THE INTERACTION DURING RECOMBINATION BETWEEN CLOSELY LINKED ALLELIC FRAMESHIFT MUTANT SITES IN *ASCOBOLUS IMMERSUS*

II. A AND B TYPE MUTANT SITES

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SUMMARY

One hundred and eight single or multi-point crosses involving frameshift mutants in the A group of intragenic suppression were analysed. The frequencies of coconversion were always very high (>0.94). Two parameters were considered: the frequency of conversion and the disparity values of conversion (*i.e.* the ratio of $6+ : 2m$ to $2+ : 6m$ asci). The pattern of variation of the disparity values agrees with an equal influence of the associated heterozygous sites in determining the direction of conversion.

This suggests that the excision process needed for heteroduplex correction proceeds in both directions from the one stranded nick provoked by the mismatch, *i.e.* that there is no important excision polarity. It also suggests that recognition of the mismatch prior to nicking is not part of a polar process.

The positive correlation observed between the disparity value and the frequency of conversion is considered as new evidence for frequent symmetrical distribution of hybrid DNA in the region studied.

1. INTRODUCTION

PREVIOUS studies in *Ascobolus immersus* show that a correction process is triggered by specific mismatches arising from hybrid DNA formation during meiotic recombination. This correction process has been studied particularly in relation to frameshift mutants corresponding to small additions or deletions of probably one base-pair (Leblon, 1972, 1979; Leblon and Paquette, 1978). Such mutants give one of two conversion patterns, in both of which all the convertant asci show 6 : 2 segregation. In pattern A there is an excess of $6+ : 2m$ over $2+ : 6m$ and in pattern B a converse excess of $2+ : 6m$ over $6+ : 2m$. A third pattern C is shown by certain other mutants; in this pattern there are numerous post-meiotic segregations, *i.e.* 5 : 3 asci. Patterns A and B are interpreted as indicating a high frequency, and pattern C a comparatively low frequency of mismatch correction.

Some of the characteristics of the conversion process are similar to those involved in UV excision-repair. The analysis of crosses heterozygous for two allelic sites indicates that the correction may span the neighbouring sites. In a cross of the $BC \times ++$ type, the 6 : 2 segregations at the C site are greatly increased at the expense of 5 : 3, suggesting that correction initiated at the B site may include C, thus greatly increasing the normally low frequency of correction at the latter site (Leblon and Rossignol, 1973). A general study of two-point crosses involving one mutant of type A (or B) and one of type C showed a decrease of the probability that correction

initiated at A or B will overlap C as the distance between the two sites increased (Rossignol and Haedens, 1978). These studies involved a small region defined by the A group of mutually suppressing mutants and restricted to the right part of the *b2* locus (Leblon and Paquette, 1978). Within this region the occurrence of recombinants seems to be mainly due to the repair tract rather than the hybrid DNA ending between the two sites and all data are consistent with the frequent formation of homologous hybrid DNA on both participating chromatids (Leblon and Rossignol, 1973; Paquette and Rossignol, 1978; Rossignol and Haedens, 1978).

In this same *b2* region, the interaction of a type A with a type B mutant was also studied in a $AB \times ++$ cross. In control crosses of each mutant to wild type, the type A mutant showed 10 times more $6+ : 2m$ than $2+ : 6m$ whereas type B showed 10 times more $2+ : 6m$ than $6+ : 2m$; in the $AB \times ++$ cross, the two sites were almost always coconverted and the disparity disappeared, with the number of conversions from wild to mutant becoming nearly equal to the number from mutant to wild. These results suggest that the excision repair almost always overlaps the neighbouring site and can be triggered at either site with similar probability (Leblon and Rossignol, 1973).

The availability of five distinct sites in the A group of intragenic suppression allowed a systematic study of the interaction between type A and B mutants in crosses involving up to four distinct sites.

2. MATERIAL AND METHODS

(i) *Ascospore pigmentation mutants*

The crosses involved seven distinct mutations mapped in six distinct sites of the *b2* pigmentation locus. These mutants belong to one group of intragenic suppression (Leblon and Paquette, 1978) and have been called *b2A0*, *b2A1*, *b2A2*, *b2A3*, *b2A4*, *b2A5* and *b2A6*. They will respectively be referred to as: 0, 1, 2, 3, 4, 5 and 6. Each of these mutations leads to a white spore phenotype and is a frameshift (Leblon, 1979). Two-point crosses established the order of the mutant sites (Leblon and Paquette, 1978) and three-point crosses confirmed it (Leblon, 1979). This order and the sign of the phase shift of each mutant are indicated in fig. 1. The mutants 1, 2, 3

FIG. 1.—Genetic map of group A of intragenic suppressors, sign of the phase shift of each suppressor and frequencies of coconversion among all conversions. * From cross $231 \times +++$ (for detailed frequencies of single site conversion see Leblon, 1979). ** From cross $20 \times ++$ (for detailed frequencies of conversion see Leblon and Rossignol, 1973). *** From cross $26 \times ++$ (assuming that all 6 coloured : 2 white asci arise from single site conversion).

and 6 show a type A conversion pattern. They will be named A mutants. The mutants 0 and 5 which are at the same site, show a type B conversion pattern. They will be named B mutants. When present, the mutant 4 was always in the homozygous state.

(ii) *Mutant strains*

Sixty-nine single, double and triple *b2* mutant and wild type strains were used. The genotypes of these strains can be deduced from table 1. For the origin of the double and triple mutant strains, see Leblon (1979).

(iii) *Crosses and confrontation types*

One-hundred-and-eight crosses involving distinct strains were carried out. The size of the observed sample of asci was always larger than 1200. The 38 distinct crosses are shown in table 1. In every cross, the two parents have different phenotypes. In certain cases, the presence of another mutant in homozygous condition was necessary for the distinction to be made.

The conversion pattern of a mutant is not altered by a homozygous mutant sequence in its neighbourhood (Leblon, 1979). Therefore, when considering the genotypes of the two strains crossed, only the heterozygous sites will be taken into account. Within the group of intragenic suppression studied, the conversion pattern is homogeneous among type A mutants and also among type B mutants. Consequently for every heterozygous site only the type (A or B) of the mutant involved need be taken into account. On this basis, each cross can be represented in the form of a simplified confrontation. For example, cross XXXVI is $\frac{4+ + 0}{4 \ 2 \ 3 \ +}$ where 4 is homozygous, 2 and 3 are type A and 0 is type B. The simplified confrontation is $\frac{+ + \ B}{A \ A \ +}$. Thus crosses with distinct genotypes may show the same confrontation. Crosses with identical confrontations are grouped within table 1.

(iv) *Establishing the conversion pattern*

Two kinds of crosses are performed, differing in the parental phenotypes. Crosses I, XXXI, XXXVI and XXXVII involve two parents with differently coloured spores. In these crosses, the various conversion events can be recognised as departures from the usual 4 : 4 : 0 (dark : light : white) pattern. The parental genotypes can be distinguished from each other and from the recombinant genotypes leading to white spores in the progeny (table 2). The frequency of coconversion involving both sites can be directly estimated from the frequency of 6 : 2 : 0 and 2 : 6 : 0 asci. Fig. 1 gives the value of the coconversion frequency among all convertant asci for the various pairs of type A and B sites whenever it could be determined. Since the frequency of coconversion is always higher than 0.943, a good approximation of the conversion pattern of each mutant site can be obtained from the coconversion asci alone, neglecting conversions that lead to recombinant asci (with white spores).

TABLE 1

Characteristics of the crosses grouped in confrontation types and classes, and values of the conversion parameters in progenies. F.C.: frequency of conversion, i.e. frequency of 6 : 2 : 0 plus 2 : 6 : 0 (*) or 6 : 2 plus 2 : 6 : 0 (**) times 10%. D.V.: ratio of most frequent direction of conversion/least frequent direction. N symbolizes an allele site which tends to be converted to the alternative, and n an allele to which the alternative tends to be converted. Thus for type A mutants, N is mutant and n wild, while for type B mutants the converse is the case

Cross number	Parental genotype	Parental spore colour	F.C.	D.V.	Average D.V.	Confrontation type	Class	No. of mut. sites	N ratio
I*	$\frac{2\ 0}{+ +}$	Light Dark	79	1.3	1.3	$\frac{A\ B}{+ +}$	$\frac{Nn}{nN}$	2	1
II*	$\frac{2\ 3\ +}{2\ +\ 6}$	White Brown	58 83 77 70	1.5 2.3 2.4 2.6	2.2	$\frac{A\ +}{+ A}$			
III*	$\frac{2\ +\ +\ 6}{+ 3\ 1\ +}$	Brown White	93 69 70 97	2.1 2.4 2.5 3.0	2.5	$\frac{A\ +\ +\ A}{+ A\ A\ +}$	$\frac{NNnn}{nnNN}$	4	1
IV*	$\frac{3\ +\ 0}{+ 1\ +}$	Brown White	124 108 133 138 109 92	5.6 5.7 7.4 7.7 8.2 13					
V*	$\frac{2\ +\ 0}{+ 3\ +}$	Brown White	90 81 88 77 77 84	5.4 5.6 5.8 6.6 6.9 7.4	6.7	$\frac{A\ +\ B}{+ A\ +}$			
VI*	$\frac{2\ +\ 0}{+ 1\ +}$	Brown White	120 111 126	3.7 5.0 5.8				3	
VI*	$\frac{+ 3\ 0}{2\ +\ +}$	Brown White	129 126	6.1 8.0					
VIII*	$\frac{+ 1\ 0}{3\ +\ +}$	Brown White	105 110 140 114 142 135	4.5 5.7 6.8 6.9 7.1 8.8	6.5	$\frac{+ A\ B}{A\ +\ +}$			
IX*	$\frac{+ 1\ 0}{2\ +\ +}$	Brown White	181 116 132 119 114 145	4.4 5.5 6.3 6.4 6.6 8.2					

X**	$\frac{++}{2} \frac{10}{3} \frac{0}{0}$	Brown White	125 111	5-4 5-6	5-5	$\frac{++}{A} \frac{A}{A} \frac{+}{+}$	$\frac{Nnn}{nNN}$	3	2	
XI**	$\frac{++}{2} \frac{++}{3} \frac{++}{0}$	Dark Brown White	102	3-8	3-8	$\frac{++}{A} \frac{A}{A} \frac{B}{B}$				
XII**	$\frac{++}{2} \frac{++}{3} \frac{++}{6}$	White Brown	77 79 72	4-0 5-2 6-8	7-0	$\frac{++}{A} \frac{+}{A} \frac{+}{A}$				
XIII**	$\frac{++}{2} \frac{++}{1} \frac{++}{6}$	White Brown	113 67 141	5-8 9-3 11			$\frac{Nnnn}{nNNN}$	4		
XIV**	$\frac{++}{2} \frac{++}{3} \frac{++}{0}$	Brown White	123 138 141	8-3 11 15	11	$\frac{++}{A} \frac{A}{A} \frac{B}{B}$				
XV**	$\frac{++}{2} \frac{++}{3} \frac{++}{0}$	Brown White	132	9-8	9-8	$\frac{++}{A} \frac{++}{A} \frac{B}{A}$				
XVI**	$\frac{++}{2} \frac{++}{3} \frac{++}{1} \frac{++}{6}$	White Dark Pink	130 100 125 122 94	5-6 5-8 6-8 7-1 8-1	6-7	$\frac{++}{A} \frac{++}{A} \frac{+}{A}$	$\frac{n}{N}$	1		
XVII**	$\frac{5}{+}$	White Dark Brown	66	7-8						
XVIII**	$\frac{20}{2} \frac{++}{+}$	Brown White	107	12	11	$\frac{B}{+}$				
XIX**	$\frac{10}{1} \frac{++}{+}$	Brown White	153	11			$\frac{n}{N}$			
XX**	$\frac{0}{+}$	White Dark Brown	166	13						
XXI**	$\frac{++}{3}$	Dark Brown White	136 126 114 149	10 12 13 13						
XXII**	$\frac{++}{1}$	Dark Brown White	186 147 146	9-6 11 12			$\frac{n}{N}$			
XXIII**	$\frac{++}{2}$	Dark Brown White	161 112 137 105	13 9-4 13 14	12	$\frac{+}{A}$				
XXIV**	$\frac{++}{2} \frac{++}{3} \frac{++}{1}$	White Dark Pink	149	11						
XXV**	$\frac{++}{6}$	Dark Brown White	195	11			$\frac{n}{N}$			
XXVI**	$\frac{++}{2} \frac{++}{6}$	White Brown	128	12						

TABLE 1 *contd.*

Cross number	Parental genotype	Parental spore colour	F.C.	D.V.	Average D.V.	Confrontation type	Class	No. of mut. sites	N ratio
XXVII**	$\frac{+30}{23+}$	Brown White	113	8.7	14	$\frac{+B}{A+}$	$\frac{nn}{NN}$	2	∞
			125	12					
			83	21					
			108	21					
XXVIII**	$\frac{3+0}{31+}$	Brown White	128	14					
			148	24					
XXIX**	$\frac{+10}{31+}$	Brown White	137	14					
			137	14					
XXX**	$\frac{2+0}{23+}$	Brown White	95	4.9					
			132	9.6					
			110	12					
			94	15					
XXXI*	$\frac{++}{26}$	Dark Light	162	9.6					
			129	12					
			96	15					
			149	7.5					
XXXII**	$\frac{+3+}{231}$	White Dark Pink	117	14					
			131	17					
			194	12					
			149	31					
XXXIII**	$\frac{++}{31}$	Dark Brown White	142	36					
			119	8.1					
			155	9.3					
			117	13					
XXXIV**	$\frac{++}{23}$	Dark Brown White	123	13					
			205	11					
			87	10					
			61	17					
XXXV**	$\frac{++1}{231}$	White Dark Pink	54	21					
			122	8.7					
			138	9.6					
			103	12					
XXXVI*	$\frac{++}{231}$	Dark Light	128	22					
			122	8.3					
			189	18					
			122	8.3					
XXXVII*	$\frac{++}{231}$	White Dark Pink	189	18					
			122	8.3					
			189	18					
			122	8.3					

TABLE 2

White-spored recombinants arising from crosses with differently coloured parental phenotypes

Cross number	Parental		White spored recombinant genotype
	genotype	phenotype	
I	2 0	Light	+ 0, 2 +
	+ +	Dark	
XXXI	+ +	Dark	2 +, + 6
	2 6	Light	
XXXVI	4 + + 0	Light	4 + 3 0, 4 2 + 0 4 2 3 0, 4 + + + 4 + 3 +, 4 2 + +
	4 2 3 +	Dark	
XXXVII	+ + +	Dark	+ + 1, 2 + +, + 3 + + 3 1, 2 3 +, 2 + 1
	2 3 1	Light	

In all the other crosses one parent exhibits a coloured spore phenotype and the other a white spore phenotype. In such crosses, the coconversions always lead to 6 : 2 (coloured : white) or 2 : 6 asci, distinguishable from the common parental 4 : 4 asci. Again the low frequency of single-site conversion in comparison with coconversion means that a good approximation of the conversion pattern of each mutant site can be obtained from the frequency of 6 : 2 and 2 : 6 asci.

(v) *Culture media and crossing techniques*

The details of media and culture techniques were previously described (Rizet *et al.*, 1960; Lissouba *et al.*, 1962; Yu Sun, 1964).

3. RESULTS

(i) *Coconversion in crosses involving two parents with coloured spores*

In such crosses, coconversions involving all heterozygous sites lead to 6 : 2 : 0 and 2 : 6 : 0 asci. The analysis of 12 6 : 2 : 0 and 11 2 : 6 : 0 asci in cross I (2 0 × + +) showed that all these asci did actually arise from coconversion (Leblon and Rossignol, 1973). In the other crosses involving parents with coloured spores (XXXI, XXXVI, XXXVII), it was assumed that the frequencies of 6 : 2 : 0 and 2 : 6 : 0 asci directly reflected the frequencies of coconversion. The resulting frequencies of coconversion among total convertant asci are shown in fig. 1 for the different pairs of sites; nearly all the conversions correspond to coconversion.

(ii) *Disparities in the direction of conversion*

Table 1 reports the disparity values (D.V.) obtained in the 108 crosses, according to the genotype of the parental strains. The D.V. corresponds to the ratio of the more frequent direction of conversion to the less frequent one. It shows variation from cross to cross from 1.3 to 36. Referring to control-one-point crosses, whenever a change is observed in the D.V. for multi-point crosses, it seems to correspond to a decrease rather than an increase.

(a) *Grouping the crosses*

In crosses with identical parental genotypes, the observed D.V. are similar (table 1). In crosses with distinct parental genotypes, similar D.V. are observed in the two following situations:

1. The parental genotypes differ only by one or two homozygous mutant site(s), e.g. $\frac{0}{+}$, $\frac{1\ 0}{1\ +}$ and $\frac{2\ 0}{2\ +}$.

2. One heterozygous mutant site is replaced by an other heterozygous site giving the same type of conversion pattern (A or B) as long as it is in the same array with the other sites, e.g. $\frac{+3\ 0}{2\ 3\ +}$, $\frac{3\ +\ 0}{3\ 1\ +}$ and $\frac{+1\ 0}{3\ 1\ +}$ where 2 may be replaced by 1 or 3.

These results suggest that the D.V. are similar within each group of crosses of similar confrontation pattern. Therefore the average D.V. was calculated for every confrontation (table 1).

(b) *Grouping confrontation types into classes*

Similar D.V. are observed in crosses XXXVI $\left(\frac{4\ +\ +\ 0}{4\ 2\ 3\ +}; \frac{+ +\ B}{A\ A\ +}\right)$ and XXXVII $\left(\frac{+ +\ +}{2\ 3\ 1}; \frac{+ +\ +}{A\ A\ A}\right)$ (table 1). Between these crosses, the difference in heterozygosities is that of a B mutant site in *trans* as opposed to an A mutant site in *cis*. Since both confrontation types lead to similar disparity, this suggests that two mutant sites with opposite conversion patterns in *trans* affect the disparity in the same way as two mutant sites with similar conversion patterns in *cis*.

Similar D.V. are observed in crosses X $\left(\frac{+ +\ +\ 1\ 0}{2\ 3\ +\ 0}; \frac{+ +\ A}{A\ A\ +}\right)$ and XI $\left(\frac{+ +\ +}{2\ 3\ 0}; \frac{+ +\ +}{A\ A\ B}\right)$ (table 1). Between these crosses, the difference lies in the substitution of an A mutant site in *trans* for a B mutant site in *cis*. Since both confrontation types lead to similar disparity, this suggests that two mutant sites with similar conversion patterns in *trans* affect the disparity in the same way as two mutant sites with opposite conversion patterns in *cis*.

Similar D.V. are observed in crosses V $\left(\frac{2\ +\ 0}{+3\ +}; \frac{A +\ B}{+ A\ +}\right)$ and VII $\left(\frac{+3\ 0}{2\ +\ +}; \frac{+ A\ B}{A\ +\ +}\right)$ (table 1). In these crosses, the number and types of conversion patterns of the mutant sites in each parental genotype are the same. The difference lies in the relative order of the heterozygous sites in the confrontation type. This suggests that the order of the heterozygous sites does not affect the D.V.

On the basis of the three last observations, the 18 confrontation types were put into eight classes (table 1). In every class, each allelic site enjoying a conversion "advantage" (*i.e.* wild type at A sites and mutant at B sites)

is symbolised by n , and the corresponding "disadvantaged" allelic sites are referred to as N . Furthermore, the order of the heterozygous sites is not taken into account, *e.g.* both $\frac{A+B}{+A+}$ and $\frac{+AB}{A++}$ are symbolised

$\frac{Nnn}{nNN}$.

(c) *Homogeneity of the D.V. within every class*

Covariance analysis shows that in every class, there is not more D.V. variation among crosses with different heterozygous sites than among crosses with identical ones (table 3). So, every class is homogeneous with respect to D.V. The conclusions are that:

- (1) The substitution between two mutant sites showing the same type of conversion pattern (A or B) does not affect the disparity.
- (2) Type A and B mutant sites are equivalent in opposite arrays.
- (3) The D.V. is not altered by a change in the order of the heterozygous sites.

(d) *Role of the N ratio in the disparity*

The distinction between classes depends upon two criteria: the number of heterozygous sites and the N ratio, which corresponds to the ratio of N sites between the genotype showing the larger number of N and that showing the smaller one; *e.g.* in class $\frac{Nn}{nN}$ the N ratio is 1; it is 2 in class $\frac{Nnn}{nNN}$.

In order to check whether these two parameters play a role in the disparity, covariance analysis was performed in two ways. In a first step, classes with identical N ratios and different numbers of heterozygous sites were compared (table 4a); in a second step, the comparison involved classes with identical numbers of heterozygous sites and different N ratio values

TABLE 3

Covariance analysis of crosses differing in their heterozygous sites within each class. F.C. (after angular transformation; $\arcsin \sqrt{F.C.}$; Lison, 1958) is taken as an independent variate and the frequency of the most frequent direction of conversion (after angular transformation) is taken as a dependent variate. Asci have been equally numbered in every cross

Class	F value observed	P value
$\frac{Nn}{nN}$	$F_2^1 = 11.99$	$> 5\%$
$\frac{Nnn}{nNN}$	$F_{27}^9 = 2.27$	$\approx 5\%$
$\frac{Nnnn}{nNNN}$	$F_5^2 = 3.47$	$> 5\%$
$\frac{n}{N}$	$F_{11}^5 = 1.08$	$> 5\%$
$\frac{nn}{NN}$	$F_{18}^6 = 1.58$	$> 5\%$
$\frac{nnn}{NNN}$	$F_4^1 = 0.31$	$> 5\%$

TABLE 4

(a) Covariance analysis between classes with identical *N* ratios and with different numbers of heterozygous sites. (b) Covariance analysis between classes with different *N* ratios and with the same number of heterozygous sites. Legend: see table 3

(a)				
Class	Number of heterozygous sites	N ratio	F value observed	P value
$\frac{Nn}{nN}$	2	1	$F_6^1 = 1.12$	> 5%
$\frac{NNnn}{nnNN}$	4	1		
$\frac{n}{N}$	1	∞	$F_{48}^3 = 0.26$	> 5%
$\frac{nn}{NN}$	2	∞		
$\frac{nnn}{NNN}$	3	∞		
$\frac{nnnn}{NNNN}$	4	∞		
(b)				
Class	Number of heterozygous sites	N ratio	F value observed	P value
$\frac{Nn}{nN}$	2	1	$F_{28}^1 = 81.0$	< 1%
$\frac{nn}{NN}$	2	∞		
$\frac{Nnn}{nNN}$	3	2	$F_{42}^1 = 22.3$	< 1%
$\frac{nnn}{NNN}$	3	∞		
$\frac{NNnn}{nnNN}$	4	1	$F_{11}^2 = 11.4$	< 1%
$\frac{Nnnn}{nNNN}$	4	3		
$\frac{nnnn}{NNNN}$	4	∞		

(table 4b). Covariance analysis shows that within classes with identical *N* ratio values, the D.V. does not vary more among crosses with different numbers of heterozygous sites than among crosses with equal numbers of heterozygous sites. Hence the number of heterozygous sites has no significant influence on disparity. On the other hand, the covariance analysis reveals a strong effect of the *N* ratio on D.V. among classes with equal numbers of heterozygous sites. The classes can then be grouped according

to the value of the N ratio; this leads to four groups of classes with N ratio values: 1, 2, 3, ∞ . Covariance analysis (table 5) shows that all these groups differ according to their D.V.

TABLE 5

Covariance analysis between classes with different N ratios. Legend: see table 3

Classes	N ratio	F value observed	P value
$\frac{Nn}{nN}$ plus $\frac{NNnn}{nnNN}$	1	$F_{44}^1 = 89.59$	<1%
$\frac{Nnn}{nNN}$	2		
$\frac{Nnn}{nNN}$	2	$F_{44}^1 = 6.11$	<2.5%
$\frac{Nnnn}{nNNN}$	3		
$\frac{Nnnn}{nNNN}$	3	$F_{69}^1 = 13.7$	<1%
$\frac{n}{N}$ plus $\frac{nn}{NN}$ plus $\frac{nnn}{NNN}$ plus $\frac{nnnn}{NNNN}$	∞		

(iii) Disparity value and the frequency of conversion

The frequency of conversion (F.C.) varies from 54×10^{-3} to 205×10^{-3} (table 1). The existence of a correlation between this variation and the number of heterozygous sites on the one hand and the D.V. on the other hand was tested. Statistical analysis shows no relationship between the number of heterozygous sites and the F.C. (the slope of the regression line is not different from zero: $t = 1.43$; $P > 0.10$). Thus no effect of the number of heterozygous sites on the F.C. can be shown.

A low but significant positive correlation is found between the D.V. and the F.C. ($r = 0.29$; $P < 1\%$).

4. DISCUSSION

From the effect of site interaction on the conversion parameters within the *A* group of intragenic suppression the following conclusions can be drawn:

1. When they can be scored, nearly all the conversions correspond to coconversions even when extreme sites within the region studied are considered. These coconversions involve almost always all the sites (up to 3).

2. The substitution of one heterozygous site by another in the same array does not modify the disparity of conversion, provided that the two sites show the same conversion pattern (e.g. $\frac{+}{A_1} + \frac{+}{A_2}$). On the other hand, the substitution of one heterozygous site by another in the opposite array does not modify the disparity of conversion, provided that the two sites show opposite conversion patterns (e.g. $\frac{+}{A} + \frac{+}{A}$ and $\frac{+}{A} + \frac{+}{A}$).

3. A change confined to the relative order of the heterozygous sites (+/A or B/+) does not modify the disparity of conversion $\left(\text{e.g. } \frac{A+B}{++} \right.$ and $\left. \frac{+A+B}{++} \right)$.

4. The number of heterozygous sites in the confrontation type modifies neither the disparity of conversion nor its basic frequency.

5. A decrease in the N ratio leads to a decrease in disparity. The N ratio is defined as the ratio of allelic sites showing a conversion "disadvantage" between the confronted genotypes.

6. A decrease in disparity is connected with a decrease in the basic frequency of conversion.

(i) *Formation of hybrid DNA in a multimarked region*

The very high frequencies of coconversion show that when formed, hybrid DNA rarely ends within the marked region. This conclusion is supported by a previous study in the same region involving two-point crosses which associate one type A (or B) and one type C mutant site (Rossignol and Haedens, 1978).

The absence of any correlation between the basic frequency of conversion and the number of heterozygous sites indicates that the formation of hybrid DNA is not prevented by the accumulation of "non-matchings" (up to 4) in a short region.

Furthermore, since most conversions are coconversions even in multi-point crosses (at least three-point crosses), the propagation of hybrid DNA is not impaired either.

The frameshift mutants used are known to reverse and to correspond to complementary types (Leblon and Paquette, 1978). Therefore, they should correspond to small additions/deletions of probably one base pair. As all data are consistent with a frequent formation of homologous hybrid DNA on both participating chromatids (Leblon and Rossignol, 1973; Paquette and Rossignol, 1978; Rossignol and Haedens, 1978; see also section iii in this discussion), it is reasonable to assume that the hybrid DNA in this region frequently involves a "Holliday structure" (Holliday, 1964). If this is true, then the accumulation of non-matchings of probably one base does not prevent the propagation of a Holliday structure. This is compatible with the proposed mechanism of propagation of hybrid DNA by branch migration driven by rotary diffusion (Meselson, 1972).

(ii) *Correction of multiple non-matching in hybrid DNA*

a. *Excision is triggered at only one site at the time*

The following interpretation was proposed to account for the differences between the conversion patterns in terms of the nature of mutations, and particularly to explain the opposite disparities observed with small addition or deletion mutants used in this study (Leblon, 1972, 1979). A correction mechanism would efficiently recognise non-matching formed in hybrid DNA

at addition/deletion sites. Since this correction mechanism seems to be specific for addition/deletion mutants, it is named "ADEL correction" which means "Addition-DEletion correction" (Leblon, 1974). It is assumed that, during this process, one strand within the hybrid DNA is preferably nicked. The location of the extra base on either strand would determine which one is nicked. It was pointed out in a previous study that B type mutants probably arise from base pair additions and A type mutants from base pair deletions (Leblon, 1972). If these assumptions are correct, the shortest strand would be preferably nicked, *i.e.* for an addition mutant, a nick occurs on the wild type strand, leading to the restoration of a homoduplex mutant sequence. An excess of $2+ : 6m$ over $6+ : 2m$ convertant asci is thus expected. Conversely deletion-type mutants will show an excess of $6+ : 2m$ asci resulting from a preferential nicking of the mutant strand (fig. 2).

FIG. 2.—Preferential direction of conversion for A or B type mutant explained using ADEL correction.

According to this interpretation, a preferential nicking involving the shortest strand could occur at every heterozygous site (A or B) in hybrid DNA, *e.g.* in the confrontation $\frac{A + + B}{+ A A +}$, four preferential nicking points are formed in hybrid DNA in the following array:

In such a case nicking in one point is sufficient to yield a coconversion event assuming that excision repair spans all the other heterozygous sites.

In the above example, there are three preferential nicking points on one strand and one on the other, making a ratio of 3 to 1 between the more loaded strand and the less loaded one. This value is the same as the N ratio value since the above structure corresponds to the class $\frac{Nnnn}{nNNN}$, each N corresponding to one preferential nicking point.

In the case of one nick being sufficient to lead to a coconversion, (the correction triggered at one site sweeps over the whole region), the observed variation of the disparity between coconversions as a function of the N ratio is expected, provided that each heterozygous site is equally likely to be involved in preferential nicking. This variation indicates that one heterozygous site is more or less equivalent to another, so that the disparity value is only determined by the relative numbers of preferential nicking points in the two strands involved in hybrid DNA.

The concordance between N ratio and disparity value is not absolute. This could be due either to a slightly larger influence of certain sites on the triggering process, or to the existence of a small fraction of conversions resulting from a process other than hybrid DNA formation followed by ADEL correction.

If excision starts simultaneously at all heterozygous sites, one would expect that excision proceeding along strands of both polarities would usually lead to breaks in the DNA duplex where the regions of excision overlap. This would result in much lower frequencies of conversion than are currently observed. The hypothesis of a simultaneous nicking was previously dismissed for this reason (Leblon and Rossignol, 1973). The absence of a correlation between the frequency of conversion and the number of heterozygous sites also strongly disagrees with this hypothesis. The following explanation is then proposed: excision is triggered at one N site, usually sweeping over the whole region and correction is completed, *before* there is much chance of excision being triggered at a second or third site, thus avoiding overlapping excision and chromatid breaks. In other words, excision-correction is very fast compared with the total time during which it can occur. The chance of an N site on one strand triggering excision before an N site on the complementary strand does so, depends on the ratio of N sites between the two strands.

b. Absence of polarisation of the ADEL correction process

The polar excision mechanism during mismatch correction was discovered in *Escherichia coli* by Wildenberg and Meselson (1975) and further characterised by Wagner and Meselson (1976). A similar polar excision was proposed by Fincham (1976) in order to account for polarity in allelic crosses. Sobell (1972) proposed polar recognition of mismatches connected with hybrid DNA formation. The crosses studied here bring no support for either the existence of an absolute strand polarity of excision or a polar recognition process of the mismatches.

The hypothesis of polar excision was discussed previously (Leblon and Rossignol, 1973). It was concluded that whether it is a double-stranded polarity (determined by some kind of orientation of the hybrid DNA region) or a single-stranded polarity (determined by the 5'-3' orientation), it is not consistent with the existence of very high frequencies of coconversion. Additional evidence against strict polar excision is obtained from the present study. It consists of the fact that a modification in the order of the hetero-

zygous sites does not affect the disparity value; e.g. $\frac{+ + A}{A A +}$ and $\frac{+ A +}{A + A}$ lead to similar disparity values.

This argues in favour of excision sweeping across the region in both directions from the N triggered nick. It also argues against polar recognition of the mismatches, meaning that the middle site is as important as the outside sites in triggering the correction process. The correction probably does not start before the hybrid DNA has spanned all sites. Our data do not agree with the idea that correction is instantly triggered as soon as a non-matching is formed. They also conflict with the idea that a progressive recognition system starting from either end of the hybrid DNA would trigger a correction when it reaches the first non-matching.

(iii) *The source of variation in the frequency of conversion*

A positive correlation between the disparity value and the frequency of conversion is observed. Such a correlation is expected if hybrid DNA frequently occurs on two homologous chromatids and if correction on the two chromatids occurs independently. Indeed, when symmetrical hybrid DNA is formed, 4 : 4 segregation corresponding either to a restoration of the parental genotypes in both chromatids or to a substitution of each parental genotype may be expected. The frequency of 4 : 4 segregation is expected to be highest when the frequencies of correction to either parental genotype have the same probability, that is, when the disparity value is lowest. Conversely, the frequency of detected convertant asci (*i.e.* 6 : 2), is expected to be lowest when the disparity value is lowest as we observed.

The hypothesis of symmetrical hybrid DNA frequently involved in the region studied (group A of intragenic suppression) also agrees with two previous observations:

1. The frequency of aberrant 4 : 4 asci for a type C mutant reaches from 0.41 to 0.54 among convertant asci (Paquette, 1978; Paquette and Rossignol, 1978).

2. The frequencies of the various classes of double site conversion in progeny of AC × ++ or BC × ++ crosses are very close to those expected

on the hypothesis of systematically symmetrical hybrid DNA (Leblon and Rossignol, 1973; Rossignol and Haedens, 1978).

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5. REFERENCES

- FINCHAM, J. R. S. 1976. Recombination in the *am* gene of *Neurospora crassa*. A new model for conversion polarity and an explanation for a marker effect. *Heredity*, 36, 81-89.
- HOLLIDAY, R. 1964. A mechanism for gene conversion in fungi. *Genet. Res. Camb.*, 5, 282-304.
- LEBLON, G. 1972. Mechanism of gene conversion in *Ascobolus immersus*. II. The relationships between the genetic alterations in *b1* or *b2* mutants and their conversion spectrum. *Molec. Gen. Genet.*, 116, 322-335.
- LEBLON, G. 1974. Mise en évidence et propriétés d'un mécanisme de correction mutant spécifique à l'origine de conversions chez *l'Ascobolus immersus*. Theses de Doctorat ès Sciences, Université Paris-Sud, 217 pp.
- LEBLON, G. 1979. Intragenic suppression at the *b2* locus in *Ascobolus immersus*. II. Characteristics of the mutations in groups A and E. *Genetics* (in press).
- LEBLON, G., AND PAQUETTE, N. 1978. Intragenic suppression at the *b2* locus in *Ascobolus immersus*. I. Identification of three distinct groups of suppression. *Genetics*, 90, 475-488.
- LEBLON, G., AND ROSSIGNOL, J.-L. 1973. Mechanism of gene conversion. III. The interaction of heteroalleles in the conversion process. *Molec. Gen. Genet.*, 122, 165-182.
- LISON, L. 1958. Statistique appliquée à la biologie expérimentale, p. 208. Gauthier-Villars, Paris.
- LISSOUBA, P., MOUSSEAU, J., RIZET, G., AND ROSSIGNOL, J.-L. 1962. Fine structure of genes in *Ascobolus immersus*. *Adv. Genet.*, 11, 343-380.
- MESELSON, M. 1972. Formation of hybrid DNA by rotary diffusion during genetic recombination. *J. Mol. Biol.*, 71, 795-798.
- PAQUETTE, N. 1978. Detection of aberrant postmeiotic 4 : 4 asci in *Ascobolus immersus*. *Can. J. Genet. Cytol.*, 20, 9-17.
- PAQUETTE, N., AND ROSSIGNOL, J.-L. 1978. Gene conversion spectrum of 15 mutants giving post-meiotic segregation in the *b2* locus of *Ascobolus immersus*. *Molec. Gen. Genet.*, 163, 313-326.
- RIZET, G., ENGELMANN, N., LEFORT, C., LISSOUBA, P., AND MOUSSEAU, J. 1960. Sur un Ascomycète intéressant pour l'étude de certains aspects du problème de la structure du gène. *C.R. Acad. Sci. (Paris)*, 270, 2050-2052.
- ROSSIGNOL, J.-L., AND HAEDENS, V. 1978. The interaction during recombination between closely linked allelic frameshift mutant sites in *Ascobolus immersus*. I. A (or B) and C type mutant sites. *Heredity*, 40, 405-425.
- SOBELL, H. M. 1972. Molecular mechanism for genetic recombination. *Proc. Natl. Acad. Sci. (U.S.A.)*, 69, 2483-2487.
- WAGNER, R., AND MESELSON, M. 1976. Repair tracts in mismatched DNA heteroduplexes. *Proc. Natl. Acad. Sci. (U.S.A.)*, 73, 4135-4139.
- WILDENBERG, J., AND MESELSON, M. 1975. Mismatch repair in heteroduplex DNA. *Proc. Natl. Acad. Sci. (U.S.A.)*, 72, 2202-2206.
- YU SUN, C. C. C. 1964. Biochemical and morphological mutants of *Ascobolus immersus*. *Genetics*, 50, 987-998.