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Genetics

Hybrid DNA formation during meiotic recombination

(aberrant segregation/branch migration/polarity/*Ascobolus immersus*)

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ABSTRACT **G234 is a silent mutation located in the middle of gene *b2*, which controls spore pigmentation in *Ascobolus immersus*. Its effect on the aberrant segregation patterns of white spore mutants located in the same gene was investigated. When heterozygous, G234 decreases the frequency of aberrant segregations of the mutants located on its right, toward the low conversion end. It almost completely suppresses the aberrant 4:4 asci for mutants giving postmeiotic segregation and decreases the disparity between the 6 wild-type:2 mutant and 2 wild-type:6 mutant aberrant asci for mutants giving only these types of convertant asci. These effects are polar; G234 does not change the aberrant segregation pattern of the mutants located on its left, toward the high conversion end. This behavior suggests that G234 blocks the migration of the symmetric phase of hybrid DNA that diffuses from the high conversion end but does not prevent the formation of asymmetrical hybrid DNA. Taking into account previous observations, we conclude that the high conversion end corresponds to a region of asymmetric initiation of recombination rather than to a region of preferential ending of recombination. The asymmetric hybrid DNA first formed is further changed into a symmetric phase that extends via branch migration toward the low conversion end.**

It was proposed by Holliday (1) that homologous recombination is mediated by reciprocal strand exchange between homologous DNA molecules. During this intermediate state, hybrid DNA (hDNA) is formed on the two interacting DNA molecules (symmetrical hDNA). The formation of symmetric hDNA is supported by two kinds of observations. In prokaryotes, electron micrographs visualize the presence of figure-eight plasmid dimers showing two strand-crossing junctions (2). In Ascomycetes, some genetic markers frequently yield aberrant 4:4 asci in which two of the four meiotic products give a postmeiotic segregation (3–5), thus showing the existence of two heteroduplex DNA molecules during meiosis. However, several lines of evidence suggest that asymmetrical hDNA involving only one of the two interacting chromatids is also formed during genetic recombination (6, 7). The observation of the aberrant segregation pattern of various mutants controlling spore pigmentation and lying in gene *b2* of *Ascobolus* suggested that both modalities of hDNA formation—symmetric and asymmetric—could occur in this gene. At the end of the gene at which aberrant segregation is frequent, asymmetric hDNA would preponderantly form whereas, at the other end, at which aberrant segregation is less frequent, symmetric hDNA would be formed most of the time (8, 9). Further observation of aberrant segregation occurring simultaneously at sites located one at each end of this gene suggested that the asymmetric hDNA formed at the high end is often physically associated in the same meiosis with symmetric hDNA at the low end (10). The study of sites stimulating recombination in phage λ (11) and in Ascomycetes (12–14) shows that the two interacting molecules do not need to play equal roles during the recombination act; furthermore, biochemical experiments involving *recA* protein (15) have shown the possible existence of numerous types of asymmetric interaction. Hence, although the general existence of symmetric hDNA structures, as foreseen by Holliday, seems more and more probable (16), it also becomes obvious that part of the overall process is basically asymmetric. This apparent contradiction is overcome in the Aviemore model of recombination (17), which postulates asymmetric initiation of the recombination process followed by symmetric hDNA formation through isomerization. However, as no definitive demonstration that the initiation of recombination is an asymmetric process has yet been found, recombination models postulating symmetric initiation (18, 19) still have to be ruled out. A key problem in this context is the distinction between starting and ending regions in the recombination process. Thus, two interpretations account equally well for the observations made in *b2* (10): (*i*) random symmetric initiation of recombination events followed by a switch to asymmetric hDNA distribution undergoing preferential termination in a region located in the high conversion end or (*ii*) asymmetric initiation in a preferential starting region located in the high conversion end followed by a switch to symmetric hDNA distribution and random ending toward the low conversion end.

To choose between these hypotheses, we used a cryptic mutation located in the middle of *b2*. When heterozygous, this mutation strongly perturbs the conversion pattern of closely linked mutants. It is inferred from the modifications observed in the aberrant segregation patterns that the mutant blocks propagation of symmetrical hDNA and that this propagation proceeds from the high conversion end toward the low conversion end. We infer that the initiation of hDNA formation occurs in the high conversion region where hDNA distribution is asymmetric.

MATERIAL AND METHODS

Media (20–22) and crossing conditions (9) have been described. Strains used belong to stock 28 of *Ascobolus immersus* (23) and bear the *cv2A* modifier of gene *b2* (24).

Mutants. The mutants used lying in *b2* are shown in Fig. 1. All except G234 give a white spore phenotype instead of the wild-type brown spore phenotype. The origins of type C mutants (8) and of types A and B mutants (9) have been given. G234 derives from the unstable spontaneous mutant G0 that recombines with neither G1 nor E1. In self-crosses, G0 leads to a high frequency of revertant pigmented spores. These revertants are pseudo-wild-type: either they show a pseudo-wild-type phenotype (the distribution of pigment on the spore wall is not uni-

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Abbreviations: 6+2m, etc. . . ., 6 wild type:2 mutant, etc. . . .; 6:2, 6+2m and 2+6m; hDNA, hybrid DNA; pms, asci showing postmeiotic segregation.

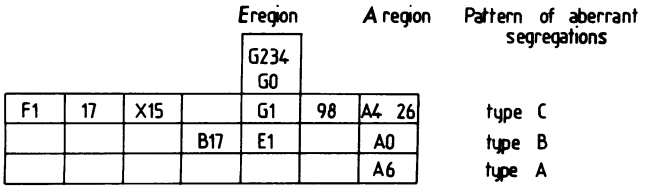

FIG. 1. Map of the *b2* mutants used (see ref. 25). Type C mutants give many pms (5:3 and aberrant 4:4 asci) in addition to 6:2 segregation. Type A and B mutants give very few pms; type A gives an excess of 6 wild type:2 mutant (6+2m) over 2 wild type:6 mutant (2+6m) convertant asci and type B gives an excess of 2+6m over 6+2m convertant asci (26). Recombinants were obtained in (G234 × B17) and (G234 × 98) crosses but not in crosses (G234 × G1). Regions *A* and *E* correspond to regions in which frame-shift mutants belonging to the same group of intrageneic suppression are located (27).

form) or they have a wild-type spore phenotype but modify the aberrant segregation pattern of G1 (28). G234 belongs to this second category of revertants: it is distinguished from $b2^+$ in crosses with *G1*. ($b2^+$ × G1) crosses give ≈80% asci showing postmeiotic segregation (pms) corresponding to 5:3 and aberrant 4:4 asci among aberrant segregations (8) whereas (G234 × G1) crosses give chiefly 6:2 asci and no or few pms among the aberrant segregations (28). G234 is located in the gene *b2* in, or close to, the *E* region; this conclusion is based on three arguments. (*i*) G0 could never by reisolated in the progeny of the (G234 × $b2^+$) crosses. (*ii*) the revertants of G0 that show a pseudo-wild-type spore phenotype do not give any wild-type brown spored recombinant when crossed with G1, suggesting that the other revertants with a wild-type spore phenotype, such as G234, do not recombine with G1 either: (*iii*) When asci showing aberrant segregation for G1 or E1 were selected from crosses of (G234 × G1) or (G234 × E1), no wild-type nor double mutant recombinant was ever found, suggesting that G234 lies very close to G1 and E1; in crosses between G234 and the white spore mutants 17, A4, 26, B17, 98, etc. . . ., asci showing an aberrant segregation were also selected—some showed a coconversion for G234 and the white spore mutant while others showed wild-type or double mutant recombinant spores, indicating that these mutations lie in sites close to G234 but distinct from it.

Double Mutants. G234-A4 and G234-26 were obtained by crossing G234 with A4 or 26, screening for 3B5W (three brown, five white spores) or 5B3W asci, and then crossing the white spore of the mixed pair with wild type. Among the progeny of this last cross, 3B5W or 5B3W asci were isolated, and the brown spore of the mixed pair was crossed with G1. The existence of brown spores with a G234 genotype was thus determined, indicating that the parental white spore was actually double mutant G234-A4 (or G234-26).

In all crosses, two additional markers were used: *mt* (mating type) and *rnd1-1* (round spores). These two markers are not linked to *b2*; *mt* is loosely linked to *rnd1* (30cM) (29). *Rnd1* was used to detect aberrant 4:4 segregation of the *b2* marker (5).

Testing the Effect of G234. The effect of G234 on the aberrant segregation pattern of a mutant m was tested by comparing crosses ($b2^+$ × m) and (G234 × m). In each comparison, the same m strain was used, the genotype of which was *b2*·m, mt^-, $rnd1^+$. Sets of $b2^+$ and of G234 strains were used as test indicators: all these strains were issued from the same ($b2^+$ × G234) cross; their genotype was $b2^+$ (or *b2*.G234), mt^+, *rnd1-1*. The m strain was crossed to each test strain ($b2^+$ or G234) and a sample of 1000 asci was counted in each cross. Thus, the comparison would take into account the variability of results among ($b2^+$ × m) and among (G234 × m) crosses. In the same way, other comparisons involved the cross of a double-mutant strain G234-m with the sets of $b2^+$ and G234 test strains.

RESULTS

The results of a series of experiments in which the aberrant segregation patterns of three type C mutants located toward the low conversion end (on the right of G234 in Fig. 1) were compared in the presence of homozygosity and heterozygosity at the G site are given in Table 1. Without exception, when G234 is heterozygous, the frequency of total aberrant segregation is significantly reduced as a consequence of the dramatic reduction of the frequency of aberrant 4:4 asci. This effect occurs in both repulsion crosses [e.g., (+ A4 × G +) compared with (+ A4 × + +)] and coupling crosses [e.g., (G A4 × + +) compared with (G A4 × G +)]. Concerning the comparison between wild-type and mutant homozygosity at G, the results of experiments 2 and 3 are similar to those of experiments 4–6, and the results of experiment 7 are similar to those of experiments 8 and 9. This suggests that the type of homozygosity (wild type or mutant) at G causes no important difference in the conversion pattern of these mutants.

The decrease in the frequency of aberrant segregations due to G234 is shown in Table 2; there is, on average, one third fewer aberrant segregations when G234 is heterozygous. The decrease in aberrant 4:4 segregations is very drastic, corresponding, on average, to 90% loss.

The influence of G234 on the aberrant segregation pattern of two mutants (one type A, one type B) located to its right and giving no pms is shown in Table 3. As in the case of type C mutants in the same region, a reduced frequency of aberrant segregation is observed when G234 is heterozygous (again, about one-third of the aberrant segregations are lost). This reduction corresponds to a decrease in the number of asci in the major aberrant segregation class (2+6m for A0 and 6+2m for A6), leading to a reduction in the disparity coefficient (the ratio

Table 1. Aberrant segregation patterns of mutants 98, A4, and 26 in the presence of homozygosity and heterozygosity at the G234 site

Exp.	Parents crossed	*n*	6:2	5:3	Aberrant 4:4*	FAS†‡
1	(+ 98 × + +)	6	51	74	16(8–25)	143 ± 8
	(+ 98 × G +)	8	45	40	0(0–2)	89 ± 5
2	(+ A4 × + +)	12	8	79	45(22–70)	132 ± 4
	(+ A4 × G +)	12	11	90	6(4–10)	107 ± 4
3	(+ A4 × + +)	8	10	85	66(53–92)	161 ± 6
	(+ A4 × G +)	8	17	99	7(2–11)	123 ± 6
4	(G A4 × G +)	4	9	89	87(71–95)	185 ± 7
	(G A4 × + +)	4	12	96	13(4–25)	121 ± 10
5	(G A4 × G +)	5	9	99	87(68–96)	195 ± 8
	(G A4 × + +)	5	13	107	6(4–14)	129 ± 7
6	(G A4 × G +)	3	10	99	79(74–86)	188 ± 9
	(G A4 × + +)	2	15	101	6(5–6)	122 ± 4
7	(+ 26 × + +)	7	10	66	65(38–83)	141 ± 6
	(+ 26 × G +)	8	10	60	6(0–10)	76 ± 3
8	(G 26 × G +)	4	12	81	52(43–68)	145 ± 11
	(G 26 × + +)	4	18	73	9(7–10)	104 ± 9
9	(G 26 × G +)	5	10	74	60(41–68)	145 ± 12
	(G 26 × + +)	5	13	66	6(1–10)	88 ± 4

Parents crossed (mutant strain × test strain). In each cross, a sample of 1000 asci was observed. Frequencies of 6:2, 5:3, and aberrant 4:4 and the total frequency of aberrant segregation (FAS) are per 1000 asci.
* Values in parentheses are extremes.
† Mean ± SEM.
‡ Includes rare segregations (i.e., 7:1 and 8:0).

Table 2. Percent of initial aberrant segregations and percent of initial aberrant 4:4 asci left when G234 is heterozygous

Exp.	% aberrant segregation	% aberrant 4:4 asci
1	62	0
2	81	13
3	76	11
4	65	15
5	66	7
6	65	8
7	54	9
8	72	17
9	61	10

Experiments 1–9 are as described in Table 1.

between the number of asci in the major class and the number of asci in the minor class).

The influence of G234 on aberrant segregation patterns was also investigated for 3 mutants located toward the high conversion end (on the left of G234 in Fig. 1). No change was observed for these mutants (Table 4).

In conclusion, when homozygous, G234 has little or no effect on the patterns of aberrant segregation. When heterozygous, G234 acts only on mutants located to its right: it decreases the frequency of aberrant segregation of the five mutants tested; it almost completely suppresses the aberrant 4:4 asci for type C mutants and it reduces the disparity between 6+2m and 2+6m for type A and B mutants.

As expected from these results, G234 has no effect on the pattern of aberrant segregation of mutants located in other spore pigmentation genes (two genes tested; data not shown).

DISCUSSION

One can reasonably assume that the patterns of aberrant segregation depend primarily on the following factors: the frequency of hDNA fromation per meiosis (γ), the probability that hDNA involves one of the interacting DNA molecules (α) or both ($1 - \alpha$) at the mutant site, and the probability of correction of the mismatches formed at this site in hDNA (P) (8). The decrease in aberrant 4:4 asci for the right-hand sites when G234 is heterozygous can be interpreted in two ways: either G234 increases mismatch correction at these sites, thus decreasing postmeiotic segregation, or G234 prevents the formation of symmetric hDNA, which is required to obtain aberrant 4:4 segregation. Two observations argue against an influence of G234 on mismatch correction. First, it has been shown that corrections triggered at one site in the A (or E) region do not (or very rarely) span the E (or A) region (30, 31): corrections triggered by the G234 mismatch in region E are thus presumed not to interfere with the correction of A4 or 26 mismatches in region A, except in the situation that the size of the correction tract triggered by G234 should be larger than the size of corrections triggered by other mutants used in previous experiments. A second, stronger, argument against an effect on mismatch correction is based on the estimated values of γ, α, and P. Our estimate is derived from the algebra developed by Paquette and Rossignol (8). The philosophy of this algebra is that, without correction, 5:3 segregations will reflect an asymmetric distribution of hDNA and aberrant 4:4 asci will reflect a symmetric distribution; when correction occurs, 6:2 convertant asci are expected to appear and the ratio 6:2/pms is expected to increase along with the mismatch correction frequency. We have calculated the values of γ, α, and P for the three type C mutants affected by G234 (Table 5): no change for the frequency of mismatch correction is detected when G234 is heterozygous. The frequencies of symmetric hDNA decrease sharply when G234 is heterozygous. However, the decrease in the frequency of hDNA per meiosis is less than that of the symmetric phase, the latter is partly compensated by an increase in the asymmetric phase.

The effect of G234 on A0 and A6 is explained in the same way. The decrease in the conversion frequency is accounted for by the lower hDNA frequency; the decrease of the disparity coefficient is expected as a consequence of the decrease in the symmetric hDNA frequency. Indeed, a disparity in mismatch correction will lead to a stronger disparity in conversion when two

Table 3. Aberrant segregation patterns of mutants A0 and A6 in the presence of homozygosity and heterozygosity at the G234 site

Parents crossed	n	6+2m*	2+6m*	DC	FAS†
(+ A0 × + +)	11	5(1–14)	89(68–119)	18	96 ± 5
(+ A0 × G +)	12	7(5–14)	44(36–64)	6	56 ± 3
(+ A6 × + +)	9	80(62–105)	6(1–7)	13	90 ± 5
(+ A6 × G +)	11	50(37–60)	11(2–11)	5	60 ± 3

Parents crossed are as in Table 1. The disparity coefficient (DC) corresponds to the ratio of the most frequent class of convertant asci to the least frequent one (i.e., 2+6m/6+2m for A0 and 6+2m/2+6m for A6). FAS, frequency of aberrant segregation.
* Values in parentheses are extremes.
† Mean ± SEM.

Table 4. Aberrant segregation patterns of mutants F1, 17, and X15 in presence of homozygosity and heterozygosity at the G234 site

Exp.	Parents crossed	n	6:2	5:3	Aberrant 4:4*	FAS†
1	(F1 + × + +)	5	67	216	12(7–17)	297 ± 3
	(F1 + × + G)	6	66	212	9(5–11)	289 ± 7
2	(17 + × + +)	6	26	269	38(22–44)	333 ± 8
	(17 + × + G)	6	32	256	43(38–50)	332 ± 5
3	(X15 + × + +)	6	44	145	34(23–44)	235 ± 8
	(X15 + × + G)	5	62	130	40(34–55)	237 ± 8

Parents crossed are as in Table 1. FAS, frequency of aberrant segregation.
* Values in parentheses are extremes.
† Mean ± SEM.

Table 5. Percent of hybrid DNA per meiosis and percent of correction per mismatch

Parents crossed	% hDNA per meiosis: Asymmetric ($\gamma\alpha$)	Symmetric [$\gamma(1-\alpha)$]	Total (γ)	% correction per mismatch(P)
(+ 98 × + +)	2.7	16.6	19.3	71
(+ 98 × G +)	14	0	14	71
(+ A4 × + +)	7.8	7.5	15.3	18
(+ A4 × G +)	11.8	0.8	12.6	21
(G A4 × G +)	7.2	12.9	20.1	20
(G A4 × + +)	12.5	1.4	13.9	21
(+ 26 × + +)	0.4	14.1	14.5	36
(+ 26 × G +)	7.7	0.9	8.6	25
(G 26 × G +)	2.4	11.8	14.2	33
(G 26 × + +)	10.4	1.1	11.5	33

Parents crossed are as in Table 1. Results are calculated for right-hand type C mutants in homozygous and heterozygous conditions at the G site, using Paquette–Rossignol algebra (8).

homologous mismatches need to be corrected in the same direction (symmetric hDNA) than when only one mismatch has to be corrected (asymmetric hDNA) (9).

Because G234 affects hDNA distribution only on its right, we conclude that symmetric hDNA progresses from left to right in *b2* and that this progression is completely (or almost completely) blocked by G234 when this mutant is heterozygous. Because symmetric hDNA has been shown to be physically associated with asymmetric hDNA on its left, near the high conversion end (10), we conclude that hDNA in the asymmetric phase is initiated at the high conversion end. Asymmetric hDNA can become symmetric when it becomes farther from the initiation point. This is consistent with the mechanism postulated in the Aviemore model (17). Models postulating that hDNA is secondarily rendered asymmetric (19) are hardly consistent with these results. If both asymmetric and symmetric hDNA were derived from symmetric hDNA previously formed, then G234 would be expected to impair both asymmetric and symmetric hDNA formation, which is obviously not the case.

The estimates in Table 5 show that $<1/10$ of symmetric hDNA remains at A4 and 26 when G234 is heterozygous (accounting for the residual aberrant 4:4 observed). This could correspond either to hDNA overlapping G234 in the asymmetric phase, which then undergoes isomerization to the symmetric phase between the G site and the A4 or 26 site, or to symmetric hDNA not blocked by G234. It could also reflect hDNA branch migrating from right to left (coming from the low conversion end).

Branch migration of symmetric hDNA was previously observed *in vitro* on figure-eight dimers (32). The present report argues for the existence of this process during meiotic recombination. Frame-shift mutants probably corresponding to one base-pair additions or deletions do not seem to affect symmetric hDNA propagation during meiosis in *b2*. G234 was shown to block this process specifically. Gross heterologies between DNA molecules, such as insertions or deletions of important size, are expected to impair branch migration of symmetric hybrid DNA but not to affect assymetric hybrid DNA formation. G234 might correspond to a deletion or an insertion located in the middle of *b2* because its origin rather suggests that it does not correspond to a point mutation (17).

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