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Large Heterologies Impose Their Gene Conversion Pattern Onto Closely Linked Point Mutations

H. Hamza,[1] A. Nicolas[2] and J.-L. Rossignol

Laboratoire IMG, Bâtiment 400, Université Paris-Sud, 91405 Orsay Cedex, France

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ABSTRACT

We have studied the meiotic non-Mendelian segregation (NMS) pattern of seven large heterologous combinations located in the *b2* ascospore gene of Ascobolus. The NMS patterns of these aberration heterozygotes widely differ from each other and from those of point mutations located in the same genetic region. They give lower gene conversion frequencies than point mutations, no postmeiotic segregations (PMS), and either parity or disparity that favors the wild type allele. Two related deletions, *G234* and *G40*, were studied for their effects on the conversion behavior of closely linked point mutations. We found that, when heterozygous, the deletions impose their own NMS pattern onto close mutations. These effects occur on both sides of the heterologies. The effects upon PMS and disparity of linked point mutations gradually disappear as point mutations become more distant. The effects on NMS frequencies and on aberrant 4:4 are polar. They persist for all mutations located downstream from the high conversion end of the gene. This last effect can reflect a blockage of symmetric hDNA formation by large heterologies, whereas the epistasis of the NMS pattern of large heterologies over that of closely linked point mutations suggests that large heterologies and point mutations undergo conversion by means of distinct pathways.

IN fungi, large heterologies are able to undergo gene conversion. In *Saccharomyces cerevisiae*, FINK (1974), FINK and STYLES (1974) and FOGEL *et al.* (1978) reported the non-Mendelian segregation (NMS) pattern of three deletions of the *his4* gene, LAWRENCE *et al.* (1975) that of a deletion spanning the *cycl* and *rad7* genes and MCKNIGHT, CARDILLO and SHERMAN (1981) that of an approximately 5-kb deletion (*H3*) involving *cyc7*. For all but the *H3* deletion few if any differences between the NMS patterns of the deletions and of point mutations in the same gene were found: deletions converted at near normal frequencies; none of them gave postmeiotic segregation; and conversion occurred equally toward wild type (3+:1m asci) and toward mutant (1+:3m). Only *H3* showed disparity (favoring 3+:1m segregation). Limited results with conversion of insertions have yielded essentially equivalent data [cited in FOGEL, MORTIMER and LUSNAK (1981)]. From these data, gene conversion in yeast is explained either by a single strand transfer of information followed by an efficient mismatch repair event (FOGEL, MORTIMER and LUSNAK 1981) or by a double strand transfer of information (SZOSTAK *et al.* 1983).

This homogeneous behavior of mutations in yeast contrasts with that observed in *Ascobolus immersus* where discrete classes of NMS patterns were described for induced point mutations (LEBLON 1972a; NICOLAS 1979) and correlated with their chemical nature (LEBLON 1972b; LEBLON and PAQUETTE 1978). Base substitution mutations give all types of NMS, |6:2| (6+:2m and 2+:6m), |5:3| (5+:3m and 3+:5m) and aberrant 4:4; mutations that give this array are classified as giving type C NMS patterns. One base-pair addition-deletion mutations give only |6:2| asci, with the type B NMS pattern (2+:6m > 6+:2m) assumed to be due to single base-pair addition mutations and the type A NMS pattern (6+:2m > 2+:6m) assumed to be due to single base-pair deletions. These Ascobolus data were best interpreted in terms of gene conversion occurring by the repair of mismatches in heteroduplex DNA. Extensive subsequent studies in the *b2* spore color gene [reviewed in ROSSIGNOL, PAQUETTE and NICOLAS (1978)] have been consistent with this view.

In this context, the existence in *b2* of several large additions and deletions (NICOLAS *et al.* 1987), together with numerous point mutations, appeared propitious for a comprehensive study of the gene conversion behavior of heterologies. The conversion behavior of complex heterologies is particularly interesting because it touches not only on mechanisms of homologous exchange but also on other topics such as the transfer of information between members of multigene families and molecular evolution (BALTIMORE 1981; DOVER 1982; KOURILSKY 1983).

In this paper, we describe the NMS patterns of seven heterologous combinations. We show that these

[1] Present address: Department of Genetics, Faculty of Agriculture, Monofya University, Shebin el Kom, Egypt.
[2] Present address: Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114.

patterns are different from each other and from those of point mutations located in the same region. Among the large nonpoint mutations, two (*G234* and *G40*) exhibit a wild type spore phenotype that allowed the study of their effects upon the NMS pattern of closely linked mutations in the same gene. We found that these two large deletions impose their own conversion behavior onto all types of linked mutations. This effect on conversion is distinct from that previously reported on heteroduplex formation (HAMZA *et al.* 1981).

MATERIALS AND METHODS

Mutant strains: The *b2* white spore point mutations *E0, E2, A6* (type A NMS pattern), *F0, B100, B17, B16, B20, E1, E3, B79, B101, A0* (type B NMS pattern) and *F1, 17, X15, G1, 24, 81, 94, 98, A4, 26* (type C NMS pattern) have been described (PAQUETTE and ROSSIGNOL 1978; NICOLAS *et al.* 1987). *E0, E1, E2* and *E3* belong to the same (*E*) intragenic suppression group and probably correspond to single base pair addition-deletion mutations (LEBLON and PAQUETTE 1978; NICOLAS *et al.* 1987). The double mutant combinations *E0 E1* and *E2 E1* both show a pink-spored phenotype distinguishable from either the brown wild type or the mutant white. The double mutant *E3 E2* has brown spores indistinguishable from wild-type spores. *G1, 24* and "*E*" mutations are very closely linked. The genetic map of *b2* mutations, as reported by LEBLON *et al.* (1982) and NICOLAS *et al.* (1987), is given on the *x*-axis of Figures 1–3.

Several nonpoint mutations have been described (GIRARD and ROSSIGNOL 1974; NICOLAS *et al.* 1987). The white spore mutations *10* and *138* were classified as large deletions because they fail to recombine with several inter-recombining mutations. *G0* is an unstable spontaneous mutation that gives rise to many distinct types of pseudo-wild-type revertants. Based on its properties, *G0* is certainly not a point mutation; it is almost certainly an insertion (NICOLAS *et al.* 1987). *G234* and *G40* are two *G0* derivatives that have wild-type spore phenotypes. They were characterized as deletions by the following criteria: when homozygous, they shorten genetic distance (*G234*) and increase co-conversion (*G234* and *G40*) between flanking point mutations; when in *cis* with the flanking mutations *E2* and *B101*, *G40* restores a pseudo-wild-type spore phenotype (pink spores), whereas the double mutant *E2 B101* has a white spore phenotype. This effect is best explained if *G40* is a deletion (NICOLAS *et al.* 1987).

The method for associating the silent mutations *G234* and *G40* with spore color mutations in the same gene was described by NICOLAS *et al.* (1987).

Media and culture techniques have been previously described (RIZET *et al.* 1960; LISSOUBA *et al.* 1962; YU SUN 1964).

Crossing conditions were as described by ROSSIGNOL and PAQUETTE (1979). Experimental conditions that allow controlled comparisons of NMS patterns have been described (ROSSIGNOL and PAQUETTE 1979). Thus, in order to randomize strain background differences between *m* × wild type and *m* × *G* crosses, the same *m* strain was crossed to a set of wild type strains and a set of *G* strains which had been isolated from a unique *G* × wild type cross (*e.g.*, *G* × + gives strains *G*i, *G*ii, . . . *G*n, +i, +ii, . . . +n; comparison crosses consist of crosses: *m* × *G*i, *m* × *G*ii, . . . *m* × *G*n, *m* × +i, *m* × +ii, . . . *m* × +n). All crosses were heterozygous for the *rnd1* ascospore shape marker in order to detect aberrant 4:4 segregations (PAQUETTE 1978). Some crosses were also heterozygous for *vag4* (mycelial growth) which makes the determination of sister spore pairs during ascus analysis easier. *rnd1* and *vag4* are unlinked to *b2* (NICOLAS *et al.* 1981).

RESULTS

NMS pattern of large heterologies: The NMS patterns of seven heterologous combinations are given in Table 1. These heterologies correspond to the heterozygosity of the *b2* nonpoint mutations with wild type or with each other. We considered only overlapping heterozygotes of nonpoint mutations (see Figure 1). The NMS pattern of *G234* could not be studied because of its wild-type phenotype. Despite the fact that *G40* also gives phenotypically wild-type spores, its NMS pattern could be established in the *E2 G40 B101* × *E2* + B101 cross, because the triple mutant *E2 G40 B101* has a pink-spore phenotype. The *G40 vs.* + segregation corresponds to pink *vs.* white ascospore segregation.

The seven NMS patterns have two qualitative characteristics in common: (1) they show no postmeiotic segregation (PMS) and (2) the NMS frequencies are lower than those of point mutations located nearby (see Figure 1 and Tables 2 and 3). In other respects the nonhomologous combinations exhibit broad diversity in their conversion patterns. The decrease in NMS frequency is variable: between one-third and one-half with *G* heterologies, but fourfold with *10* and 70-fold with *138*. With respect to the direction of conversion, five heterologous combinations exhibit parity (6+2m equals 2+6m), whereas the other two both exhibit disparity in favor of wild type: *G0* shows a threefold excess, and *138* apparently exhibits only conversion to wild type. Since *G0* is an insertion and *138* is a deletion, direction of disparity is uncorrelated with the molecular nature of the heterology. Rare conversions of *138* to wild type were also detected in heteroallelic crosses between *138* and other *b2* mutations (NICOLAS 1983).

Effect of *G234* and *G40* deficiencies upon the NMS pattern of allelic point mutations: We have tested the effect of *G234* and/or *G40* heterologies upon the NMS patterns of 23 point mutations located along the entire length of the *b2* gene. Since both deletions have phenotypically brown spores, the spore color segregation (brown *vs.* white) corresponds to the segregation of the point mutation. The results are presented in Table 2 for class A and B mutations (giving a type A or B NMS pattern) and in Table 3 for class C mutations (giving a type C NMS pattern). The analysis of NMS patterns in terms of variation between control crosses (absence of *G* heterologies) and test crosses (presence of *G* heterologies) for individual classes of NMS and related parameters deduced from NMS patterns are presented in Figures 1–3. Figure 1 illustrates the effect of *G* deficiency heterozygosity on NMS frequency for each of the mutants studied and the effect on aberrant 4:4 frequencies for

TABLE 1

NMS patterns of seven heterozygous combinations corresponding to large heterologies in gene *b2*

Crosses	n^a	Number per 1,000 asci of:				PMS[e]	DV[f]
		6C:2W[b]	2C:6W[b]	Others[c]	NMS[d]		
10 × +	4	16	19	3	39	0	1.2
138 × +[g]	10	2(2)	2(0)	1(0)	(2)	0	
G0 × +[h]	4	51	15	10	76	10[h]	3
E2 G40 B101 × *E2* + B101	11	35	41	4	80	0	1.2
10 × *G234*	4	18	23	3	44	0	1.3
G0 × *G234*	4	42	39	2	83	0	1.1
G0 × *G40*	2	36	34	0	70	0	1.1

[a] *n*: number of distinct crosses studied; in each cross a sample of 1,000 asci was counted.
[b] C: colored spores; W: white spores.
[c] Others: 5C:3W, 3C:5W, aberrant 4C:4W, 7C:1W, 8C:0W, and 0C:8W asci.
[d] NMS: total non-Mendelian segregations.
[e] PMS: percent of NMS that show postmeiotic segregation.
[f] DV: disparity value; it is the ratio (6C:2W + 5C:3W)/(2C:6W + 3C:5W) when 6C:2W + 5C:3W are the most frequent or the ratio (2C:6W + 3C:5W)/(6C:2W + 5C:3W) when 2C:6W + 3C:5W are the most frequent.
[g] In the *138* × + crosses, the numbers in parentheses give the corrected values after ascus analysis: of a sample of 11 6C:2W, 11 2C:6W, and 5 PMS, only the 6C:2W asci were confirmed as 6+:2(*138*); the others were new mutations or phenocopies.
805 [h] In *G0* × + crosses, many phenotypic 5C:3W, 3C:5W and aberrant 4:4 asci do not correspond to PMS for *G0*, but rather to PMS for a non-*G0* derivative (*G1*), a product of the instability of *G0* (Nicolas *et al.* 1987).

class C mutants; Figure 2 illustrates the effect on |5:3| frequencies for class C mutants; and Figure 3 illustrates the effect on the disparity value (DV) of class A, B and C mutants. Clearly, *G234* and *G40* modify the NMS pattern of most of the mutations tested and the magnitude of the effect is a function of the position of the point mutation relative to the deficiency.

Nearby mutations: The effect of deficiency heterozygosity is particularly strong and remarkably homogeneous on mutations that are very tightly linked to the deficiency ends, that is those mapping in the (*E0,E3*)–(*81,B79*) interval.

Heterozygosity for *G234* or *G40* has two effects on close type A and B mutations (Table 2): the frequency of NMS is reduced by approximately twofold and disparity in conversion direction is abolished or very greatly reduced.

Heterozygosity for *G234* or *G40* has three effects on close class C mutations (Table 3): as with class A and B mutations, the frequency of NMS is reduced by approximately twofold and disparity (if present) disappears, but in addition the frequent postmeiotic segregations that characterize mutations as being class C also almost completely disappear.

The effects of the two deficiencies,*G40* and *G234*, on the NMS pattern of tightly linked class A, B, and C mutations are identical, suggesting either that these effects are characteristic of deficiencies in general or else that these two deficiencies have common special features. Interestingly, the NMS pattern imposed on the nearby mutant *m* in an *m*+/+ G40 heterozygote is the same as that displayed by *G40* itself in a *G40*/+ heterozygote (Table 1), suggesting that *G40* imposes its own NMS pattern on nearby point mutations. Since *G234* has the same effect as *G40*, we infer that *G234* has the same NMS pattern as *G40* and that it also imposes its NMS pattern on nearby point mutations.

Four other issues are resolved by the data in Tables 2 and 3. First, since in crosses homozygous for *G234* or *G40* the NMS pattern of heterozygous point mutants is the same as in +/+ controls, the effects observed when these aberrations are heterozygous are due to heterozygosity *per se* rather than to some other attribute of the aberrations. Second, coupling and repulsion arrangements give the same effect. Third, since all of the point mutants under discussion here but *24* and *G1* are known to lie outside of the *G234* deficiency (Nicolas *et al.* 1987), the alterations in NMS pattern are effects at a distance. And finally, these effects are not polar; point mutants to the left and to the right are affected equally.

However, the identical effects of *G40* and *G234* heterozygotes upon NMS patterns of nearby point mutations raises the question of whether these two deficiencies are in fact different, especially considering that *G40* was identified among the progeny of a cross between *G234* and *G1*, both *G0* derivatives and therefore potentially complementing for residual instability (Nicolas *et al.* 1987). Since for both *G40* and *G234* it has been shown (Table 2) that homozygosity of the aberrations restores the wild-type pattern of NMS to nearby point mutations, we can use nearby point mutations to ask whether the *G40/G234* heterozygote behaves like the homozygotes or like heterozygotes with wild type; these data are also in Table 2, and the effect of the *G234/G40* heterozygote is intermediate between that of homozygous aberration (= homozygous wild type) and either aberration heterozygote in all respects (effect on NMS frequency and disparity reduction). This suggests that these are over-

TABLE 2

NMS pattern of 13 single and one double class A and B mutations in crosses with and without G deficiencies heterozygous

Crosses	*n*	Number per 1,000 asci of: 6C:2W	2C:6W	Others	NMS	DV
F0 × +	3	99	213	3	315	2.2
F0 × *G234*	3	112	220	4	336	2.0
B100 × +	4	66	206	2	274	3.1
B100 × *G234*	4	69	195	1	265	2.8
B17 × +	4	21	122	6	149	5.8
B17 × *G234*	6	34	94	3	131	2.8
B16 × +	5	21	152	3	176	7.2
B16 × *G234*	5	32	119	3	154	3.7
B20 × +	4	10	165	6	181	16.5
B20 × *G234*	4	41	97	6	144	2.4
B20 × *G40*	1	39	60	0	99	1.5
E0 × +	5	139	8	7	154	17.4
E0 × *G234*	5	50	48	6	104	1.0
E3 × +	3	9	156	7	172	17.3
E3 × *G234*	3	60	59	3	122	1.1
E3 × *G40*	2	60	64	1	125	1.1
E3 G234 × +	5	58	55	3	116	1.1
E3 G234 × *G234*	4	10	140	2	152	14.0
E3 G234 × *G40*	3	31	92	3	126	3.0
E2 × +	3	159	10	9	178	15.9
E2 × *G234*	5	48	46	9	103	1.0
E2 × *G40*	1	51	46	0	97	1.1
E2 G40 × +	4	48	59	2	109	1.0
E2 G40 × *G234*	4	129	34	4	167	3.8
E2 G40 × *G40*	2	159	12	2	173	13.3
E1 × +	5	8	185	9	202	23.1
E1 × *G234*	5	60	65	5	130	1.1
E1 × *G40*	1	48	45	0	93	1.1
E0 E1 × +[a]	4	48	45	7	100	1.1
E0 E1 × *G234*[a]	5	34	32	6	72	1.1
B79 × +	5	10	128	2	140	12.8
B79 × *G234*	6	46	23	2	71	2.0
B79 × *G40*	1	50	43	1	94	1.2
B101 × +	4	14	126	1	141	9
B101 × *G234*	4	26	56	1	83	2.2
A0 × +[b]	11	5	89	2	96	17.8
A0 × *G234*[b]	12	7	44	5	56	6.3
A6 × +[b]	9	80	6	4	90	13.3
A6 × *G234*[b]	11	50	11	0	60	4.6

See Table 1 for abbreviations. For each mutation crosses with + and with *G234* are comparison crosses, + and *G234* being sibs derived from the same cross (see MATERIAL AND METHODS).

[a] The spore color segregation is B:P, *i.e.*, brown spores (wild type or *G234*) *vs.* pink (*E0 E1*).

[b] From HAMZA *et al.* (1981).

lapping (since the effect is not as strong as either heterozygote *G*/+) but not identical (since there is an effect) deficiencies, an implication that is in accord with the possibility that *G40* arose from *G234* by further rearrangement.

Effect of the *G234*/+ heterology on the double point mutation *E0 E1*. Examination of the interaction of *G234* and *E0 E1* addresses two questions: (1) does the *G234*/+ heterology affect close double point mutations, here *E0 E1*, as it does with single site point mutations and (2) does the *G234*/+ heterology affect the NMS pattern of a heterology which shows parity. The results (*E0 E1* × + and *E0 E1* × *G234*, Table 2) demonstrate that there is a further decrease in the NMS frequency of *E0 E1* in the presence of *G234*/+, whereas parity is observed in both crosses.

More distant mutations: The effects on more distant mutations depend on the location of these mutations with regard to *G234* and *G40* (distance and left or right position). On the left side, there is no significant change of the NMS pattern for the five mutations *F1* to *X15*: NMS and PMS frequencies and DV are not affected. A parallel decrease of NMS frequencies and DV is seen for mutations *B17* to *E*. No conclusion can be drawn about PMS in the *X15-E* interval since no class C mutations are available.

For the seven mutations on the right (*94* to *26*), the patterns of effects are quite different for NMS and aberrant 4:4 asci frequencies on the one hand and |5:3| frequencies on the other hand. Aberrant 4:4 asci frequencies are drastically reduced (90% decrease) for all mutations. NMS frequencies are also reduced to the same extent for those mutations as for mutations close to *G234* (*e.g.*, the same reduction is seen for *E2* and *26* or *G1* and *A0*). However, we cannot exclude—when looking at the *81-26* interval—that the NMS frequency reduction tends to be less strong when mutations become farther from *G234*. Whatever it might be, the NMS frequency reduction slope contrasts sharply with that observed on the left of *G234* which is much steeper: for example, *B20* which is much closer to *G234* than mutations in region *A* undergoes a reduction of its NMS frequency which is clearly less drastic.

This long range and steady effect upon aberrant 4:4 asci and NMS frequencies contrasts with the effect upon |5:3| that increase from 0% of the control value for *81* to almost 100% for *A4* and *26*. The effect upon |5:3| is thus clearly dependent on the proximity of the mutations to the *G*/+ heterology.

The DV parameter is affected in an intermediate way: like NMS and aberrant 4:4 frequencies, the DV of rightmost mutations is still affected, but like |5:3| frequencies, the effect becomes progressively less drastic when mutations become closer to the right end, suggesting that proximity to the *G*/+ heterology plays an important role for DV, as for |5:3|.

Effect of point mutation heterozygosities on tightly linked mutations: The interaction during recombination between closely linked point mutation sites in the *A* region of *b2* gene has been reported (LEBLON and ROSSIGNOL 1973, 1979; ROSSIGNOL and HAEDENS 1978). We wished to extend this type of study to the *E* region in order to compare the effects of interactions between point mutations and *G*/+ heterologies with that of point mutations with other point mutations in the same region. This was feasible because the double mutants *E0 E1*, *E2 E1* and *E3 E2*, combinations of two intersuppressing mutations, have

TABLE 3

NMS pattern of ten class C mutations in crosses with and without *G* deficiencies heterozygous

Crosses	*n*	Number per 1,000 asci of: 6C:2W	2C:6W	5C:3W	3C:5W	4:4ab[a]	Others[b]	NMS	DV	PMS
F1 × +[c]	5	28	39	111	105	14	1	298	1	77
F1 × *G234*[c]	6	30	37	107	105	11	2	292	1	76
17 × +[c]	6	11	12	134	135	41	4	337	1	92
17 × *G234*[c]	6	11	18	116	140	45	5	335	1.2	90
X15 × +[c]	5	28	25	73	74	34	5	239	1	76
X15 × *G234*[c]	4	34	30	73	56	43	5	241	1.2	71
24 × +	4	68	14	37	10	5	2	136	4.4	38
24 × *G234*	5	44	34	0	0	1	2	81	1.3	1
24 × *G40*	1	25	40	2	0	0	0	67	1.5	1
G1 × +	4	6	5	35	50	33	1	130	1.3	91
G1 × *G234*	5	43	28	1	1	2	0	75	1.5	5
G1 × *G40*	1	37	34	0	0	0	0	71	1.1	0
81 × +	4	35	9	80	25	33	3	185	3.4	75
81 × *G234*	5	45	42	1	1	0	1	90	1.1	2
81 × *G40*	2	45	43	0.5	1	0	0	90	1	2
94 × +	2	34	11	41	24	24	0	134	2.1	66
94 × *G234*	2	25	22	6	17	3	0	73	1.3	36
98 × +[c]	6	35	16	49	25	18	3	146	2.1	63
98 × *G234*[c]	8	29	16	18	22	2	3	90	1.2	47
A4 × +[c,d]	12	3	5	41	38	45	0	132	1	94
A4 × *G234*[c,d]	12	5	6	45	45	6	0	107	1	90
26 × +[c,d]	7	5	5	38	28	67	0	143	1.3	93
26 × *G234*[c,d]	8	5	5	36	24	8	0	78	1.4	87

See Table 1 for abbreviations.
[a] 4:4 ab: aberrant 4:4; corrected values are given (see PAQUETTE 1978).
[b] Others: 8C:0W, 0C:8W, 7C:1W, 1C:7W asci.
[c] From HAMZA *et al.* (1981).
[d] With *A4* and *26*, one among several sets of comparison crosses performed by HAMZA *et al.* (1981) is shown.

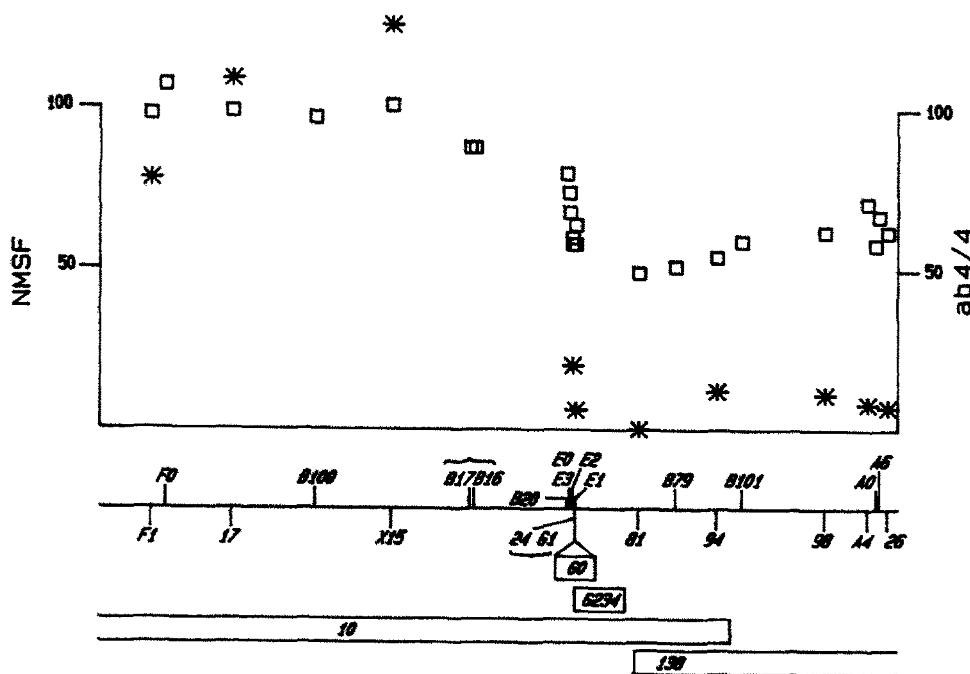

FIGURE 1. Effect of the *G234* deficiency heterozygote on NMS frequencies of 23 *b2* point mutations and on the relative frequencies of aberrant 4:4 asci for 10 type C mutations. The values plotted are experimental/control (in percent) for each point mutation; the data are from Tables 2 and 3; for *A4* and *26*, the values are the mean values of distinct comparison crosses performed by HAMZA *et al.* (1981). Genetic map: the positions of the aberrations *10*, *138*, *G0* and *G234* with respect to the point mutations are shown along the abscissa. The order of nondeficiency mutations within the *E* region is: *(E3,E0)–E2–E1,24*, *(G1,G0)*, where parentheses mean no recombination detected and commas mean that the relative order is unknown. *G234* is located between the mutations *E1* and *81*; it is not known whether or not *G234* spans *24*, *G1* and *G0*. The deficiency *G40* is not shown: it overlaps *G234* (see results); it is located between *E2* and *B101*; it is not known whether or not it spans the mutations within the *E2–B101* interval. (□) NMS, (*) aberrant 4:4.

colored spore phenotypes (see MATERIAL AND METHODS); moreover, these mutations are rarely reassociated through recombination, so most events detected probably involve co-conversions (LEBLON and PAQUETTE 1978). Crosses involving one, two or three point mutations are presented in Table 4. The inter-

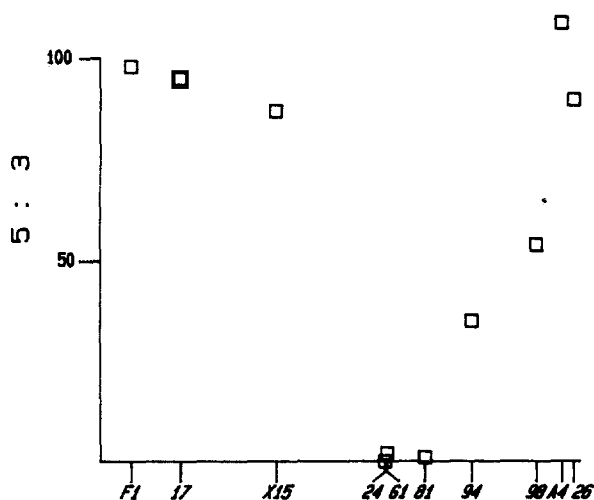

FIGURE 2.—Effect of the *G234* deficiency heterozygote on the frequency of |5:3| asci for 10 class C mutations. The values plotted are experimental/control (in percent) for each point mutation. The data are from Table 3; for *A4* and *26* see legend Figure 1.

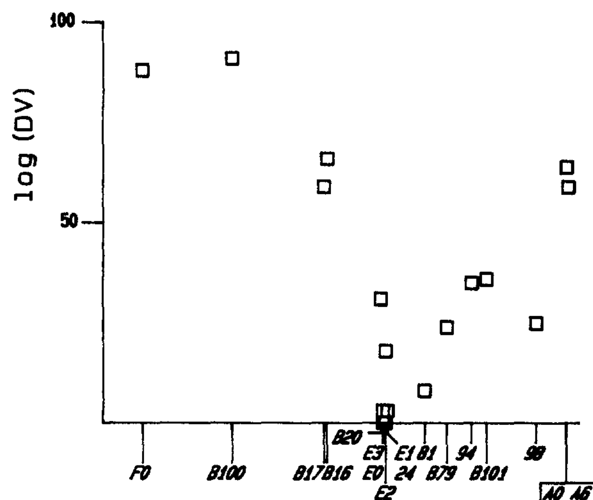

FIGURE 3.—Effect of the *G234* deficiency heterozygote on the disparity value (DV) for 17 class A, B and C mutations. The values plotted are experimental/control (in percent) for each point mutation. Only mutations with DV > 4 in control crosses are plotted. DV are calculated as defined in the legend of Table 1. The experimental/control values were calculated by using the log of the DV. The data are from Tables 2 and 3.

action of double point mutations with class C mutations was studied in crosses of *E2 E1* or *E3 E2* with *24*, *G1* or *81*. For *24* and *G1*, |6:2| are enhanced at the expense of PMS, which in turn almost completely disappear. *E2 E1* imposes its NMS pattern to *24* (no PMS, low disparity). The NMS pattern of *E3 E2* cannot be observed directly; however, it seems reasonable to assume that it is like that of other double mutant combinations composed of closely linked class A and class B mutations, (*i.e.*, no PMS and low disparity: see *E0 E1* and *E2 E1*, Table 4, and LEBLON and ROSSIGNOL (1979) for double mutants in region *A*): this suggests that in the cross *E3 E2* × *G1*, *E3 E2* imposes its NMS pattern onto *G1*. With the more distant mutation, *81*, a similar though weaker effect is observed (PMS are lowered but do not disappear).

The other crosses in Table 4 show the interaction of class A and B mutations with each other. In crosses with wild type, the disparity of the double mutants *E2 E1* and *E0 E1* is much lower than that for each single mutation *E0*, *E1* and *E2*. This is also observed in the five three-point crosses involving the interaction of three class A and B mutations, *e.g.*, in the cross *E2 E1* × *E0*, the DV is lower than that for each single mutation and it is higher than that for *E2 E1*, indicating that the three mutations mutually interact to give the observed NMS pattern.

In conclusion, this study of the interaction between point mutations in the middle region confirms previous studies involving point mutations in the *A* region: class A and B mutations mutually interact in their conversion patterns and tend to impose their own patterns on closely linked class C mutations.

DISCUSSION

The results presented in this paper bear on the mechanism of meiotic gene conversion in the filamentous fungus Ascobolus. This study of the NMS pattern of large nonpoint mutations reveals important differences from the NMS pattern of point mutations. Heterologies exhibit lower NMS frequencies than point mutations located in the same area. In contrast with class C mutations, they do not give PMS. In contrast with class A and B mutations, several of them show parity in the direction of conversion.

Nonpoint mutations also show a variety of NMS patterns. This variety may reflect the different location of these mutations with respect to the recombination signals operating in the *b2* region and/or differences in sizes of the respective mutations. The facts that *G234* (and *G40*) are epistatic to *G0* and that *10* is in turn epistatic to them suggest the existence of complex interactions and/or hierarchies.

All previous studies on the mechanism of gene conversion in *b2*, reviewed by ROSSIGNOL *et al.* (1987), strongly supported the model of MESELSON and RADDING (1975), in which gene conversion occurs by two steps: hybrid DNA formation involving either one (asymmetric hDNA) or two (symmetric hDNA) interacting duplexes and mismatch correction. Mismatches formed with class C mutations are assumed rarely to be corrected, leading to PMS. Mismatches formed with class A and B mutations are assumed always to be corrected. Class B mutations are probably one GC base pair additions. Indeed, they were induced by ICR-170 which induces the addition of one GC base pair in *Saccharomyces cerevisiae* (DONAHUE, FARABAUGH and FINK 1981) and *Neurospora crassa* (BURNS *et al.* 1986). In crosses with wild type, the preferential conversion to mutant thus indicates that it is the shorter strand which is excised and further corrected by copying the longer mutant strand. The excision of the shorter strand also accounts for the preferential

TABLE 4

Effect of double class A and B mutations upon the NMS pattern of closely linked class A, B or C point mutations

Crosses	*n*	Number per 1,000 asci of:							DV	PMS
		6C:2W	2C:6W	5C:3W	3C:5W	4:4ab	Others	NMS		
E0 E1 × +[a]	4	48	45	1	6	0	0	100	1.1	7
E2 E1 × +[a]	3	76	28	0	0	0	0	104	2.7	0
+ × *24*[b]	3	91	17	26	7	2	1	144	4.9	24
E2 E1 × *24*[b]	3	41	70	2	1	0	0	114	1.7	2
E3 E2 × *G1*	6	43	32	1	1	0	0	77	1.3	2
+ × *81*[b]	3	30	7	73	18	24	2	154	4.1	75
E3 E2 × *81*[b]	3	33	28	24	12	8	0	105	1.4	42
E2 E1 × *E3*	2	32	65	0	0	0	0	97	2	0
E0 E1 × *E3*	2	45	35	0	0	0	0	80	1.3	0
E2 E1 × *B20*	2	17	107	0	0	0	0	124	6.3	0
E0 E1 × *B20*	2	11	48	0	0	0	0	59	4.4	0
E2 E1 × *E0*	2	52	9	0	0	0	0	61	5.8	0

See Table 1 for abbreviations.

[a] The spore color segregation is B:P, *i.e.*, brown spores (wild type) *vs. pink (E0 E1* or *E2 E1*).

[b] The crosses + × *24* and *E2 E1* × *24* on the one hand, + × *81* and *E3 E2* × *81* on the other hand, correspond to comparison crosses, + and *E2 E1* (or *E3 E2*) being sibs derived from the same cross (see MATERIAL AND METHODS).

The NMS patterns of single mutations *E0, E1, E2, E3, B20* and *G1* are given in Tables 2 and 3.

conversion to wild type of type A mutations that are complementary to class B and should thus correspond to one base pair deletions (LEBLON 1972b).

The present study relies on the interaction between mutations in order to address the mechanism of conversion of large heterologies. This revealed an important difference in the conversion behavior of point mutations and *G* mutations. Both *G*/+ heterologies and class A or B mutations impose their NMS pattern onto closely linked class C mutations. This epistasis of class A and B mutations to closely linked class C mutations was also shown in region *A* by LEBLON and ROSSIGNOL (1973) and ROSSIGNOL and HAEDENS (1978). However, *G*/+ heterologies are epistatic to closely linked class A and B mutations, whereas class A and B mutations mutually interact on their NMS pattern, in region *E* (this study) as they do in region *A* (LEBLON and ROSSIGNOL 1973, 1979). Taken collectively, the differences of point and nonpoint mutations on NMS patterns and their distinct conversion behavior in interaction studies suggest that the conversion process of heterologies such as *G*/+ might be distinct from that of class A and B mutations. This raises the interesting possibility that two distinct conversion processes occur in the same genetic region. We will examine this possibility in the light of the multiple interaction studies reported here and in previous studies involving the *G234* deletion (HAMZA *et al.* 1981) as well as point mutations (NICOLAS and ROSSIGNOL 1983).

Two effects of *G*/+ heterozygosity were observed: (1) A long range effect that affects to a similar extent all mutations on the right and in the middle region. This effect involves total NMS frequencies and aberrant 4:4. (2) A local effect that affects mutations close to the *G* region, and becomes less strong as the distance between the mutations and the *G* region increases. This effect involves the DV (disparity is lowered) and |5:3| frequencies in the right region.

The simplest hypothesis to account for the long-range effect is to assume that *G*/+ heterologies block branch migration of symmetric hDNA propagating from the left end of *b2* to the right (HAMZA *et al.* 1981). This hypothesis explains why *G40* gives about one-third less NMS than closely linked point mutations and why *G234* and *G40* also reduce by about one third the NMS frequencies of point mutations in the middle region and in the right *b2* portion. The algebraic calculation by PAQUETTE and ROSSIGNOL (1978), that about one-third of hDNA formed in the middle region of *b2* was in the symmetric phase, also nicely fits the NMS pattern frequency decrease and gives support to the proposed hypothesis. This effect on symmetric heteroduplex is not specific to large heterologies. Double point mutations in the same region (*E0 E1*, *E2 E1* and *E3 E2*) also show a polar effect, but the effect is quantitatively less drastic, with aberrant 4:4 at A4 reduced by only approximately 50% (NICOLAS and ROSSIGNOL 1983).

The polar effect of *G*/+ heterologies upon heteroduplex formation does not seem to involve asymmetric hDNA, since |5:3| segregations for distant right end class C mutations are not affected by the presence of *G*/+ heterologies. The same result was also found for double point mutations (NICOLAS and ROSSIGNOL 1983). This suggests that asymmetric hDNA is not blocked by these heterologies and leaves the possibility that the conversions of *G*, like that of point mutations, occur by mismatch correction. A mismatch correction process could account for the local effect of *G*/+

heterologies upon closely linked class C mutations: the PMS decrease may reflect mismatch correction triggered at *G*/+ and spanning the class C mutations. The weaker decrease of PMS with increasing distance would reflect the limited extension of the correction tracts. The same type of effect of class A and B mutations upon class C mutations was used to argue that |6:2| segregations of class A and B mutations result from heteroduplex formation followed by single strand mismatch correction (LEBLON and ROSSIGNOL 1973). This mismatch correction process was named "ADEL" because it specifically acts upon mismatches with one-base-pair ADdition or one-base-pair DELetion mutations. The key result presented here, that *G*/+ heterologies impose their own conversion behavior to all closely linked mutations, implies that, if a process analogous to "ADEL" is responsible for *G* conversions, it differs from it in two respects: it predominates over "ADEL" and it is able to promote parity. One simple hypothesis that explains the hierarchical conversion behavior between large and smaller heterologies is that larger loops are more likely to trigger mismatch correction. The parity effect is more troublesome. The requirement that mismatch correction, instead of exacerbating its directionality when the loop becomes larger, loses it, is not straightforward. This difficulty is overcome in the models of conversion proposed by RADDING (1978) and by HASTINGS (1984), which predict parity in the direction of conversion of large heterologies. Both models retain the assumption that large heterologies become part of a heteroduplex region but they differ by its processing: RADDING supposes that DNA synthesis makes the loop double stranded and that a resolution event similar to the resolution of HOLLIDAY structures eliminates or retains the double stranded loop with equal likelihood; HASTINGS proposes that mismatch repair begins by cutting both strands, generating a double strand gap which is repaired by double strand gap repair (SZOSTAK *et al.* 1983). HASTINGS' model also can explain the influence of *G* conversion upon closely linked mutations by variable extension of the double stranded gap region.

The two hypotheses make different predictions about the fate of the *G* heterology when heteroduplex spans it. RADDING's model predicts that conversion to the invader genotype will equal restoration to the resident one (if replication and isomerization are independent of which strand is the invader). HASTINGS' model predicts that all events will lead to conversion to the invader genotype if the double strand gap is always repaired from one of the two homologous chromatids, never from the sister. These two hypotheses were tested in experiments using *G234* (ROSSIGNOL *et al.* 1984; HAMZA *et al.* 1986). There are significantly more than 50% (between 72 and 89%) of the events leading to conversion. This is more in agreement with the prediction of HASTINGS' model than with that of RADDING.

Alternatively, the conversion of *G* heterologies may not involve a heteroduplex intermediate at all. It could occur *de novo* via a double strand gap repair event (SZOSTAK *et al.* 1983) initiated in its vicinity. Since closely linked class C mutations exhibit no PMS, this model requires that *G*/+ heterologies prevent heteroduplex formation in their vicinity as well as at the site of heterology itself. Since we suppose that hybrid DNA begins at the left end of *b2* and propagates rightward (PAQUETTE and ROSSIGNOL 1978; HAMZA *et al.* 1981), we would expect that *G*/+ heterologies would abolish all hDNA (asymmetrical as well as symmetrical) to the right. They do not: asymmetric hDNA formation is not affected. For this reason, we prefer the former hypothesis of hDNA being a prerequisite for *G* conversion.

If, as seems the most likely, *G*/+ heterologies prevent the rightward propagation of symmetric hDNA, the present results suggest that this effect begins at a distance, to the left of the deficiency heterozygosity, rather than at its very border. If the effect began at the border, then symmetrical hDNA should span *E* mutations, an hypothesis which leads to the following three predictions: (1) the drop in NMS frequencies should not be as strong for *E* mutations as for mutations farther on the right, (2) frequent arrest of hDNA between *E* and *G* should lead to frequent recombination via single site conversion involving the *E* mutation, and (3) mismatch correction of *E* mutations within the symmetrical hDNA fraction should not be under the influence of the heterology and thus retain partial disparity. None of these predictions are met: with *G*/+, *E* mutations have their NMS frequencies as strongly reduced as mutations on their right, reassociate extremely rarely with *G* ($\sim 10^{-4}$) and have a DV that drops to 1. We therefore conclude that the effect on symmetric hDNA formation begins left of the *G*/+ heterology and at a distance (although probably rather close). If true, this might suggest that either the blockage of the HOLLIDAY junction, or the strand cutting mediating its resolution, is not adjacent to the start of the heterologous region.

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