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Multiple recombinational events within the *84W* locus of *Ascobolus immersus*

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SUMMARY

Multiple recombinational events within a gene were studied by tetrad analysis of multipoint intragenic crosses. It was found that a considerable proportion of double-site conversions can arise as two separate but correlated events. The same was true for conversion and crossing-over associated with it. The data point to a possibility of multiple recombinational events involving both conversions and cross-overs, occurring as successive rounds of recombination within a single recombinational process. Some of the results suggest that more than two chromatids may be involved in such a process. The results are discussed in terms of different recombination models.

1. INTRODUCTION

It has become clear during the last couple of years that intragenic recombination in eukaryotes is basically non-reciprocal. This type of recombination, called gene conversion, was shown to be a recombinational event involving a chromosome segment. It is manifested in double-site conversions. Such conversions observed in tetrad analysis of fungal intragenic crosses (Lissouba *et al.* 1962; Rizet & Rossignol, 1963; Rossignol, 1964, 1967; Kruszewska & Gajewski, 1967; Mousseau, 1966, 1967; Fogel & Mortimer, 1969; and others) give rise to a phenomenon named by Holliday (1964) 'map expansion'. Recently Fogel, Hurst & Mortimer (1970), using point mutations within *arg4* locus of *Saccharomyces cerevisiae*, demonstrated that the frequency of simultaneous conversions correlates with the physical distance between mutant sites. Demonstration of simultaneous conversions involving sites from neighbouring cistrons (Case & Giles, 1964; Paszewski, 1967; Putrament, 1967; Murray, 1970; Touré & Marcou, 1970) suggests that conversion is a type of recombination observed in recombination studies within short segments of genetic material, but not necessarily coinciding with genes. The second feature of gene conversion is its high fidelity. It was first inferred from numerous observations of the behaviour of converted segregants in backcrosses, then at the enzymic level (Zimmerman, 1968), and directly by finding that conversions of wild-type alleles to ochre mutations give ochre segregants (Fogel & Mortimer, 1970). The third important feature of gene conversion is its association with reciprocal recombination of flanking markers. This association became a basis for DNA hybrid models proposed by Whitehouse (1963) and Holliday (1964) for genetic recombina-

tion. These models and their later versions postulate a common mechanism for both gene conversion and crossing-over. Although the existence of a common mechanism for these two types of recombination seems very probable, the concrete proposals of these models are not compatible with some of the experimental data, mainly with the absence (or rarity) of reciprocal conversions. The latter should occur in 50 % of instances where mismatched base correction proceeds in opposite directions in both chromatids involved.

To account for the absence of reciprocal conversion Whitehouse (1967) postulated that hybrid DNA can be regularly formed at a mutant site in one chromatid only. This postulation raises immediately another serious problem: how to explain the different fate of two nucleotide-chains which break and unwind symmetrically in two homologous chromatids.

The demonstration that simultaneous conversions or conversion and crossing-over within a gene or within two neighbouring cistrons can occur as apparently separate but correlated events (Rossignol, 1967; Marcou, 1969; Paszewski & Prażmo, 1969; Stadler & Kariya, 1969; Touré & Marcou, 1970), suggests a novel approach to the problem of the association between two conversions or conversion and crossing-over. We undertake this problem in the present work, which is a continuation of the previous one (Paszewski & Prażmo, 1969). The term 'co-conversion' introduced by David Stadler (quoted after Fogel *et al.* 1970) will be used here to describe correlated conversions occurring as a single event and the term 'associated conversions' to describe two correlated conversions occurring as separate events.

2. MATERIAL AND METHODS

Mutants affecting ascospore pigmentation, from the locus *84W* of *Ascobolus immersus* were used in this work. Four of them; that is, *936*, *84W*, *787 and 1043*, have been already used in our previous work (Paszewski & Prażmo, 1969). A new mutant, used now, was found among the progeny of the cross *84W . 1043* × wild type. An octad with all spores white was isolated and the spores were backcrossed. Four of them had genotype *84W . 1043*; the other four carried a new mutation which was denoted *201*.

The media and techniques used were the same as described by Paszewski, Surzycki & Mańkowska (1966).

3. RESULTS

(i) *Characterization of the mutants and genetic map of the* 84W *locus*

The mutant *201* was crossed with the wild-type to establish its basic frequencies of conversion (Table 1). The frequencies given in the table were calculated after having analysed samples of the 2w:6d and 6w:2d asci to find a proportion of false aberrant segregations. It is evident that the frequencies of conversion in either direction are similar.

The results of crosses between *201* and the four allelic mutants are given in Table 2. It may be noted that conversion of *201* to the wild-type allele in crosses

936 × *201* and *201* × *1043* was higher than the basic frequency of conversion found in the one-point cross. This is in agreement with previous observations that conversion frequency of a mutant can be markedly increased by an allelic mutant with which it is crossed. Polarity is weak or non-existent with the exception of cross 1, where it results from a very low conversion frequency of *936*.

Table 1. *Results of the cross 201 × wild type*

Type of ascus	No. of asci scored	No. of asci tested	Genotype confirmed	Frequency ($\times 10^3$)
4w:4d*	20229	—	—	—
6w:2d	179	16	7	3·8 (2·7–4·9)†
2w:6d	116	21	13	3·5 (3·2–3·8)

* w, White; d, dark. † 95 % confidence interval.

The basic frequencies of conversion for all the five mutants utilized in the present work, their arrangement and recombination frequencies in two-point crosses are shown in Fig. 1. The arrangement of the mutant sites is based on the data presented in the previous paper and the results of the present work, especially of the three- and four-point crosses described below.

(ii) *Two-point crosses in coupling*

A simple method of studying simultaneous conversions at two sites is by crossing a double mutant with the wild-type and looking for simultaneous conversions to the wild-type alleles.

Five such double mutants were obtained and crossed with the wild-type (Table 3). Attention was paid here only to the 2w:6d asci as they could result only from double-site conversions. There is reason to assume that co-conversion will depend on the distance between mutant sites and the basic frequencies of conversion in the direction from mutant allele to the wild-type for the two mutants used in a given cross. In other words, two closely situated mutants may show lower frequency of double-site conversion than more distantly located ones, if the basic frequencies of conversion of the former are lower than those of the latter. Thus, to account for such a possibility, a double-site conversion coefficient (E_{dc}) may be introduced, calculated from the following formula:

$$E_{dc} = \frac{f_{dc}\,(m_1 m_2 \rightarrow ++)}{\frac{1}{2} f_{m_1} \times f_{m_2}},$$

where f_{dc} is frequency of double-site conversions observed in a two-point cross in coupling, f_{m_1} and f_{m_2} are the basic frequencies of conversion for mutants m_1 and m_2, respectively.

Theoretically this coefficient should better reflect the relationship between the distance of mutant sites and their simultaneous conversion rates than the frequencies of double-site conversion only.

Table 2. *Results of crosses between* 201 *and its allelic mutants*

Cross $a \times b$	No. of asci scored	No. and frequency of 6w:2d asci ($\times 10^3$)	No. of asci tested	Genetic structure and frequencies ($\times 10^3$) of recombinant asci: Conversion $a \rightarrow +$	Conversion $b \rightarrow +$	Crossing-over
201 × 936	23249	262 (11·2, 9·9–12·6)	28	27 (10·8, 10·0–12·6)	—	1 (0·4, 0·1–0·2)
201 × 84W	31391	405 (12·9, 11·6–14·1)	41	11 (3·3, 1·6–5·0)	29 (8·7, 7·0–10·1)	1 (0·3, 0·08–1·6)
201 × 1043	34696	546 (15·7, 14·4–17·0)	29	16 (8·6, 5·9–10·5)	11 (5·6, 2·4–8·4)	2 (1·0, 0·6–1·4)
201 × 787	25238	252 (10·0, 8·7–11·2)	28	15 (5·35, 3·6–7·1)	13 (4·6, 2·8–6·4)	—

As it is clear that recombination frequencies found in interallelic crosses cannot be used as a measure of the physical distance between mutant sites, we expected that in our case double-site conversion coefficients would reflect at least the order of the sites in the locus which was established fairly well, if other factors besides the basic frequencies of conversion and the distance between mutant sites deter-

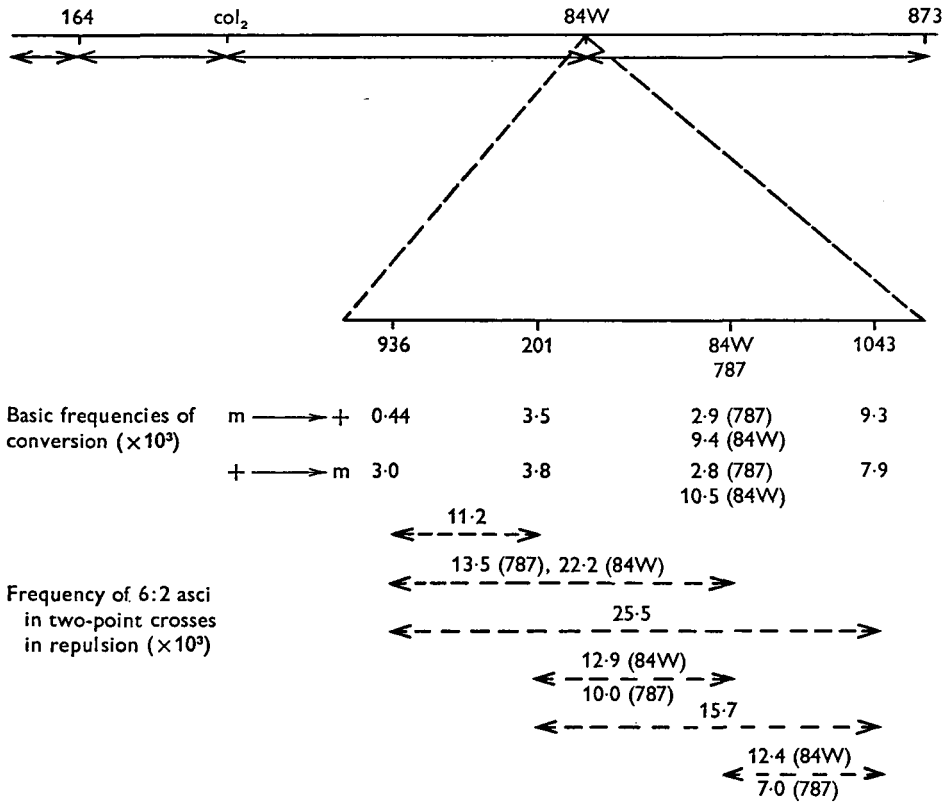

Fig. 1. Map of locus 84W.

Table 3. *Double-site conversions to the wild-type alleles in two-point crosses in coupling*

Cross	No. of asci scored	No. of 2w:6d asci	No. of 2w:6d tested	Genotype confirmed	Frequency (× 10³)	Double-site conversion coefficient
936.84W × + +	27130	28	27	7	0·14 (0·03–0·36)	68
936.201 × + +	30590	45	16	0	?	?
201.84W × + +	2214	10	6	6	4·5 (1·0–5·9)	272
201.1043 × + +	18401	32	18	12	1·1 (0·65–1·6)	68
84W.1043 × + +	53970	128	70	38	1·3 (1·0–1·6)	30

mine double-site conversion frequency. Our findings suggest that such factors do exist and we will return to this problem later.

It should be noted that double-site conversions may arise from a single recombination event (co-conversion) or from two separate but associated events (associated conversions). In crosses such as those described in this section one cannot distinguish between these two modes of double-site conversion. An estimation of the proportion of associated conversions is, however, possible in a three-point cross of the type $a+c\times+b+$ or even better in a four-point cross of the

Table 4. *Results of three-point and four-point crosses*

	Cross	No. of asci scored	No. of 6w:2d asci	Frequency of 64:2d asci ($\times 10^3$)
1.	936 + 84W × + 201 +	15827	45	2·8 (2·0–3·6)
2.	936 + 787 × + 201 +	45593	0	?
3.	936 + + 1043 × + 201 . 84W +	10236	42	4·1 (2·8–5·3)
4.	936 + 84W + × + 201 + 1043	270000	54	0·2 (0·15–0·25)

type $a+c+\times+b+d$. In such crosses double-site conversion of a and c or b and d must result from separate events, at least at some steps of the recombinational process, as converted sites are separated by a site which segregates normally. An experimental approach to this problem will be presented in the following section.

(iii) *Multi-point crosses*

Results of two three-point and two four-point crosses are given in Table 4. Eighteen tested recombinant asci from the first cross were all of the following genotype:

936	+	*84W*
936	+	*84W*
+	+	+
+	*201*	+

It is evident that the conversion frequency of *201* in this cross is about equal to the basic frequency of conversion (see Table 1) but lower than observed in the *936* × *201* cross.

It was a surprise not to find any recombinant asci in the cross *936* + *787* × + *201* + in spite of the relatively high recombination frequencies observed in two-point crosses *936* × *201* and *201* × *787* (see Table 2).

Two types of ascus were found in a sample of 12 tested from the cross *936* + + *1043* × + *201*. *84W* + (Table 5). The frequency of simultaneous conversion of the middle sites equals that of simultaneous conversions found in the cross *201*. *84W* × wild-type. It means that if there is conversion associated with crossing-over, it must occur outside the *936–1043* region. In the case of its direct connexion with conversion – that is, its appearance within the studied region – the number of detectable conversions of *201*. *84W* → ± in this four-point cross should

be considerably lower than in the corresponding two-point cross. Besides, one reciprocal conversion was observed.

Table 5. *Genetic structure of analysed recombinant asci from cross 936.1043 × 201.84W*

Ascus type ...	936	+	+	1043	936	+	+	1043
	936	+	+	1043	936	201	+	1043
	+	+	+	+	+	+	+	+
	+	201	84W	+	+	201	84W	+
		I				II		
Number		11				1		
Frequency ($\times 10^3$)		3·75 (3·1–5·3)				0·34 (0·08–1·3)		

In the fourth cross the same mutants were used, but in different configuration. The genetic structure, numbers and frequencies of various types of asci observed in a sample of 39 asci tested are given in Table 6. The majority of recombinant asci arose from simultaneous conversions of *201* and *1043* to the respective wild-type alleles (ascus type V). Almost as frequent were asci resulting from conversion and crossing-over (types III and IV). Three asci were due to simultaneous conversions to the wild-type of mutants *936* and *84W* (VI). A relatively high proportion of the asci tested were those of type VII. In these asci at least three chromatids were involved in recombination. Asci of types VIII and IX must have arisen from very complex rearrangements of genetic material and the appearance of new mutations. Question-marks in the table indicate that it is not known whether the respective spores carry the new mutations (*X* or *Y*) as the relevant intra-ascal crosses were not fertile.

The recombinant asci of types III–VI resulted from at least two recombinational events. These events had, however, to be correlated. This is shown in Table 7, where the observed frequencies of these asci are compared with the expected ones calculated on the assumption of independent occurrence of these events.

(iv) *Analysis of 4d:4w asci from cross* 936 × 1043

Analysis of 4w:4d asci from a two-point cross in repulsion provides another way of studying complex recombinations. The cross *936* × *1043* was chosen as the frequency of such asci was relatively high. (Data concerning 6w:2d asci in this cross have been already published, Paszewski & Prażmo, 1969). Forty six 4w:4d asci were found among 85397 asci scored and 33 of them were analysed (Table 8). Three types of asci were found. When their frequencies are compared with the expected ones, calculated as previously on the assumption of independent occurrence of recombinational events leading to the formation of particular types of ascus, one observes that asci of types X and XI are more frequent than expected. This indicates a correlation of recombinational events giving rise to these asci. On the other

hand the frequency of the ascus type XII is about equal to the expected value, suggesting that conversions in the sister chromatids have occurred independently.

4. DISCUSSION

(i) *Double-site conversion – one or two events?*

It is generally believed that double-site conversions arise from a single recombinational event that is co-conversion. Our results indicate that while the majority of double-site conversions can be interpreted as such, there may be a considerable proportion of associated conversions resulting from two separate but correlated events. If we compare the frequency of type V asci (Table 6) with that of 2w:6d asci from the cross *201.1043* × wild-type (Table 3), we find that at least 8·2 % of simultaneous conversions at 201 and 1042 results from separate events. If we include asci of type VII, this proportion will be even higher, but still underestimated, since in the cross *936* + *84W* + × *201* + *1043* all co-conversions *201* → + or *1043* → + with + → *84W* produced a mutant segregant, thus they remain undetected in our system. It is quite possible that in the case of the *201.1043* pair as much as 20% of double-site conversions results from two separate events. An even higher proportion of associated conversions was observed by Stadler & Kariya (1969) in *mtr* locus of *Neurospora*.

Double-site conversion frequency depending on the physical distance between sites and the basic frequencies of conversion is to be expected. As in our case the physical distances between particular mutant sites cannot be estimated, we expected to obtain a relation between the order of the sites and the respective double-site conversion coefficients: the closer are the sites, the higher the coefficient. In fact such a relation has not been found. This suggests that some other factors, besides the two mentioned above, determine co-conversion.

Mutants used in this work are very likely to be structural ones – deletions or additions. They do not revert and in some crosses they behave in quite an unexpected way (for instance, the absence of wild-type recombinants in cross *936* + *787* × + *201* + and the absence or a very low rate of double-site conversion in cross *936.201* × wild type). Such mutants may cause conformational alterations in a fraction of conjugated chromatids which, in turn, may stimulate or restrict recombination. Some of these alterations probably caused specifically by a given set of mutants, may abolish the physical contact of chromatids on a certain length.

(ii) *Analysis of the results on DNA hybrid models*

The results of the cross *936* + *84W* + × + *201* + *1043* (Table 6) indicate that a high proportion of conversion-associated cross-overs occur as separate events. In terms of Holliday's model (1964, 1968), these would be independent corrections of mismatched bases within a DNA hybrid segment. This is shown in Fig. 2. To obtain a wild-type recombinant, independent corrections of the pairs *201*/+ and +/*84W* to the wild-type alleles in the chromatid 2 are necessary, while correction in the homologous chromatid can proceed in either direction. According to the model,

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Table 6. *Genetic structure of analysed recombinant asci from cross 936.84W × 201.1043*

Ascus type ...	936	+	84W	+	936	+	84W	+	936	+	84W	+			
	936	201	+	1043	936	+	84W	1043	936	+	84W	+			
	+	+	+	+	+	+	+	+	+	+	+	+			
	+	201	+	1043	+	201	+	1043	+	201	+	1043			
			III				IV				V				
Number			6				7				16				
Frequency ($\times 10^3$)			0·03 (0·012–0·06)				0·035 (0·015–0·075)				0·082 (0·056–0·11)				
Ascus type ...	936	+	84W	+	936	+	84W	+							
	+	+	+	+	936	+	84W	+							
	+	201	+	1043	+	+	+	+							
	+	201	+	1043	+	201	84W	1043							
			VI				VII								
Number			3				5								
Frequency ($\times 10^3$)			0·014 (0·003–0·04)				0·024 (0·008–0·056								
Asci with new mutations	936	201	+	1043	?	+	+	+	+	Y					
	+	+	+	+	X	+	+	+		Y					
	+	+	+	+	+	+	+	+	+	+					
	+	+	+	+	+	+	+	+	+	+					
	+	201	+	1043	?	+	201	+	1043	?					
	+	201	+	1043	?	+	201	+	1043	?					
	+	201	+	1043	?	+	201	+	+	?					
	+	201	+	1043	?	+	201	+	+	?					
			VIII				IX								
Number			1				1								

two corrections involving one nucleotide chain are more likely than corrections involving two chains. According to the segment excised, $+-84W$ or $201-+$, one or the other set of alleles is restored. It is likely that excision of a segment from each chain occurs with equal frequency, so the frequencies of asci III and IV

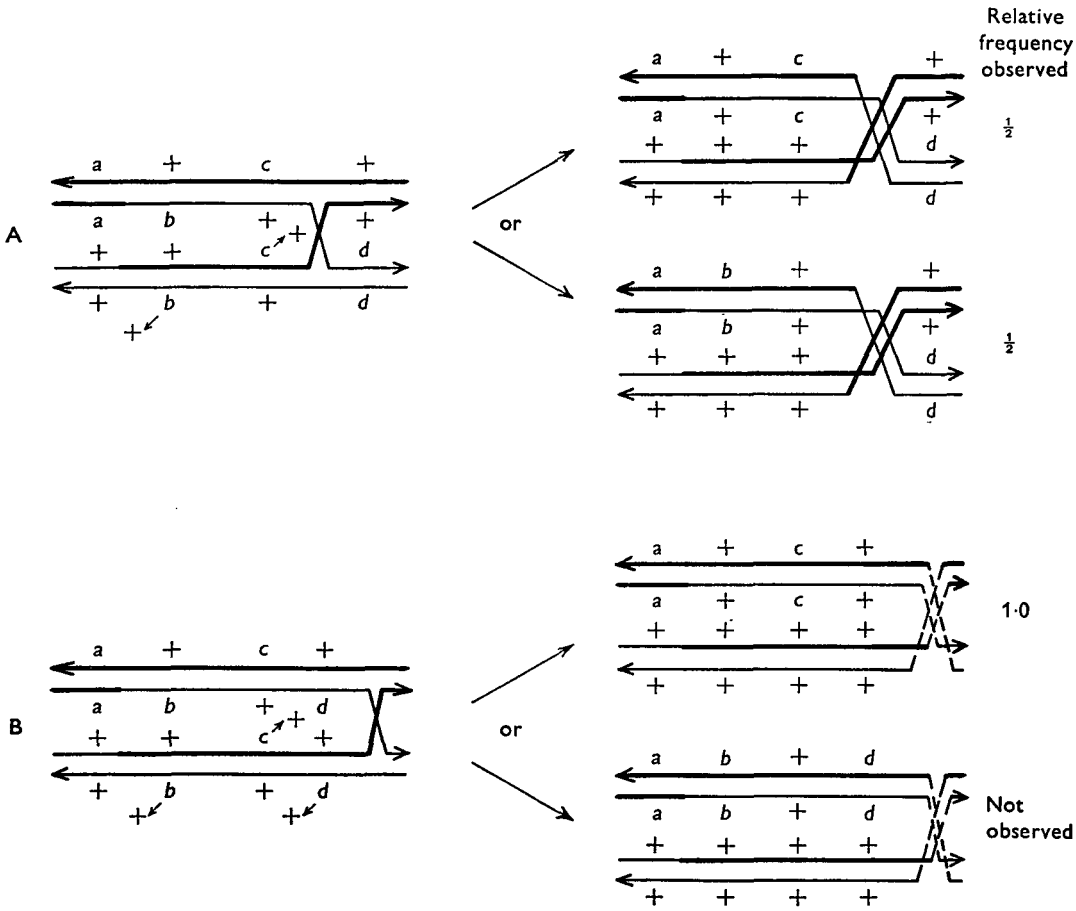

Fig. 2. Interpretation of the origin of ascus types IV(A) and V(B) in terms of Holliday's model. Letters *a*, *b*, *c* and *d* represent mutants 936, 201, 84W and 1043, respectively. Only two out of four chromatids are shown. See text for explanation.

should be equal. (This is the simplest assumption one can make, especially when the mutants involved in the DNA segment show the same rate of conversions in opposite directions in one-point crosses.) In fact they were found to be so, thus these results are in agreement with the model.

However, with the same set of postulations that have been made to explain the origin of asci III and IV, this model fails to give a satisfactory explanation for the ascus type V, as no type of ascus 'complementary' to it has been found (Fig. 2B). There are thus two alternatives: there is one basic mechanism for genetic recombination in eukaryotes, but the model is wrong, or there are two or many mechanisms and the model corresponds to one of them only.

It should be noted that similar analysis on Whitehouse's original model (Whitehouse, 1963) leads to similar conclusions. The modified version of this model (Whitehouse, 1967) postulating hybrid DNA at a mutant site confined to one chromatid only allows us to explain asci of type V, but as we have pointed out earlier, it is difficult to reconcile this postulation with the general idea of the model.

Table 7. *Observed and expected frequencies of asci with complex recombination patterns from cross 936.84W × 201.1043*

Ascus type	Frequency observed (*a*)	Frequency expected (*b*)	Ratio *a*:*b*
III	0·03	0·0017	17·6
IV	0·035	0·00084	41·6
V	0·082	0·012	6·8
VI	0·014	0·002	7·0

Frequencies of single events for the calculation of the expected frequencies of complex recombination patterns were taken from Table 4, cross 11 (Paszewski & Prażmo, 1969) and from Table 2, cross 1, and Table 4, cross 1, this paper. In all cases the expected frequency equals one-half the product of the respective single-event frequencies.

Table 8. *Analysis of 4w:4d asci from the cross 936 × 1043*

Ascus type	936 + 936 + + +	+ 1043 + +	936 + + + + 1043	+ + + +	936 + 936 + + +	+ + + +
	X		XI		XII	
Number	25		6		2	
Frequency observed ($\times 10^3$), *a*	0·4 (0·32–0·48)		0·095 (0·032–0·15)		0·032 (0·004–0·1)	
Frequency expected ($\times 10^3$)*, *b*	0·078		0·004		0·11	
Ratio *a*:*b*	5		23		0·30	

* Frequencies of single event for calculation of the expected frequencies were taken from Table 4, cross 3 (Paszewski & Prażmo, 1969).

(iii) *Analysis of the data on the donor–recipient model*

Boon & Zinder (1969) proposed a mechanism for recombination in phages, by which one parent and one recombinant is formed. A very similar donor–recipient hypothesis was suggested to explain gene conversion in fungi (Paszewski, 1970). The formation of ascus types IV and V according to this hypothesis is given in Fig. 3A and B, respectively. A break in a single nucleotide strand in one chromatid, followed by its partial degradation and unwinding, starts the process. The strand with a free end which is formed triggers off an involvement of the homologous chromatid into recombination. Such a mechanism for the initiation of a certain type of recombination following u.v.-irradiation in bacteria has been suggested by Howard-Flanders, Theriot & Stedeford (1969). The chromatid involved in recombination can serve both as template for repair-type replication of the partly

degraded strand and as material donor for the recipient chromatid. Successive steps in material transfer are shown in Fig. 3A (I–V). They involve two cuts in the donor strand, and the excision of a single-stranded segment from the recipient chromatid. The gap in the donor chromatid is repaired by the normal repairing system.

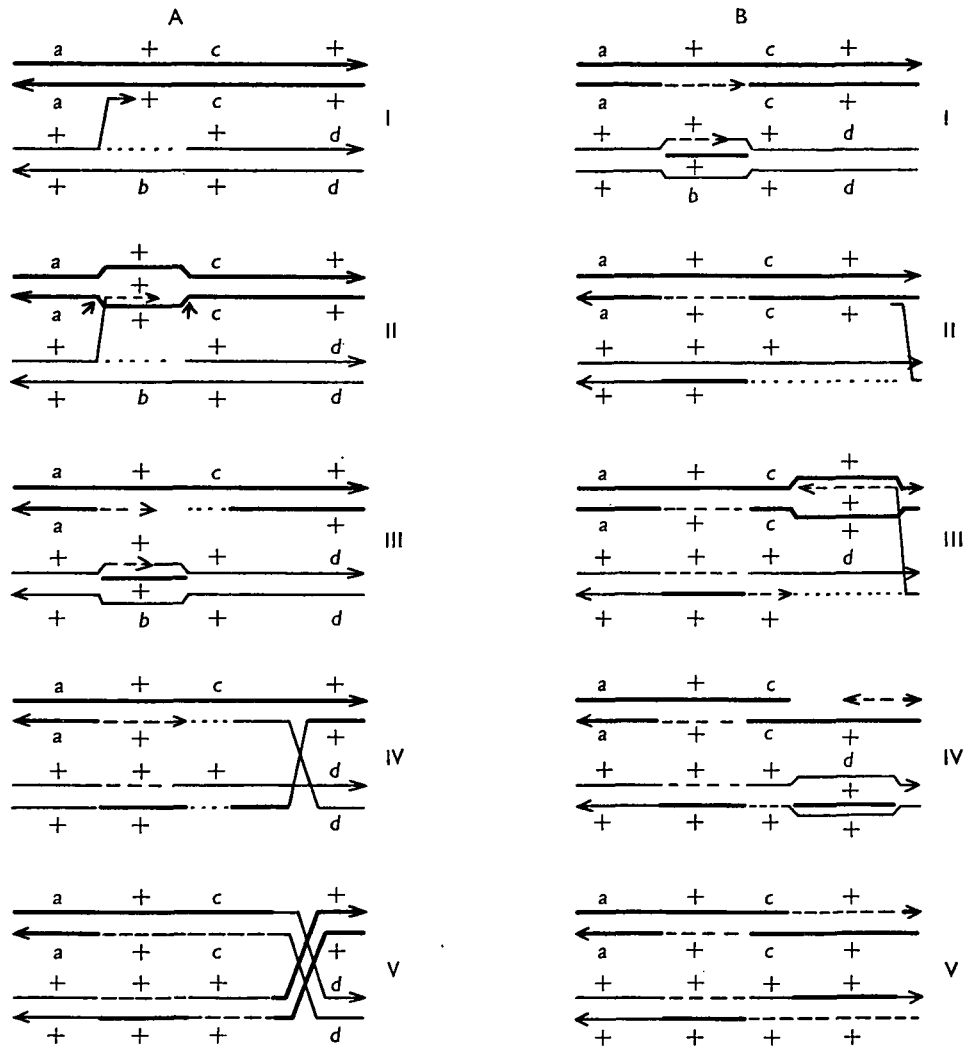

Fig. 3. Interpretation of the origin of ascus types IV(A) and V(B) in terms of the donor–recipient hypothesis. See text for explanation. ·····, Degraded DNA; ----, newly synthesized DNA.

As shown in Fig. 3A (IV), at a certain stage of the process nucleotide-chains of the same polarity are broken at correspondingly points in both the chromatids. They can be partly degraded and unwound. After having been separated from their complementary strands they can anneal with corresponding strands of homologous chromatids, giving rise to a configuration identical to that postulated

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by Holliday, which, as in Holliday's model, can lead to crossing-over, provided appropriate chain-breaks occur.

A different possibility is shown in Fig. 3B. Here, for one reason or another, after the integration of a transferred fragment into the recipient chromatid, it is only this chromatid which has at the moment a single-stranded DNA with a free end. This single-stranded DNA interacting with the homologous chromatid may initiate a new round of reactions similar to that shown in Fig. 2A, leading to gene conversion in the adjacent region (Fig. 3B). Consequently associated conversions take place.

The schemes given in Fig. 3 represent hypothetical steps in the formation of ascus types IV and V, but corresponding schemes based on the same principles can be drawn for the III and VI types.

It should be noted here that the hypothesis on which the schemes are drawn is based on two principal assumptions: (*a*) that conversion results from a donor–recipient process, and (*b*) that there can be several rounds of recombination within the same recombinational process. We are quite aware that a number of different models can be elaborated based on these two assumptions.

It would seem that the donor–recipient hypothesis allows us to solve the dilemma of reconciling a generally observed non-reciprocality of gene conversion with its marked association with crossing-over. According to this hypothesis, conversion and conversion-promoted crossing-over occur as separate events occurring, however, within the same recombinational process. It is not clear yet whether all cross-overs are associated with conversion. It should be stressed that in a four-point intragenic cross such as described here, only a part of the multiple recombinational events is detected as they result in wild-type segregants. For instance, crossing-over associated with conversion *201* → + must occur in the *84W–1043* interval to be detected. Similarly, one can observe conversion-promoted second conversion occurring as a separate event. Thus, double-site conversions for a given pair of mutants can fall into one of the three categories: (*a*) co-conversion only, (*b*) co-conversion and associated conversions, (*c*) associated conversion only. Simultaneous conversions observed for the mutant pairs *936–84W* and *201–1043* would thus belong to the second category.

The physical distance between mutant sites might be expected to be a predominant parameter in determining the kind of simultaneous conversions involving these sites. However, the character of the mutant used or the specificity of a given set of mutants and the relative position of the preferential opening points can probably strongly influence the pattern of recombination. This may take place particularly in the case of structural mutants.

Baranowska (1970) observed a correlation not only between conversion and crossing-over, but also between cross-overs: one in a studied locus and another in the adjacent region (i.e. true negative interference). Thus, with the growing amount of data pointing to a possibility of the clustering of various recombinational events, we are returning to the original concept of Pritchard (1960) although in a modified form.

The 4w:4d asci from cross *936* × *1043* resulted from multiple recombinational events, in some of which at least three chromatids must have been involved. There is abundant evidence suggesting that only two chromatids may participate in a simple recombination. Therefore when we find a three-chromatid event, we must postulate at least two simple recombinations. These recombinations appear to be correlated. It could mean that an 'activated' donor chromatid may initiate a new round of recombination with the same homologous chromatid for which it has served as a material donor, or with its sister chromatid. Asci type X represent the second possibility. This degree of freedom in choosing a partner for a second round of recombination is consistent with the concept of multiple recombinational events giving rise to complex recombination patterns. We do not know what proportion of XI type asci resulted from two- and what from three-chromatid recombinational processes.

The donor–recipient hypothesis avoids the need of postulating the correction of mismatched bases, but does not exclude such a possibility. It was proposed in the hypothesis that during repair synthesis of the DNA strand degraded following primary breaks (see Fig. 3A) a homozygous state is reached automatically. There is no reason to suppose that DNA is degraded only after breaks connected with correction of mismatched bases, i.e. secondary breaks as postulated in Holliday's model (Fincham & Holliday, 1970). Whitehouse (1963) assumes that some degradation may take place after primary breaks as well.

Formation of hybrid DNA over shorter or longer fractions of chromatids has, on the other hand, to be postulated also in the donor–recipient hypothesis, and thus such hybrid DNA may include mutant sites. Whether mismatched bases are corrected or not depends most likely on the character of the mutation.

Leblon & Rossignol (1970) found that ascospore-colour mutants in *Ascobolus* induced by nitroso-guanidine (which is believed to induce base substitutions) when crossed with the wild-type gave a higher frequency of postmeiotic segregation than conversion. On the other hand, conversion dominated in acridine ICR 170-induced mutants. This mutagen is believed to cause frame-schift mutations, i.e. deletions or additions. These observations could mean that the repair system in *Ascobolus* is not very efficient, so point mutations can give conversions mainly through resynthesis of the DNA fraction degraded following the primary break, and not through the correction of mismatched bases in hybrid DNA. The structural mutants, on the other hand, give predominantly conversion, since correction of mispairing (or rather non-pairing) can take place also after the formation of hybrid DNA involving the mutant site. In case of structural mutants the structure of conjugated chromatids or of hybrid DNA may be considerably altered. Single-stranded loops may be formed which may be attacked by enzymes acting on single-stranded DNA and homozygotization may take place.

Any model for gene conversion must explain two phenomena: (*a*) the existence of mutants having different frequencies of conversion in opposite directions, and (*b*) 'map expansion'. The former can be explained by assuming that primary breaks occur with different frequency in homologous chromatids, especially in the

case of structural mutants. We assume here, in contrast to the DNA hybrid models, that mutant specificity can play a role not only at the hybrid DNA stage of the recombinational process but also before it. The latter phenomenon, which results, in principle, from co-conversion can be explained by any model postulating an involvement of a segment of DNA in conversion, provided appropriate assumptions are made. This aspect of intragenic recombination has been well worked out only for Holliday's model (Fincham & Holliday, 1970). We find it difficult, however, to explain our data by this model. Maybe a part of the algebraic formulations elaborated by Fincham and Holliday could be applied in the donor–recipient hypothesis, especially for interpreting simple recombination patterns. This would require, however, some additional assumptions; for instance, that a fragment of DNA strand which is transferred from the donor chromatid can be shorter than the fragment degraded in the recipient chromatid. Such an assumption seems quite reasonable. It seems, however, that very speculative considerations on this problem are of limited value before basic features of the recombination mechanism(s) in eukariotes will be established.

It is evident that Boon and Zinder's model can be used as well to explain the data given in this paper provided that some additional, minor postulates are advanced. To apply Stahl's model (1969) one must postulate an increased number of cross-overs within sex-circles. Donor–recipient-type models seem, however, to account for various aspects of gene conversion better than does the Stahl's model; for instance, for the asymmetry of conversion in two directions observed in the case of many mutants.

Like all other models of recombination, this one is difficult to check experimentally. One important feature, however, is likely to be tested experimentally, i.e. the initiation of conversion by a single break in one chromatid only. If this general assumption is true (apart from the concrete proposal given here), stimulation of chain breaks occurring in one chromosome – for instance, by its heavy labelling with ^{32}P prior to meiosis – should change the frequency of conversion in one direction more than in the opposite, i.e. from mutant allele to the wild-type or vice versa, depending on which chromosome was labelled. Yeast seems to be, among fungi, the most convenient material for such an experimental approach.

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