* serves to validate the scheme we have devised to allow screening for duplications of vital loci linked to *lev-1(x22)*. The method is powerful; it was possible to easily identify adult mobile hermaphrodites in a background of approximately 24 000 hypercontracted and paralyzed or developmentally retarded individuals. Our scheme should, in theory, be adaptable to accomodate screens for duplications of lethal mutations that map anywhere within the *nT1(IV;V)* balanced region. Because of the pseudolinkage imposed by *nT1(IV;V)*, this includes not only the right half of chromosome IV but also the left half of chromosome V (Ferguson and Horvitz 1985; Clark et al. 1988). Lethal mutations in the latter region would appear pseudolinked to *lev-1(x22)(IV)*.

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