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Increased intragenic recombination and non-disjunction in the Rec-1 strain of *Caenorhabditis elegans*

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Summary

The Rec-1 strain of *Caenorhabditis elegans* increases recombination frequency three-fold. In this paper, we have investigated the effect of Rec-1 on the intragenic recombination phenomena of crossing-over and gene conversion. These events were increased two- to three-fold as was *X*-chromosome non-disjunction. All of the recovered recombinants were independent events, indicating that Rec-1 does not act pre-meiotically. The pattern of recombination in the Rec-1 strain resembles a meiotic pattern more than a radiation expansion. We conclude from this result that the Rec-1 enhancement of recombination is not the result of an increased number of DNA lesions randomly distributed along the chromosome. The increased recombination frequency of Rec-1 was not accompanied by any detrimental effects on growth, progeny number or spontaneous mutation rate. In this regard, the results may have implications for models which propose either selective advantage or disadvantage accompanying increased recombination.

1. Introduction

The only known strong enhancer of recombination frequency exists in the hermaphroditic nematode *Caenorhabditis elegans* (Rose & Baillie, 1979*a*). Rec-1 strains increase recombination frequency three- to four-fold for markers on all six linkage groups. In yeast and *E. coli*, mutants that increase mitotic recombination have been described. These are usually repair defective and hyper-mutable (Holliday *et al.* 1976; Glickman & Radman, 1980; Malone & Hoekstra, 1984). In *Drosophila*, mutants that increase meiotic recombination dominantly are generally chromosomal rearrangements which exhibit a phenomenon known as the interchromosomal affect (Schultz & Redfield, 1951). To date, the only mutant which segregates in a simple Mendelian manner and increases meiotic recombination is Rec-1 in *C. elegans*. In order to understand the mechanism of recombination enhancement in the Rec-1 strain, we have more fully characterized the Rec-1 phenotype with regard to growth, spontaneous mutation rate, *X*-chromosome non-disjunction and intragenic recombination. Our results show that both intragenic recombination and *X*-chromosome nondisjunction were increased in the Rec-1 strain. These increases have little or no effect on growth or spontaneous mutation rate.

In *Drosophila* analysis of intragenic recombination using the *rosy* locus has demonstrated a series of parallels between crossing-over and gene conversion (Hilliker & Chovnick, 1981; Hilliker, Clark & Chovnick, 1987). Their results are consistent with a model that all recombination involves conversion (non-reciprocal transfer of information) in the immediate region of the exchange event. Two mutants, *mei-9* (Hilliker & Chovnick, 1981; Carpenter, 1982, 1984) and *mei-218* (Carpenter, 1982, 1984) have increased conversion frequencies with an accompanying decrease in crossing-over. The authors have suggested that these gene products play an essential role in completing the process leading to the production of flanking marker exchange. We have investigated gene conversion and crossing-over in the Rec-1 strain and found both of these meiotic processes to be increased.

2. Materials and methods

(i) *General*

C. elegans were grown using culture plates containing nematode growth medium (NGM) streaked with *Escherichia coli*, strain OP50 as a food source (Brenner 1974). The wild-type strain N2 and mutant strains were obtained from either the Medical Research Council stock collection in Cambridge, England, the Simon Fraser stock collection Burnaby, British Columbia or the Caenorhabditis Genetics Center (CGC) at the University of Missouri, Columbia, Missouri.

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(ii) *Origin of a high recombination strain*

The high recombination mutation was originally isolated from a heterozygous strain, *dpy-5(e61) unc-15(e73)* + / + + *unc-13(e51)* in the autumn of 1977 (Rose & Baillie, 1979*a*).

(iii) *Measurement of recombination frequency*

The frequency of recombination between linked markers in the hermaphrodite was determined by counting progeny produced from *cis*-heterozygotes (e.g. + + /*dpy-5 dpy-14*). All progeny were counted and removed, and the recombination frequency, p, was calculated according to the formula $p = 1-\sqrt{(1-2R)}$, where R is the fraction of recombinant phenotypes (Brenner, 1974). The total number of progeny was calculated as 4/3 (the number of wild type plus half the recombinants) in order to correct for reduced viability of the segregating double mutant (Rose & Baillie, 1979*b*). The number of recombinant phenotypes was calculated as twice the number of Dpy-5 progeny, since their viability is comparable to wild type. Unless otherwise specified standardized conditions of temperature and parental age were used as recommended by Rose & Baillie (1979*b*).

3. Results

(i) *Growth rate*

Does a mutant like Rec-1 which has three-fold increased recombination have any deleterious affect on the fitness of the individual? In order to measure the fecundity of the Rec-1 strain relative to that of N2, crescent stage hermaphrodites of both genotypes (*rec-1/rec-1* and + +) were transferred to separate culture plates, self-crossed and brooded. Cultures were maintained at 20 °C and the number of progeny counted. In N2, an average of 276 with a standard deviation of 38·5 was observed in 1657 progeny counted. In the Rec-1 strain (BC313), an average of 281 with a standard deviation of 39·9 was observed in 4774 progeny. No difference in fecundity was observed between the two strains.

Further, an experiment was performed in which Rec-1 hermaphrodites competed with N2 worms in a food-limited environment. Three crescent stage hermaphrodites of each genotype (*rec-1/rec-1* and + / +) were placed on 100 mm culture plates containing NGM covered with a lawn of *E. coli*. The population was allowed to grow until starvation symptoms appeared in the progeny (i.e. dauer larvae were produced). The population was transferred to fresh culture plates. A 2 × 2 cm piece of agar containing about 100 worms was cut from the old culture and placed onto a new plate. As the worms rapidly overgrew the plate, they were regularly exposed to a starvation stress. At the end of 2 months (approx. sixteen generations) the genotypes of ten hermaphrodites were determined by out-crossing to *dpy-5 dpy-14 rec-1*/ + + *rec-1* males and then scoring the recombinants in the F2 generation. Four Rec-1 and six N2 individuals were present. Under these conditions, there was no substantial difference in the survival of the two strains.

In addition, no difference was observed between the developmental times of the Rec-1 and N2 strains during the maintenance of male cultures. Male cultures were maintained by transferring males to fresh hermaphrodites each generation. Both wild-type and Rec-1 strains have been transferred in parallel over a number of years. Individuals from both strains were found to mature at the same rate.

(ii) *Spontaneous mutation rate*

Mutation rate in the Rec-1 strain was estimated using the reciprocal translocation, *eT1* (Rosenbluth & Baillie, 1981). The translocation *eT1* produces pseudo-linkage between *dpy-18* and *unc-46* and suppresses recombination within the translocated regions. The effect of *rec-1* on the mutation rate was examined by scoring for spontaneous occurrence of recessive lethal mutations in the 40 map unit region balanced by *eT1*. The occurrence of a lethal mutation in the area balanced by *eT1* would result in the absence of Dpy-18 Unc-46 worms. Any plate with two or less Dpy Unc worms was progeny-tested to confirm the existence of a lethal mutation. No lethal mutations were isolated from the 1877 screened chromosomes of the Rec-1 strain. The upper 95% confidence limit (calculated from Stevens, 1942) is $1{\cdot}6 \times 10^{-3}$ spontaneous recessive lethal mutations per 40 map units, which does not differ significantly from the previously reported frequency of 6×10^{-4} spontaneous lethals in the N2 strain (Rosenbluth, Cuddeford & Baillie, 1983). Rosenbluth *et al.* (1983) recovered two lethals in 3198 tested chromosomes.

We further tested whether the recombinational suppression of *eT1* could be reversed by *rec-1*. Heterozygotes of the genotype, + *dpy-18(III)*; *unc-46(V)/eT1* + + ; *rec-1/rec-1* were allowed to self for several generations. These heterozygotes proved to be stably balanced by *eT1*. No Dpy-18 or Unc-46 individuals were recovered amongst their progeny. That is, the pseudo-linkage imposed by the cross-over suppression of *eT1* was maintained in the high recombination strain. Crossing-over did not occur within the translocated region.

(iii) *Intragenic recombination*

In order to test whether the *rec-1* mutation increased recombination within genes, the *unc-13* gene was studied using methods modified from Rose & Baillie (1980). Uncoordinated, high recombination hermaphrodites were crossed with Rec-1 males. Heterozygous

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Table 1. *The effect of Rec-1 on intragenic crossing-over and gene conversion*

Strain	Chromosomes screened	Crossing-over		Conversion	
		Observed[a]	Frequency	Observed	Frequency
N2	2×10^6	5	5×10^{-6} (1·6–11)[b]	3	$1·5 \times 10^{-6}$ (0·3–5·5)
Rec-1	3×10^6	22	15×10^{-6} (13–17)	12	4×10^{-6} (2·1 – 6·6)

[a] Doubled to calculate recombination frequency.
[b] Figures in parentheses are 95% confidence intervals.

Table 2. *The effect of Rec-1 on* X*-chromosome non-disjunction*

Strain	Temp. (°C)	Progeny	Males	%	95% C.I.[a]
N2	20	1657	3	0·2	0·03–0·5
Rec-1	20	4896	19	0·4	0·3–0·5
N2	26	1933	17	0·9	0·7–1·1
Rec-1	26	1825	32	1·8	1·5–2·1

[a] C.I., Confidence intervals.

Table 3. *Rec-1 and radiation increased recombination frequency*

Markers	Wild type (% *R*)	Rec-1 (% *R*)	Radiation (% *R*)
dpy-5 dpy-14(I)	1·5 (3780)	4·5 (1790)	2·9 (861)
bli-3 unc-35(I)	1·9 (1960)	4·9 (1410)	1·2 (3174)

Note. The numbers of progeny scored are indicated in parentheses. Radiation data from Kim & Rose (1987).

male progeny (i.e. *unc-13(e450)/+ ; rec-1/rec-1*) were mated to *dpy-5 unc-13 unc-29 rec-1* hermaphrodites to produce individuals heterozygous for the flanking loci and heteroallelic for *unc-13*. Uncoordinated hermaphrodites (Unc-13 phenotype) were placed individually on plates and allowed to self. All plates were screened for two generations. The normal segregants included Dpy-5 Unc-13 and Unc-13 individuals (Unc-13 is epistatic to Unc-29). Some Dpy-5 Unc-13 worms were removed to avoid overcrowding.

Exceptional progeny consisting of Wild-type (e.g. normal movement) and Unc-29 (e.g. slightly uncoordinated) were isolated from the background of Unc-13 (severely uncoordinated). Progeny testing to observe exchange of flanking markers was done in order to distinguish between crossing-over and conversion (apparent double cross-overs) (see Moerman & Baillie, 1979). Wild-type and Unc-29 progeny carrying a chromosome produced by a single intragenic cross-over event segregated the expected Unc-29 progeny, unlike either parental or conversion genotypes. These results were as expected based on the map position of *e51 e450* determined by Rose & Baillie (1980). Wild-type and Dpy-5 Unc-29 progeny carrying a chromosome produced by conversion segregated as expected. The total number of chromosomes screened was estimated from the mean number of progeny per hermaphrodite per generation. Half of the recombinants (Wt and Unc-29) were easily distinguished, whereas half were indistinguishable from the parental types. Two separate experiments were performed. The results of the replicates were consistent and have been combined (Table 1). The frequency of both crossing-over and conversion increases two to three-fold in the Rec-1 strain. The 22 recombinants recovered from the Rec-1 experiment were independent events. No evidence of clustered or multiple events was observed, indicating that Rec-1 did not act premeiotically.

(iv) X-*chromosome non-disjunction*

Other meiotic phenomena, such as the stability and disjunction of the *X*-chromosome, were examined. The amount of *X*-chromosome non-disjunction was assayed by the number of males (i.e. XO individuals) produced in the self-progeny of a hermaphrodite. Crescent-stage hermaphrodites were individually placed on culture plates, transferred at regular intervals, and their progeny counted. A two-fold higher incidence of *X*-chromosome non-disjunction was observed in the Rec-1 strain (Table 2).

(v) *Chromosome region*

In order to test whether the increased recombination in the Rec-1 strain was similar to that observed after treatment with radiation, recombination frequencies in two chromosomal regions were compared. Table 3 shows the results for a region within and one outside the gene cluster of linkage group *I*. Recombination frequency increases in both intervals approximately three-fold in the Rec-1 strain, but only the interval within the gene cluster was expanded with radiation.

Discussion

Unlike previously described meiotic mutants, Rec-1 recessively increases recombination three-fold with no

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detrimental effect on growth rate, and no elevation in spontaneous mutation rate. The *rec-1* gene product appears to function in meiosis and have an affect on both flanking marker exchange, conversion and proper disjunction.

It is unlikely that accumulated DNA lesions are the primary cause of the high recombination phenotype because the regional pattern of enhancement is unlike that for radiation. In *C. elegans*, radiation increased recombination frequency in the region of the gene cluster (centrally located in the chromosome), but not outside the cluster (Kim & Rose, 1987). Rec-1, on the other hand, increased recombination frequency in both regions three-fold. Radiation presumably causes DNA lesions which can act as stimuli for the initiation of cross-over events. A consequence of this, in *Drosophila*, is the occurrence of radiation-induced map expansion in heterochromatic regions. In these regions there is very little meiotic recombination per unit of DNA. In *Caenorhabditis*, radiation causes map expansion in the region of a meiotic map cluster (Kim & Rose, 1987). If the Rec-1 phenotype were the result of an increased number of DNA lesions, we would expect the regional pattern of enhancement to be like that of radiation. Yet no increase in recombination was observed in the *bli-3 unc-35* interval after treatment with radiation, whereas a three-fold increase in that region was observed in the Rec-1 strain. It seems therefore, that Rec-1 increases recombination without distorting the normal meiotic map.

Rec-1 does not have an increased mutation rate and conversion is not decreased. Both these facts are consistent with the idea that Rec-1 is not the result of a major repair defect. Furthermore, Rec-1 is not radiation sensitive (Hartman & Herman, 1982).

Since both recombination and conversion events are increased, Rec-1 does not alter the proportion of heteroduplexes that resolve into crossovers. Rather, it is as if more heteroduplexes are formed or more are recombinagenic. The formation of a three-fold higher number of cross-over events may be related to the disruption of proper segregation. The increased frequency of non-disjunction may be either a consequence or a cause of the increased recombination. An important question in this regard is whether non-disjunction of all the chromosomes is increased.

Since the Rec-1 phenotype is recessive, it is unlikely that the increased recombination is a consequence of chromosomal rearrangement. Nor is Rec-1 likely to be a *cis*-acting overproducer of a recombination enzyme. It could however affect some control point in the regulation of recombination frequency, such as a defective repressor of the recombination machinery, for example.

Rec-1 may provide a valuable system for the study of recombination in higher eukaryotes. The increased conversion frequency could be used to test mechanisms of cross-over suppression. Inversions in *Drosophila*, for example, reduce the recovery of cross-over events, but do not disrupt pairing or gene conversion (Chovnick, 1973). In *C. elegans* such rearrangements would be expected to demonstrate both wild-type and Rec-1 increased conversion. On the other hand, the reciprocal translocation, *eT1*, has been proposed to suppress crossing-over in the heterozygote due to a lack of homologue pairing (Rosenbluth & Baillie, 1981). Rec-1 does not relieve the cross-over suppression of *eT1* and one might predict that conversion would not be detected.

A number of authors have proposed that increasing recombination frequency would either increase or decrease the fitness of the individual (see Maynard-Smith, 1978). The fact that Rec-1 generally increases meiotic exchange events with very few other affects on the organism may make it a powerful model system for testing the effects of increased recombination on an organism.

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