MASTER

RADIATION INDUCED ASYMMETRIES IN MITOTIC RECOMBINATION:
EVIDENCE FOR A DIRECTIONAL BIAS IN THE FORMATION OF ASYMMETRIC HYBRID
DNA IN YEAST

by

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Key words:

(genetic recombination, radiation induced asymmetries, mitotic recombination,
yeast genetics)

GENETICS

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ABSTRACT:

We have examined radiation induced mitotic recombination using two alleles (his1-36, his1-49) in the his1 gene. When the haploid containing his1-36 is irradiated with varying doses of γ -rays and then mated with the unirradiated strain containing his1-49, analyses of the selected prototrophs show them to be primarily + +/+ 49. If, on the other hand, the haploid strain containing his1-49 is the irradiated parent, the prototrophic diploids are primarily + +/36 +. In control experiments, where either both strains are irradiated or not irradiated, no such asymmetries are found. These data indicate that the irradiated haploid chromosome tends to be the recipient of genetic information.

We interpret these results as indicating a directional bias in the formation of hybrid DNA in radiation induced mitotic recombination, and discuss these results in terms of current models of genetic recombination.

In recent years, there has been a growing interest in understanding the molecular mechanism of genetic recombination. Although the Holliday model (1,2) provides a conceptual framework to understand the latter stages of the recombination process in eukaryotes, little is known about the sequence of events involved in the initiation of recombination. In principle, initiation can be either a symmetric or an asymmetric event. By a symmetric event, we mean the equal participation of both chromatids to give, initially, the reciprocal formation of hybrid DNA. Such a start could then be followed by a nicked translation mechanism to give both nonreciprocal and reciprocal regions of hybrid DNA (3). An asymmetric event, on the other hand, implies unequal participation of both chromatids to give rise, initially, to hybrid DNA on only one chromatid (i.e., this corresponds to nonreciprocal hybrid DNA formation). The subsequent formation of reciprocal hybrid DNA is required to explain the patterns of post-meiotic segregation and the occurrence of flanking marker exchange (4-7).

We have examined radiation induced mitotic recombination in yeast using two alleles (his1-36, his1-49) in the his1 gene. When the haploid containing his1-36 is irradiated with varying doses of γ -rays and then mated with the unirradiated strain containing his1-49, analyses of the selected prototrophs show them to be primarily + +/ + 49. If, on the other hand, the haploid strain containing his1-49 is the irradiated parent, the prototrophic diploids are primarily + +/ 36 +. In control experiments, where both strains were either irradiated or not irradiated, no such asymmetries are found. These data indicate that the irradiated haploid chromosome tends to be the recipient of genetic information.

We interpret these results most easily as indicating a directional bias in the formation of hybrid DNA in radiation induced mitotic recombination, and discuss these results in terms of current models of genetic recombination.

MATERIALS AND METHODS:

Strains:

The strains used here have been constructed from crosses involving strains obtained from R. Snow (8), F. Sherman and S. Lieberman (personal communication).

These are:

Z279A-1197a α hom3 his1-36 ARG ade TYR

Z279A-1198b α hom3 his1-36 ARG ADE TYR

Z352AV-2b a HOM his1-49 arg6 ade tyr7-1

His1-36 and his1-49 are nonsuppressible alleles which do not complement each other. His1-36 is scored by complementation with tester strains carrying his1-61 (8). His1-49 can be identified since it forms prototrophs with his1-36 tester strains after irradiation with near u-v light.

Media:

Media used are: glucose nutrient agar (GNA), synthetic complete (SC), SC minus a particular amino acid (omission media) and potassium acetate sporulation media (KAC). For details, see ref. 9.

Experimental procedure:

Haploid strains were grown up overnight in liquid GNA at 30 C. Aliquots were then put into scintillation vials and exposed to γ -rays from a Co_{60} source for varying lengths of time. Cell counts on the irradiated haploids were done by plating diluted samples on GNA to estimate the percent survival. To form diploids and to select for prototrophs, undiluted samples of haploid strains of opposite mating types were placed together on histidine deficient media and then spread over the plate with a metal spreader. Prototrophic recombinants appear 48 hours later. These were first tested to determine their phenotypes, and then sporulated on KAC plates. Random spore analysis was done to determine the geno-

types of the haploid spores (10) and, from this, the genotypes of the diploids could be inferred.

RESULTS:

Figure 1 shows the survival curves after γ -radiation for the three haploid strains. One can see that strains Z352AV-2b and Z279A-1198b have similar radiation sensitivities, while strain Z279A-1197a may be somewhat more radiation sensitive.

Figure 2 shows a plot of the number of prototrophic recombinants recovered per viable haploid cells plated as a function of the γ -ray dose. As can be seen, there is an initial increase in this ratio at low doses (0.6-2.0 krads), followed by a leveling off in this ratio at higher doses (4.0-6.0 krads). It is evident therefore that there is a substantial increase in the level of mitotic recombination stimulated by the doses of γ -radiation used in these experiments. The initial increase (i.e., at 0.6-2.0 krads) is not accompanied by an excessive loss in parental viability (see Figure 1).

Table 1 summarizes the genotypes of the prototrophic recombinants with respect to the surviving alleles in the his1 gene. It can be seen that the prototrophs arise primarily from a single site conversion event. With no irradiation (or with equal doses of γ -radiation to both parents) there are roughly equal numbers of + +/ 36 + and + +/ + 49 diploids (we attribute the small departure from equality in crosses 1197a x 2b to be due to the differences in radiation sensitivity of these two strains). However, if only one of the parents is irradiated, the prototrophic recombinants found show a definite bias. If the irradiated parent contains his1-36, then the primary conversion class is + +/ + 49. If, on the other hand, the irradiated parent contains his1-49, then the primary class is + +/ 36 +. This bias has been observed for all radiation doses that have been examined.

We also show data regarding the arrangement of flanking markers in the prototrophic recombinants (see Table 2). These data indicate that the major class of events involves no flanking marker exchange. The remaining classes include reciprocal flanking marker exchanges and gene conversion events that involve flanking markers. It should be noted that classes F2 and G2 (which could arise by nondisjunction) form less than 3% of the total number of prototrophs, and therefore, nondisjunction does not play a major role in causing the asymmetries observed.

DISCUSSION:

Interpreted in terms of hybrid DNA models for genetic recombination, our data are consistent with the following possible mechanisms:

- 1) In the absence of radiation, hybrid DNA forms symmetrically and spans both sites (see Fig. 3A). Generally, only one of the four mismatches is corrected and this gives rise to $+ +/36 +$ and $+ +/+ 49$ diploids with equal frequency. The low frequency of $+ +/36 49$ argues against many events that involve multiple mismatch corrections and against many events where hybrid DNA involves only one site. In the presence of radiation, two possible events can occur. Hybrid DNA initially forms symmetrically and spans both sites; correction (via nick translation) involves the elimination of irradiated hybrid DNA spanning both sites (see Fig. 3B and C). Subsequent mismatch correction then allows prototroph formation. Alternatively, hybrid DNA initially forms symmetrically and spans only one site; correction (via nick translation) involves the elimination of irradiated hybrid DNA spanning this site (Fig. 3B and C). No further correction is necessary.

- 2) In the absence of radiation, hybrid DNA forms symmetrically and spans either both or only one site. One of these hybrid DNA regions is then removed

by a nick translation mechanism to give rise to asymmetric hybrid DNA (see Fig. 3B and C). If hybrid DNA spans both sites, subsequent mismatch correction is necessary to give a prototroph; if hybrid DNA spans only one site, no further correction is necessary. In the presence of radiation, we envision a similar mechanism to occur except that there is a directional bias introduced when forming asymmetric hybrid DNA. Unlike the first mechanism, however, radiation does not alter the process of genetic recombination -- it simply adds an element of bias to a preexisting mechanism.

3) In the absence of radiation, hybrid DNA forms asymmetrically and spans either both or only one site. As before, if asymmetric hybrid DNA spans both sites, a mismatch correction step is needed to form a prototroph; if hybrid DNA spans only one site, however, no further correction is necessary. The effect of radiation is to bias the directionality of asymmetric hybrid DNA formation -- again perturbing a preexisting mechanism. Unlike the other mechanisms that have been discussed, however, radiation alters the initiating event in the recombination process.

Although the frequency of prototrophic diploids increases with radiation dose, the degree of bias does not. This can be interpreted in one of two ways. If a major fraction of induced recombination events is unbiased while a minor fraction of events is strongly biased and the relative proportions of these events remain constant with dose, then the degree of bias is expected to be relatively independent of dose. Alternatively, if all recombination events had a moderate bias independent of dose then this would also explain the data.

The bias we have observed in these mitotic recombination studies is, in many respects, similar to that observed by Gutz (11) in meiotic studies of the

M26 mutation in Schizosaccharomyces pombe. This mutation gives rise to an enhanced frequency of gene conversion events that show profound asymmetry in the direction of conversion. A similar type of mutation has been observed by Catcheside in Neurospora crassa (12). This mutation (cog) acts to enhance recombination in the his3 gene and gives rise to a definite directionality in the conversion products when cog is heterozygous.

Acknowledgements:

This work has been supported by grants from the National Institutes of Health, the American Cancer Society and the Department of Energy (DOE). This paper has been assigned report no. UR 0000-00 at the DOE, the University of Rochester. We thank Karen Gargano for her expert technical assistance. We are grateful to Dr. R. K. Mortimer for sharing his unpublished results from similar experiments that are consistent with the finds presented here.

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TABLE CAPTIONS:

Table 1. Genotypes of the recombinant diploids with respect to the surviving his1 alleles. It should be noted that the haploid strains used in these experiments show no significant his1 revertants when tested under the experimental conditions previously described.

Table 2. Genotypes of the prototrophs that arise by single site conversion events including the flanking marker arrangements.

FIGURE CAPTIONS:

Figure 1. Haploid survival as a function of the γ radiation dose for strains used in these experiments. (A) Z352AV-2b (B) Z279A-1197a (C) Z279A-1198b. Different symbols denote data points obtained in different experiments, while $\times$ denotes averaged values.

Figure 2. Number of prototrophic recombinants recovered per viable haploid cells plated as a function of the γ ray dose. (A) Z279A-1197a $\times$ Z352AV-2b (B) Z279A-1198b $\times$ Z352AV-2b. $\blacktriangle$ indicate data points obtained when both parents are irradiated, $\bullet$ indicate data points obtained when the Z279A parent is irradiated, $\circ$ indicate data points obtained when Z352AV-2b is irradiated. Line 1 connect averaged $\blacktriangle$ data points, line 2 connects averaged $\circ$ data points and line 3 connects averaged $\bullet$ data points. See text for discussion.

Figure 3. Schematic diagram illustrating the possible arrangement of hybrid DNA in his1 and the expected prototrophs produced. (A) Reciprocal hybrid DNA formation. (B) Nonreciprocal hybrid DNA formation that occurs in the + 49 parent DNA duplex. (C) Nonreciprocal hybrid DNA formation that occurs in the 36 + parent DNA duplex. See text for discussion.

ARRANGEMENTS OF HYBRID DNA IN *his 1*

POSSIBLE PROTOTROPHS PRODUCED

+	+
+	49

+	+
36	49

+	+
36	+

+	+
+	49

+	+
+	+

+	+
36	49

+	+
36	+

+	+
36	49

NONE

+	+
36	+

+	+
36	+

+	+
+	49

+	+
+	49

NONE

GENOTYPE OF
RADIATION DOSE PROTOTROPHIC RECOMBINANTS

(Krad/s)					
1197a	2b	++/36+	++/49	++/++	++/36 49
0	0	39	48	2	0
.6	.6	3	10	0	0
2	2	16	30	5	0
4	4	20	27	2	0
Total		39	67	7	0
.6	0	7	21	0	0
1	0	6	26	1	0
2	0	4	17	2	0
4	0	2	11	1	0
Total		19	75	4	0
0	.6	17	8	1	1
0	1	16	9	0	1
0	2	26	10	2	4
0	4	23	4	2	1
Total		82	31	5	7
1198b	2b				
0	0	18	26	2	2
2	2	17	15	2	0
4	4	20	22	3	0
Total		37	37	5	0
.6	0	2	26	2	1
1	0	6	28	0	0
2	0	4	12	1	0
4	0	2	19	0	3
Total		14	85	3	4
0	.6	25	6	0	0
0	1	18	9	0	0
0	2	25	15	0	0
0	4	27	9	1	3
Total		95	39	1	4

1197a x2b

1198b x2b

Genotypes of Prototrophs		Neither irradiated	Both irradiated	<i>his</i> 1-36 irradiated	<i>his</i> 1-49 irradiated	Neither irradiated	Both irradiated	<i>his</i> 1-36 irradiated	<i>his</i> 1-49 irradiated
A 1.	- 36 + +	14	15	4	29	10	12	5	31
	+ + + -								
2.	- + + +	24	22	31	11	11	8	36	17
	+ + 49 -								
B 1.	+ + + +	4	2	4	2	0	1	7	3
	- + 49 -								
2.	+ + + +	3	14	11	0	5	8	15	3
	+ + 49 -								
C 1.	- + + -	2	4	2	4	0	2	5	3
	+ + 49 +								
2.	- + + -	3	2	3	1	0	0	2	1
	+ + 49 -								
D 1.	+ 36 + +	3	1	0	4	1	1	1	5
	- + + -								
2.	- 36 + +	4	1	0	15	0	4	1	19
	- + + -								
E 1.	- 36 + -	2	8	1	10	1	5	2	10
	+ + + +								
2.	- 36 + +	8	3	5	8	2	4	2	10
	+ + + +								
F 1.	+ + + -	0	3	4	3	2	1	2	2
	- + 49 +								
2.	+ + + -	0	1	1	1	2	2	2	1
	+ + 49 -								
G 1.	+ 36 + -	0	5	3	5	2	0	0	5
	- + + +								
2.	- 36 + +	0	1	1	2	0	2	0	2
	- + + +								
H 1.	+ + + +	1	1	1	0	0	0	0	3
	- + 49 +								
2.	+ + + -	0	1	3	0	1	0	1	1
	+ + 49 +								
3.	- + + -	0	2	0	2	1	0	0	0
	- + 49 +								
4.	- + + +	1	0	0	0	0	1	2	1
	- + 49 +								
5.	- + + +	0	0	1	0	1	0	2	2
	- + 49 -								
6.	+ + + -	2	3	1	1	0	2	2	0
	+ + 49 +								
7.	- + + +	2	2	2	3	0	2	1	2
	+ + 49 +								
8.	+ + + +	0	3	3	1	0	3	3	0
	+ + 49 +								
9.	- + + -	0	1	1	1	1	0	0	0
	- + 49 -								
10.	+ + + -	0	2	0	0	0	0	0	0
	- + 49 -								
11.	+ +/+ 49	6	4	7	1	2	7	5	0
I 1.	+ 36 + +	0	0	0	1	0	0	0	2
	+ + + -								
2.	+ 36 + -	0	0	0	0	0	1	0	1
	+ + + +								
3.	- 36 + -	1	0	0	1	1	1	0	0
	+ + + -								
4.	- 36 + -	1	0	0	1	0	0	0	5
	- + + +								
5.	+ 36 + +	1	2	1	0	1	2	0	0
	- + + +								
6.	- 36 + -	0	0	0	1	0	1	0	0
	- + + -								
7.	+ 36 + +	2	1	1	0	0	0	2	0
	+ + + +								
8.	+ 36 + -	0	0	0	0	0	1	0	1
	- + + -								
9.	+ +/36 +	3	2	3	5	0	3	1	4