Reciprocal Exchanges Instigated by Large Heterologies in the *b2* Gene of Ascobolus Are Not Associated With Long Adjacent Hybrid DNA Stretches

T. Langin, H. Hamza,[1] V. Haedens and J. L. Rossignol

Laboratoire Interactions Moléculaires Génomiques, Bâtiment 400, Université Paris-Sud, 91405 Orsay Cedex, France

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ABSTRACT

In the gene *b2* of *Ascobolus immersus*, large heterologies increase the frequencies of reciprocal exchanges on their upstream border (corresponding to the high non-Mendelian segregation side). Tests were made to determine whether these reciprocal exchanges, instigated by large heterologies, resulted from the blockage of a Holliday junction bordering a hybrid DNA tract extending from the end of the gene to the heterology. Three types of experiments were performed to answer this question. In all cases, results did not correlate the presence of reciprocal exchanges instigated by large heterologies with the presence of adjacent hybrid DNA tracts. These reciprocal exchanges were rarely associated with postmeiotic segregation at upstream markers, they were not associated with gene conversion of a marker within the interval and their frequency was not decreased by decreasing the frequency of hybrid DNA formation in the gene. These results led to the proposal of the existence of a precursor to reciprocal exchange different from a single branch-migrating Holliday junction. This precursor migrates rightward and its migration is dependent on the DNA sequence homology. The existence of this precursor does not exclude that reciprocal exchanges resulting from the maturation of single Holliday junctions bordering adjacent hybrid DNA tracts could also occur.

THE current models of recombination assume that reciprocal exchanges (RE) come from the maturation of HOLLIDAY junctions (HOLLIDAY 1964) bordering recombination areas. In the AVIEMORE model (MESELSON and RADDING 1975) recombination is initiated by a single-strand nick promoting an asymmetric strand transfer which is followed by a transition to reciprocal strand transfer. This leads to heteroduplex DNA (HDNA) ending with a HOLLIDAY junction. The resolution of this HOLLIDAY junction can lead to reciprocal exchange. In the double-strand break repair model (SZOSTAK *et al.* 1983), recombination is initiated by a double strand gap followed by a gap filling using the homologous duplex as a template. In the process, the two interacting DNA molecules are connected by heteroduplexes on both sides of the gap. In this model RE come from the resolution of the two HOLLIDAY junctions flanking the recombination area. The characteristics of genetic recombination in the *b2* gene of *Ascobolus immersus* allowed us to test the hypothesis of crossing-over occurring at the border of the recombination event (*e.g.*, heteroduplex formation or gene conversion). The HDNA formation in gene *b2* is reflected by non-Mendelian segregations (NMS) observed in mutant × wild-type crosses (PAQUETTE and ROSSIGNOL 1978). HDNA is initiated mostly near the left end (high NMS frequency end) (KALOGEROPOULOS 1986) and extends toward the right. Experiments involving heterozygous large deletion or large insertion, located in the middle part of the gene, have shown an increase of the frequency of RE upstream the large heterology (LH) (NICOLAS 1983) and a decrease of the frequency of aberrant 4:4 asci (reflecting the symmetric HDNA which corresponds to HDNA formed on both interacting duplexes) downstream the LH (HAMZA, NICOLAS and ROSSIGNOL 1987). In the AVIEMORE model, these two results could be explained by the blockage of the HOLLIDAY junctions upstream the LH followed by their maturation. In this hypothesis, RE should be associated with NMS for markers located upstream the RE, on their high NMS side. Moreover, we should be able to correlate the frequency of RE with that of HDNA formation. Three types of experiments were performed to test the relationship between HDNA and RE and all led to the same conclusion that RE instigated by LH were not associated with the extension of HDNA between the left end of the gene and the site of the RE.

MATERIALS AND METHODS

Crossing conditions and media were previously described (RIZET, ROSSIGNOL and LEFORT 1969; ROSSIGNOL and PAQUETTE 1979).

Strains used belong to stock 28 (RIZET, ROSSIGNOL and LEFORT 1969). The map of the *b2* mutants used is shown in Figure 1. All single mutants but *G234* give a white

[1] Present address: Monofya University, Faculty of Agriculture, Department of Genetics, Shebin el Kom, Egypt.

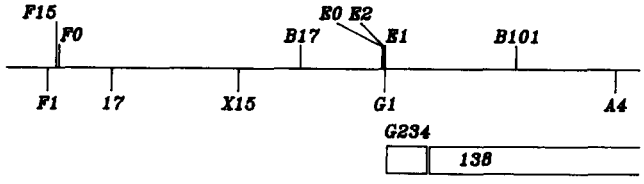

FIGURE 1.—Genetic map of *b2*.

ascospore phenotype. They exhibit different NMS patterns previously defined by LEBLON (1972). *F0, B17, E1* and *B101* are class B mutations [they give a type B NMS pattern with no postmeiotic segregations (PMS) and an excess of convertant asci with two wild-type and six mutant spores (2+:6m) over convertant asci with six wild-type and two mutant spores (6+:2m)]. *F15, E0* and *E2* are class A mutations [they give a type A NMS pattern with no PMS and an excess of 6+:2m over 2+:6m]. *F1, 17, X15, G1* and *A4* are class C mutations (they give a type C NMS pattern with numerous PMS).

F0 and *F15* on the one hand and *E1, E0* and *E2* on the other hand belong to the same intragenic suppression group named "F" and "E," respectively. They are putative 1 base pair additions (*F0* and *E1*) and putative 1 base pair deletions (*F15, E0* and *E2*) corresponding to class B and class A mutations, respectively (LEBLON and PAQUETTE 1978). The double mutants *E2 E1* and *E0 E1* show a pink spore phenotype distinguishable from both the brown wild-type and the white mutant spore phenotypes. The double mutant *F0 F15* exhibits brown spores, identical to wild type. *138* and *G234* are two large deletions (HAMZA, NICOLAS and ROSSIGNOL 1987). *G234* is a revertant of the unstable mutation *G0* and exhibits a brown spore phenotype, identical to wild type (NICOLAS *et al.* 1987).

Experimental conditions accurate for the comparison of NMS patterns and recombination asci frequencies were devised by ROSSIGNOL and PAQUETTE (1979). To randomize the strain background differences between *m1* × *m2* and *m1* × *m3* crosses the same *m1* parental strain was crossed to a set of *m2* parental strains and to a set of *m3* parental strains which had been isolated from an unique *m2* × *m3* cross. When comparing crosses *m1* × *m3*, *m1* × *m4*, *m2* × *m3* and *m2* × *m4*, the sets of *m1* and *m2* parental strains were isolated from an unique *m1* × *m2* cross and the sets of *m3* and *m4* parental strains were isolated from an unique *m3* × *m4* cross. Significant differences between recombinant asci frequencies correspond to probabilities smaller than 0.05, calculated by the Student test.

Ascus analysis: all crosses were heterozygous for *mt* (mating type) *rdn1* (ascospore shape marker) and *vag8* (mycelial growth marker). This allowed the determination of sister spore pairs during ascus analyses. *rnd1, vag8* and *mt* are unlinked to *b2* (NICOLAS *et al.* 1981). *mt* and *vag8* are closely linked to their centromere.

Analysis of recombinant asci: the individual ascospores were germinated and the strains were analyzed for their genotype by appropriate back-crosses. The presence of *E2 E1* (or *E0 E1*) in white spores was detected by crossing with *G1* (which does not recombine with *E2 E1*): the recombinant spores were all pink when *E2 E1* (or *E0 E1*) was present, and all brown when it was absent. *F0 F15* modifies the NMS pattern of *F1:* in crosses *F1* × *F0 F15*, no PMS are observed. This allowed to test for the presence of *F0 F15* by crossing with *F1* [when *F0 F15* was associated with a white spore mutation, the test crosses with *F1* lead to only 2 Colored:6 White (2C:6W) recombinant asci instead of both 2C:6W and 1C:7W recombinant asci].

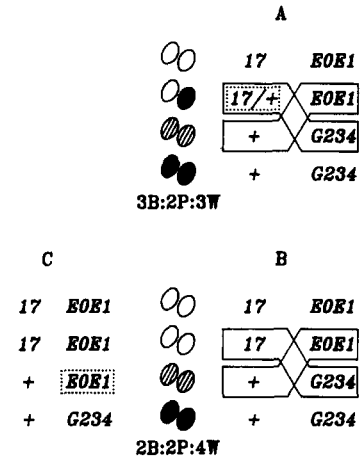

FIGURE 2.—Two types of RE can be visualized in *17 E0 E1* × *+G234* (or *17 E0 E1* × + + +) crosses. A, RE between *17* and *E0 E1* associated with a PMS at *17*. B, RE between *17* and *E0 E1* with a Mendelian segregation at *17*. C, 2+:6m conversion at *E0 E1*.

RESULTS

Reciprocal exchanges instigated by large heterology are not associated with PMS for upstream marker: We have tested the hypothesis of RE associated with the extension of HDNA by using class C mutations located upstream the deletion *G234*. Class C mutations rarely undergo mismatch correction. If RE are associated with HDNA spanning the class C mutation, they should be associated most of the time with a PMS at this mutant site. We have compared crosses *17 E0 E1* × + with crosses *17 E0 E1* × *G234*. *17* is unefficiently corrected: this mutation gives more than 90% PMS among NMS (PAQUETTE and ROSSIGNOL 1978). *E0 E1* gives pink spores and *G234* gives wild-type brown spores. Since *E0 E1* and *G234* are tightly linked, the pink or brown color of the spores indicates the alternative *E0 E1* or *G234* genotypes. Two types of RE can be visually detected (Figure 2): RE with a Mendelian segregation at *17* (2B:2P:4W asci; where B = brown, P = pink, W = white) and RE with a 5+:3m segregation at *17* (3B:2P:3W asci). In the hypothesis, we expect only the 3B:2P:3W asci to be enhanced in the presence of *G234*. The results (Table 1) show a significant increase of the 2B:2P:4W asci but a nonsignificant increase of the 3B:2P:3W asci. A sample of 2B:2P:4W asci was analyzed to determine the size of the fraction corresponding to RE. This analysis showed that indeed RE were strongly increased with *G234*. The same type of crosses was performed using *X15* instead of *17* as left class C mutation (Table 1). Again, only the 2B:2P:4W class showed a significant increase. We conclude that RE instigated by *G234* are mainly associated with a Mendelian segregation at *17* and *X15*.

TABLE 1

Effect of *G234* on frequencies of 3B:2P:3W and 2B:2P:4W asci in crosses with *17 E0 E1* and *X15 E0 E1*

Crosses		n^a	Number per 1000 asci			
				2B:2P:W[b]		
			3B:2P:3W RE with a PMS (Fig. 2A)	RE with a Mendelian segregation (Fig. 2B)	Conversion of *E0 E1* (Fig. 2C)	Total
I	*17 E0 E1* × + + +	4	9	2	10	12
II	*17 E0 E1* × + *G234*	5	16	24	4	28[c]
III	*X15 E0 E1* × + + +	4	6			9
IV	*X15 E0 E1* × + *G234*	5	10			32[d]

Crosses I and II on the one hand and crosses III and IV on the other hand were made in conditions of comparison.
[a] n = Number of distinct crosses studied; in each cross a sample of 1000 asci was counted.
[b] A sample of 17 and 16 2B:2P:4W asci were analyzed in crosses I and II, respectively.
[c] The frequency is significantly higher in crosses II than in crosses I.
[d] The frequency is significantly higher in crosses IV than in crosses III.

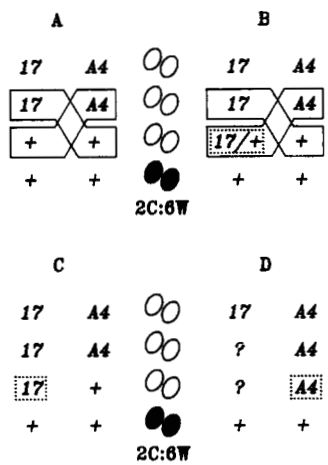

FIGURE 3.—Ascus genotypes of the 2C:6W asci in the *17 E2 E1 A4* × *+E2 E1* + and *17 E2 E1 A4* × *+G234* + crosses. The genotype for *E2 E1* and *G234* is not shown. The segregation of *A4* was first checked; when a NMS at *A4* was found the segregation of *17* was not further studied (see D). A, RE between *17* and *A4* associated with a Mendelian segregation at *17*. B, RE between *17* and *A4* associated with a PMS at *17*. C, 2+:6m conversion at *17*. D, 2+:6m conversion at *A4*.

We have also tested if RE instigated by *G234* could be associated with a 3+:5m segregation at *17*. This was done by comparing crosses *17 E0 E1 A4* × *E0 E1* with crosses *17 E0 E1 A4* × *G234*. In these crosses RE occurring between *17* and *G234* lead to 2C:6W asci when they are associated with a Mendelian segregation at *17* and when they are associated with a 3+:5m segregation at *17* as well (Figure 3). The results (Table 2) show a strong increase of RE associated with a Mendelian segregation at *17* but no (or little) increase for RE associated with a 3+:5m segregation at *17*. In both experiments (Table 1 and Table 2) the absolute frequency of the increase of RE associated with a Mendelian segregation at *17* was close to 2% of total meioses.

Since mismatch correction at *17* is too rare to account for these RE we conclude that RE instigated by *G234* are not associated with HDNA spanning *17*.

Reciprocal exchanges instigated by large heterologies are rarely associated with conversion in upstream interval: Another prediction of the hypothesis of an association between RE and the extension of HDNA is the following: if we select for RE in an interval, we should find associated gene conversion for a marker lying within the interval. The deletion *138* was used in these experiments (the point mutation *B101* being used in control crosses). The left mutation was *B17*. In crosses VII and VIII the silent double mutation *E2 E1* was used as middle marker (Table 3). A strong increase of the 2C:6W recombinant asci frequency was observed in test crosses as compared to control crosses. Ascus analysis showed that this increase was not a consequence of 6+:2m conversions of *B17* or *138*. As a matter of fact, the contribution of conversions to 2C:6W recombinant asci was lower in test crosses because *138* does not undergo 6+:2m conversion. The enhancement of recombinant asci was entirely due to an enhancement of RE. The RE responsible for this enhancement were located between *E2 E1* and *138* and did not show conversion at *E2 E1*. This indicates that most of the RE instigated by *138* are not associated with gene conversion at *E2 E1*. The increase of RE associated with a Mendelian segregation at *E2 E1* is sufficient alone to account for the increase of 2C:6W recombinant asci. The same crosses were performed in the absence of *E2 E1* (crosses IX and X, Table 3). A similar enhancement of 2C:6W asci corresponding to an enhancement of RE between *B17* and *138* was observed. This shows that *E2 E1* has no or little effect upon RE between *B17* and *138*.

The same type of crosses was performed using *F0* instead of *B17* as left marker (Table 4). In these crosses we were able to distinguish two types of 2C:6W asci: 2P:6W asci where the two colored spores

TABLE 2

Effect of *G234* on frequencies of 2C:6W recombinant asci in crosses with *17 E2 E1 A4*

Crosses		n^a	Number per 1000 asci				
				Genotypes of 2C:6W asci[b]			
			2C:6	RE with a Mendelian segregation at *17* (Fig. 3A)	RE with a PMS at *17* (Fig. 3B)	Conversion of *17* (Fig. 3C)	Conversion of *A4* (Fig. 3D)
V	*17 E2 E1 A4* × + *E2 E1* +	5	49	5	12	6	26
VI	*17 E2 E1 A4* × + *G234* +	6	91[c]	25[d]	15[e]	8	43

Crosses V and VI were made in conditions of comparison.

[a] n = Number of distinct crosses studied; in each cross a sample of 1000 asci was counted.

[b] A sample of 94 and 97 2C:6W asci were analyzed in crosses V and VI, respectively.

[c] The frequency is significantly higher in crosses VI than in crosses V.

[d] Among 27 asci, 25 showed a Mendelian segregation for *G234*; 24 of them enjoyed RE between *17* and *G234* and 1 between *G234* and *A4*.

[e] Among 16 asci, 15 showed a Mendelian segregation for *G234*; 14 of them enjoyed RE between *17* and *G234* and 1 between *G234* and *A4*.

TABLE 3

Effect of *138* on frequencies of 2C:6W recombinant asci in crosses with *B17 E2 E1*

Crosses		n^a	Number per 1000 asci						
				6+:2m conversion		RE			
			2C:6W	*B17*	*B101* (*138*)	*B17-E2 E1* interval	*E2 E1-B101* (*138*) interval	Conversion at *E2 E1*	Total
VII	*B17 E2 E1* + × + + + *B101*	3	42[b]	17	12	3.5	6	4	13.5[c]
VIII	*B17 E2 E1* + × + + + *138*	3	73[d,e]	18	0	6	40	9	55
IX	*B17* + + + × + + + *B101*	3	55[f]	31	14				10
X	*B17* + + + × + + + *138*	3	95[g,h]	25	0				68

Crosses VII and VIII on the one hand and crosses IX and X on the other hand were made in conditions of comparison. In crosses VII and VIII, the distinction between 2P:6W and 2B:6W asci was not made when scoring 2C:6W asci.

[a] n = Number of distinct crosses studied; the number of asci counted in each cross was 2000 in crosses VII and 1000 in crosses VIII, IX and X.

[b] In crosses VII, among 42 asci analyzed, 17 showed a 6+:2m conversion at *B17*, 12 a 6+:2m conversion at *B101* and 13 showed a RE between *B17* and *B101*.

[c] The segregation of *E2 E1* in crosses VII was studied on 31 asci with a RE, starting from a much larger sample of 2C:6W asci.

[d] The frequency is significantly higher in crosses VIII than in crosses VII.

[e] In crosses VIII, the frequencies of 6+:2m conversion and the frequencies of the various types of RE and the segregation of *E2 E1* are deduced from the analysis of 48 2C:6W asci.

[f] A sample of 16 2C:6W asci was analyzed in crosses IX.

[g] The frequency is significantly higher in crosses X than in crosses IX.

[h] A sample of 15 2C:6W asci was analyzed in crosses X.

TABLE 4

Effect of *138* on frequencies of 2C:6W recombinant asci in crosses with *F0*, in the presence of *E2 E1*

Crosses	n^a	Number per 1000 asci							
					6+:2m conversion		RE		
		Total 2C:6W	2P:6W	2B:6W	*F0*	*B101* (*138*)	*F0-E2 E1* interval	*E2 E1-B101* (*138*) interval	Conversion at *E2 E1*	Total
XI *F0* + + + × + *E2 E1 B101*	4	154[b]	26	128[c]	112	21	14	7	0	21
XII *F0* + + + × + *E2 E1 138*	4	193[d,e]	102[d]	91	112[f]	0	20[g]	91.5[h]	0	111.5

Crosses XI and XII were made in conditions of comparison.

[a] n = Number of distinct crosses studied; in each cross a sample of 2000 asci was counted.

[b] A random sample of 22 2C:6W asci was analyzed in crosses XI.

[c] The frequency is significantly higher in crosses XI than in crosses XII.

[d] The frequency is significantly higher in crosses XII than in crosses XI.

[e] A random sample of 19 2C:6W asci was analyzed in crosses XII.

[f] Among 11 asci, 2 also showed a RE in the *E2 E1-138* interval.

[g] Among 2 asci, 1 also showed a RE in the *E2 E1-138* interval.

[h] Among 9 asci, 2 also showed a 6+:2m conversion of *F0* (see footnote *f*) and 1 also showed a RE in the *F0-E2 E1* interval (see footnote *g*).

TABLE 5

Effect of *F0 F15* on recombination between *B17* and *138* (or *B101*)

Crosses	n^a	Number per 1000 asci		
		Total 2C:6W	2P:6W	2B:6W
XIII + + *B17* + + + × + + + *E2 E1 B101*	4	51[b]	29[b]	22[b]
XIV *F0 F15 B17* + + + × + + + *E2 E1 B101*	4	27	17	10
XV + + *B17* + + + × + + + *E2 E1 138*	4	87[c]	71[c]	16
XVI *F0 F15 B17* + + + × + + + *E2 E1 138*	4	122[d]	109[d]	13
XVII + + *B17* + + + × *F0 F15* + *E2 E1 138*	3	80[e]	73[e]	7
XVIII *F0 F15 B17* + + + × *F0 F15* + *E2 E1 138*	2	50	45	5

Crosses XIII, XIV, XV and XVI on one hand and crosses XVII and XVIII on the other hand were made in conditions of comparison.

[a] n = Number of distinct crosses studied; the number of asci counted in each cross was 500 in crosses XIII, XIV, XV and XVI abnd 2000 in crosses XVII and XVIII.

[b] The frequency is significantly higher in crosses XIII than in crosses XIV.

[c] The frequency is significantly higher in crosses XV than in crosses XIII and XIV.

[d] The frequency is significantly higher in crosses XVI than in crosses XIII, XIV and XV.

[e] The frequency is significantly higher in crosses XVII than in crosses XVIII.

have a *E2 E1* genotype and 2B:6W asci where the two colored spores have a wild-type genotype. In crosses involving *138*, the 2C:6W asci were increased and this increase was restricted to the 2P:6W asci. The analysis of a random sample of 2C:6W recombinant asci showed that this increase corresponded to an increase of RE between *E2 E1* and *138* which actually correspond to 2P:6W asci. None of the analyzed RE was associated with a conversion at *E2 E1*. Among the nine asci with a RE between *E2 E1* and *138*, two showed a conversion at *F0*. The meaning of such asci showing noncontiguous RE and gene conversion events will be considered in the discussion section.

In conclusion the rarity of gene conversion at *E2 E1* among RE instigated by *138* argues against the existence of associated HDNA spanning *E2 E1*.

Decreasing HDNA formation does not decrease RE instigated by large heterologies: If RE were associated with the formation of HDNA, decreasing the frequency of HDNA formation should decrease the frequency of RE. To test this prediction, we used double-point mutations located in the left *b2* region (F region). When present in one parent, these mutations decrease the frequency of HDNA in the gene: all the classes of NMS are depressed by at least one third for all studied markers (NICOLAS and ROSSIGNOL 1983). We tested the effect of the double mutation *F0 F15* upon RE in crosses *B17* × *E2 E1 138* and *B17* × *E2 E1 B101* (Table 5). *F0 F15* which shows a brown (wild-type) spore phenotype does not interfere with the detection of 2C:6W recombinant asci between the two white spore mutations *B17* and *B101* (or *138*). The 2C:6W asci corresponded to two types of asci: 2P:6W where the two colored spores have a *E2 E1* genotype and 2B:6W where the two colored spores have a wild-type genotype. RE instigated by *138* are expected to be located between *E2 E1* and

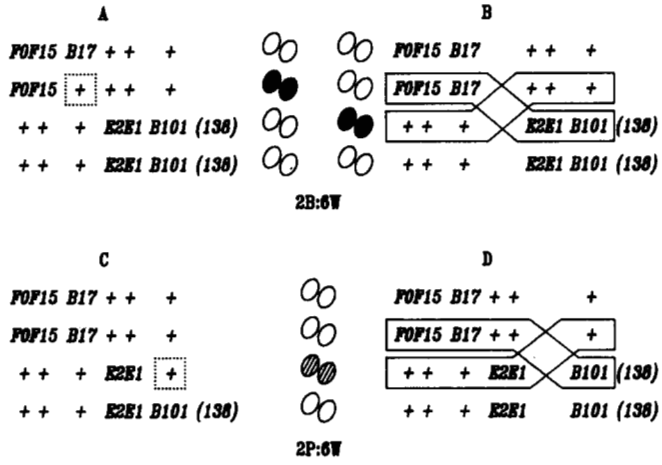

FIGURE 4.—Ascus genotypes reflecting the main events that lead to 2C:6W asci in crosses *F0 F15 B17* + + + × + + + *E2 E1 B101* (or + + + *E2 E1 138*). A, 6+:2m conversion at *B17*. B, RE between *B17* and *E2 E1*. C, 6+:2m conversion at *B101* (or *138*). D, RE between *E2 E1* and *B101* (or *138*).

138, and to be mostly associated with a Mendelian segregation of *E2 E1* (Tables 3 and 4). Such events lead to 2P:6W recombinant asci (Figure 4). Indeed, in the absence of *F0 F15*, *138* specifically stimulates the 2P:6W asci (compare crosses XIII and XV, Table 5). In crosses involving *B101* (compare crosses XIII and XIV) *F0 F15* decreases the frequencies of the two classes of recombinant asci (2P:6W and 2B:6W). This was expected since *F0 F15* should decrease the frequency of conversion of *B17* (giving 2B:6W asci) and *B101* (giving 2P:6W asci) (Figure 4). In crosses involving *138* (compare crosses XV and XVI), *F0 F15* does not depress the frequency of 2P:6W recombinant asci. This suggests that *F0 F15* does not decrease the RE instigated by *138*. Actually the presence of *F0 F15* leads to an enhancement of the frequency of 2P:6W asci. This effect is due to the *F0 F15* heterozygosity: when *F0 F15* is homozygous

TABLE 6

Origin of 2P:6W recombinant asci in crosses + + *B17* + + + × *F0 F15* + *E2 E1 138* (crosses XVII)

Total 2P:6W	2P:6W asci origin[a]			*E2 E1* segregation[b] among RE		*F0 F15* ssegregation[b] among RE		
	6+:2m conversion							
	B17	*138*	RE	6+:2m	4+:4m	6+:2m	2+:6m	4+:4m
73	8	2	63	0	63	16	3	44

All the numbers are absolute frequencies given per 1000 asci.
[a] A sample of 35 2P:6W asci was analyzed.
[b] The number of asci analyzed with a RE was 24. All correspond to RE located in the *E2 E1-138* interval.

the frequency of 2P:6W asci is smaller than when it is heterozygous (compare crosses XVII and XVIII). We have confirmed that the majority of 2P:6W asci corresponds to RE by analyzing a sample of 2P:6W asci in crosses XVII (Table 6). This analysis has confirmed the absence of association between RE instigated by *138* and conversion at *E2 E1*. All the RE were located in the *E2 E1-138* interval. In the same way as in crosses *F0* × *E2 E1 138* (Table 4), some asci ($^7/_{24}$) with RE between *E2 E1* and *138* showed a conversion for the F markers (*i.e., F0 F15*). This confirms that RE instigated by LH are not associated with contiguous gene conversion, but may be associated with noncontiguous gene conversion.

In conclusion, *F0 F15* has two antagonist effects: it depresses HDNA formation in the gene and it overstimulates the formation of RE instigated by the large deletion *138*.

DISCUSSION

We have tested if RE instigated by LH in *b2* were due to the maturation of HOLLIDAY junctions bordering HDNA tracts extending from the left end. We addressed this question by three types of experiments which lead to negative answers: (1) RE instigated by LH are rarely associated with PMS for upstream markers with low efficiency of mismatch correction, (2) RE instigated by LH are not associated with gene conversion for a middle marker, and (3) decreasing the frequency of HDNA in *b2* does not decrease the frequency of RE instigated by LH. Thus in all experiments we were unable to correlate RE instigated by LH with the adjacent extension of HDNA.

In the frame of the current recombination models two alternative hypotheses can explain these results.

In the first hypothesis, the RE may be associated with HDNA tracts which are normally initiated within the *E2 E1-138* interval. To account for the high frequency of RE instigated by *138* (up to 9% of meiosis), this secondary HDNA initiations should be very frequent. The study of the HDNA distribution in the *b2* gene (KALOGEROPOULOS 1986) shows that a very small part of HDNA tracts, if any, are initiated in this interval. This makes this hypothesis very unlikely.

In the second hypothesis, large deletion (or insertion) mutations trigger new recombination events leading to RE at their border. One way to trigger RE was proposed by HASTINGS (1984): mutations involved in a heteroduplex could be corrected via a double-strand break repair mechanism taking an homologous duplex as a template. This repair process would convert the mutation and could generate RE (SZOSTAK *et al.* 1983). A double-strand break repair mechanism has been evoked to explain the correction properties of the *G234* deletion (HAMZA *et al.* 1986). However it is clear that RE instigated by *G234* do not result from a double-strand break repair mechanism. Indeed these RE are very rarely associated with gene conversion for *G234* (Table 2). Another way to trigger RE would be the creation of some signal resulting from the mutation (deletion or insertion). This can be excluded. Indeed, NICOLAS (1983) and one of us (HAMZA 1985) have shown that *G234* had no effect upon RE when it was homozygous. The effect is not the result of a peculiar heterology since it was observed with five LH, located in two distinct areas of *b2* (NICOLAS 1983; H. HAMZA, unpublished results). In all the cases the increase was limited to the upstream interval (on the left side): this rather suggests the existence of a polar component to RE which comes from the left *b2* end. The existence of such a component is confirmed by the following experiments: cumulating two LH in the same cross suppresses the increase of RE at the upstream border of the right heterology but does not suppress the increase of RE at the upstream border of the left heterology (NICOLAS 1983). The only way to account for the results is to postulate the existence of a precursor to RE which propagates rightward from the left region. The migration of this precursor is dependent on the DNA sequence homology. The blockage of this precursor by the LH would be followed by its maturation in full RE. All these properties are the same as that expected from a single

branch migrating HOLLIDAY junction. However the failure to detect any association between RE and contiguous HDNA clearly excludes this explanation. The precursor could be viewed either as a transitory homologous duplex-to-duplex pairing step (MCGAVIN 1971) allowing RE via direct cutting and rejoining of DNA strands, or as a short HDNA segment flanked by two co-migrating Holliday junctions as in the model proposed by SOBELL (1972) or as in the Interwrapping model proposed by POTTER and DRESSLER (1978). DNA joint molecules which could be associated by this last type of junctions have been visualized by BELL and BYERS (1982) on chromosomal DNA during meiosis in yeast.

The absence of association between RE instigated by LH and contiguous HDNA does not exclude the possibility that HDNA which is not contiguous to the RE may be associated with them. As a matter of fact, the crosses involving *F0* or *F0 F15* as a left marker showed some conversions for these markers associated with a RE between *E2 E1* and *138*. The occurrence of conversion at the left end, with Mendelian segregation at *E2 E1* suggests that RE can be associated with non-contiguous HDNA tracts. Results obtained by SANG and WHITEHOUSE (1983) at the *buff* locus in *Sordaria brevicollis* also favor the existence of noncontiguous association between RE and HDNA. HOLLIDAY (1974) pointed out that the mismatch correction of markers transiently involved in a heteroduplex, bordered by two co-migrating HOLLIDAY junctions, could explain such noncontiguous associations. Together with the observation that it is the heterozygosity of *F0 F15* which is responsible for the overstimulation of RE, the existence of associated gene conversions of F markers suggests that precursors to RE instigated by *138* are related in some way with the formation of HDNA in the left end of the gene.

All the RE do not obligatory come from the maturation of the postulated precursor, RE resulting from the maturation of Holliday structures bordering long extending HDNA tracts as described in the Aviemore model might also occur: this could account for the existence in control crosses (not involving LH) of RE associated with gene conversion of the middle marker *E2 E1*. This could also explain the occurence in control crosses of asci showing a PMS at *17* and a RE between *17* and the right marker.

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