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ANOTHER TWO GENES CONTROLLING MITOTIC INTRAGENIC RECOMBINATION AND RECOVERY FROM UV DAMAGE IN *ASPERGILLUS NIDULANS*

IV. GENETIC ANALYSIS OF MITOTIC INTRAGENIC RECOMBINANTS FROM *uvs⁺/uvs⁺*, *uvsD/uvsD* AND *uvsE/uvsE* DIPLOIDS

J. J. H. FORTUIN

Genetisch Instituut der Rijksuniversiteit, Opaalweg 20, Utrecht (The Netherlands)

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SUMMARY

This paper presents the results of a genetic analysis of a number of spontaneous mitotic *p*-aminobenzoic acid-independent recombinants from *uvs⁺/uvs⁺*, *uvsD53/uvsD53* and *uvsE82/uvsE82* diploids that are heteroallelic at the *pabaA* locus. Intragenic recombination in each of the three strains is largely nonreciprocal and may usually result from gene conversion. Gene conversion may be strongly correlated with crossing-over between outside markers in the *uvsE82/uvsE82* diploid. Conversion shows no polarity in the *uvs⁺/uvs⁺* diploid; in the *uvsE82/uvsE82* diploid there is almost no conversion of the distal *pabaA* allele; the proximal allele probably converts more often in *uvsE82/uvsE82* but this is obscured by the high back-mutation rate of the proximal allele. Whether there is any polarity of gene conversion in the *uvsD53/uvsD53* diploid cannot be inferred from the results. More than half of the recombinants from the *uvsD53/uvsD53* diploid have an apparently complex origin and may have arisen by double exchanges or by second-order recombination. No such complex recombinants have arisen from the *uvsE82/uvsE82* diploid and their frequency among recombinants from the *uvs⁺/uvs⁺* strain is 16%. The frequency of appearance of these complex recombinants in our strains seems to be correlated with the frequency of intragenic recombination in the particular strains, as influenced by the *uvs* allele they bear.

INTRODUCTION

Models of recombination in fungi have to account for phenomena like nonreciprocity and polarity of recombination, which are particularly known from interallelic crosses. Meiotic and mitotic interallelic recombination in the *pabaA* gene of *Aspergillus nidulans* has been studied extensively[13,15]. Nonreciprocity of mitotic

Abbreviations: CM, complete medium; PABA, *p*-aminobenzoic acid; PFP, *p*-fluorophenylalanine.

recombination in the *pabaA* gene has been deduced from the observation that double mutant strands were present in only a minority of the PABA-independent recombinants obtained from diploids heteroallelic at the *pabaA* locus[13]. The polarity of mitotic recombination in the *pabaA* gene manifested itself in the fact that *paba*+ strands with the outside-marker configuration accompanying the distal *pabaA* allele in the parental strain were more frequent than were *paba*+ strands with the other parental type of outside-marker arrangement[13].

A workable and attractive model of fungal recombination has been developed by HOLLIDAY[6,7]. In his model (as in another model of fungal recombination[17]) nonreciprocal recombination is considered to result from gene conversion. Gene conversion and its apparent polarity are explained as follows. Enzymatically induced single-strand breaks occur at fixed sites (recombinators) in the DNA of homologous chromatids. The broken strands unwind and may reunite to form a single-stranded half-chiasma. This half-chiasma is resolved by secondary breaks, either in the strands forming the half-chiasma or in the sister strands. In the former case a region of hybrid DNA is formed without crossing-over between markers to the left and right of the hybrid region; in the latter case the formation of hydrid DNA is associated with crossing-over between the outside markers. If the region of hybrid DNA spans a mutant site, mispairing of bases ensues. Gene conversion may follow, owing to excision of a nucleotide stretch spanning the site of mispairing, and subsequent repair replication. The polarity of gene conversion is taken to depend on the distance between the mutant sites and the recombinator, the site lying nearer to the recombinator having a better chance of being included in a region of hybrid DNA.

When this model is applied to mitotic recombination in the *pabaA* gene of *Aspergillus nidulans*, the following specifications can be made. The recombinator that is more frequently involved in mitotic recombination in the *pabaA* gene lies distal to the *pabaA* gene; therefore hybrid DNA will more frequently enter the *pabaA* gene from the distal end[6]. These assumptions are based upon the direction of the polarity observed in mitotic recombination in the *pabaA* gene[13]. It must, however, be borne in mind that the conversion frequency of a particular mutation may not only depend upon the frequency of its being included in a stretch of hybrid DNA but also upon its chemical properties so that polarity of gene conversion may also be due to the difference in chemical properties between the two mutations in the cross[1,8,11].

This paper presents the genetic analysis of spontaneous mitotic intragenic recombinants from *uvs*+/*uvs*+, *uvsD53*/*uvsD53* and *uvsE82*/*uvsE82* diploids heteroallelic at the *pabaA* locus. We were interested in learning the pattern of recombination in these diploids in the hope of gaining some deeper insight into the function of the *uvsD* and *uvsE* genes. In two previous papers[3,4] we reported on the UV sensitivity of the *uvsD* and *E* mutants, and the enhancement and decrease of spontaneous mitotic intragenic recombination in *uvsD53*/*uvsD53* and *uvsE82*/*uvsE82* diploids, respectively. It was suggested that the *uvsE* gene is involved in recombination repair of UV-damaged DNA and that the *uvsD* gene is involved in excision of UV-induced lesions from the DNA. In a third paper[5] evidence was presented supporting the latter supposition.

Genetic analyses of recombinants from strains that are abnormal in recombination have been made as yet only by LOW[12]. He used *recA* and *recB* strains of *Escherichia coli* and found that *recA* mutants do not give rise to recombinants in the proper

sense and that the recombinants formed in a *recB* mutant show a pattern of exchanges that is quite similar to that found for recombinants from a *rec*+ strain. LOW has concluded that some step in recombinant production, which either allows or prevents the formation of a finished recombinant cell with a normal number of genetic exchanges, is defective in *recB* cells. It may be that our *uvsE* mutant is defective in a similar function, and that the pattern of recombinants that arise from the *uvsE/uvsE* diploid will be like that found for the *uvs*+/*uvs*+ strain. It should be noted that we examined the results of intragenic recombination events, and LOW the results of intergenic exchanges.

No analyses of recombinants have been carried out for strains that show enhanced recombination frequencies. The *uvs1/uvs1* strain of *Ustilago maydis*, which belongs to the latter category, has been suggested by HOLLIDAY[7,8] to be defective in gene conversion and to carry out intragenic recombination by means of crossing-over. The latter process is, according to him, abnormal and leads to chromosome breakage and subsequent deletion. Whether the recombinants obtained from the *uvsD/uvsD* diploid will bear evidence of similar defects cannot be predicted. It may be that the assumed excision defect of the *uvsD* strain only influences the frequency of intragenic recombination in *uvsD/uvsD* diploids but leaves the mode of intragenic recombination, *i.e.* gene conversion, unaffected.

MATERIALS AND METHODS

Strains

The recombinants analysed were obtained from diploids heteroallelic at the *pabaA* locus and homozygous for *uvs*+, *uvsD53* or *uvsE82*. For the genotype of these diploids, see Table I; for their synthesis, see ref. 4.

Media

The media have been described in previous papers[3,4].

Selection and analysis of PABA-independent recombinants

From each of the heteroallelic diploids *uvs*+/*uvs*+, *uvsD53/uvsD53* and *uvsE82/uvsE82* a number of PABA-independent recombinants were collected in the following way. Diploid colonies were grown according to standard procedures[4] on plates of CM, one plate for each recombinant to be selected. Conidia were harvested from the plates and plated in a selective medium without PABA. After 3 days of incubation at 37° one PABA-independent colony was isolated from each plating. In this way it was ascertained that the PABA-independent recombinants isolated had arisen independently of each other. The genetic analysis of the recombinants was as follows. For each recombinant we isolated 5–20 haploid segregants from colonies grown on CM containing PFP (200 mg/l for *uvs*+/*uvs*+ and *uvsE/uvsE* recombinants, and 50–100 mg/l for *uvsD/uvsD* recombinants). These haploids were purified and tested for colour, growth factor requirements and UV sensitivity. Their genotype was used to reconstruct the genotype of the original PABA-independent recombinants with respect to chromosome I. To characterize the *pabaA* allele(s) present on the nonselected chromosome in the original recombinants, suitably marked PABA-requiring haploid segregants were crossed with the tester strains UT 448 (*pabaA125*) and UT 449

TABLE I

GENOTYPES OF PABA-INDEPENDENT RECOMBINANTS ORIGINATING FROM HETEROALLELIC *uvs*⁺/*uvs*⁺, *uvsD53*/*uvsD53* AND *uvsE82*/*uvsE82* DIPLOIDS

Genotype of the parental diploid	*proA1 + + pabaA125 + bi-1* / *+ adF9 pabaA3 + y +*	*pyro-4* / *+*	*uvs-p* / *uvs-q*	
Chromosome	*I*	*IV*	*V*	
Recombinant class No.	*Genotype of the recombinants with respect to chromosome I*	*Numbers of the recombinants obtained from the diploids*		
		uvs⁺/*uvs*⁺	*uvsD*/*uvsD*	*uvsE*/*uvsE*
1	*pro + + + + bi* / *+ ad pabaA3 + y +*	17	4	
2	*pro + + + + bi* / *+ ad pabaA3 pabaA125 + bi*	1	1	
3	*pro + + + + bi* / *+ ad pabaA3 pabaA125 y +*			1
4	*pro + + + + bi* / *+ ad pabaA3 + + bi*		1	
5	*pro + + + + bi* / *+ ad + pabaA125 y +*		1	
6	*pro + + pabaA125 + bi* / *+ ad + + y +*	15		17
7	*pro + pabaA3 + y +* / *+ ad + + y +*		5	
8	*pro + + pabaA125 y +* / *+ ad + + y +*		2	
9	*+ + + pabaA125 + bi* / *+ ad + + y +*	2		
10	*pro + pabaA3 pabaA125 y +* / *+ ad + + y +*		1	
11	*pro + + + y +* / *+ ad pabaA3 + y +*	4	12	9
12	*pro + + + y +* / *+ ad pabaA3 + + bi*	4		1
13	*pro + + + y +* / *+ ad pabaA3 pabaA125 + bi*	1		2

(*pabaA3*). (For the genotype of these strains, see ref. 4.) From the frequency of the PABA-independent progeny from these crosses it was inferred whether *pabaA3* or *pabaA125* or both alleles were present on the nonselected chromosome in the original PABA-independent recombinants.

RESULTS AND DISCUSSION

About 50 PABA-independent isolates were obtained from each of the heteroallelic diploid strains *uvs*⁺/*uvs*⁺, *uvsD53*/*uvsD53* (*uvsD*/*uvsD*) and *uvsE82*/*uvsE82* (*uvsE*/*uvsE*). All isolates were of independent origin and their genotypes were deter-

TABLE I (Continued)

Genotype of the parental diploid	*proA1 + + pabaA125 + bi-1*	*pyro-4*	*uvs-p*
	+ adF9 pabaA3 + y +	*+*	*uvs-q*
Chromosome	*I*	*IV*	*V*

Recombinant class No.	*Genotype of the recombinants with respect to chromosome I*	*Numbers of the recombinants obtained from the diploids*		
		uvs⁺/uvs⁺	*uvsD/uvsD*	*uvsE/uvsE*
14	*pro + + + y +* / *+ ad + pabaA125 y +*	2		
15	*pro + + + y +* / *+ ad + pabaA125 + bi*			1
16	*pro + + pabaA125 + bi* / *+ ad + + + bi*		2	1
17	*pro + pabaA3 + y +* / *+ ad + + + bi*	1		
18	*pro + pabaA3 pabaA125 + bi* / *+ ad + + + bi*		1	
19	*pro + pabaA3 + + bi* / *+ ad + + + bi*	1		
20	*pro ad + + + bi* / *+ ad pabaA3 + + bi*		1	
21	*pro ad + + y +* / *+ + pabaA3 + y +*		1	
22	*pro + + pabaA125 + bi* / *+ + + + y +*			1
23	*pro + + + y +* / *+ ad + + y +*	2	2	
24	*pro + + + y +* / *+ + + + y +*		3	
25	*pro + + + + bi* / *+ + + + + bi*		1	
26	*pro ad + + y +* / *+ ad + + y +*		1	
Total		*50*	*39*	*33*

mined as described in the section on MATERIALS AND METHODS. Some of the isolates were found to be haploids, others could not completely be analysed. These strains were omitted from the list of the results given in Table I.

Do the results give a reliable picture of recombination in the uvs^+/uvs^+, *uvsD/uvsD* and *uvsE/uvsE* diploids? PFP, which was used to haploidize the diploid recombinants, is considered not to affect the arrangement of linked markers[13]. The picture of recombination in *uvsE/uvsE* may have been obscured, however, by reverse mutation of *pabaA3* because PABA-independent segregants from *uvsE/uvsE* arose with a frequency ($3 \cdot 10^{-8}$ to $3 \cdot 10^{-7}$) that was very close to the frequency of PABA-independent reverse mutants in a haploid *uvsE82* strain carrying *pabaA3* ($3–6 \cdot 10^{-8}$) (ref. 4). The possible consequences of this fact will be considered later on. The picture

of recombination in *uvsD/uvsD* may have been confused by second-order recombination. Upon haploidization of the recombinants from *uvsD/uvsD*, we often found more than two types of haploid segregants with respect to chromosome I, though two types were always clearly in the majority. The majority types were held to represent the original constitution of the chromosome pair I. Mostly the minority types of haploid segregants could be explained as having arisen from the original recombinant by second-order segregation. This matter will also be dealt with more extensively later on. Upon haploidization of the recombinants from the uvs^{+}/uvs^{+} and *uvsE/uvsE* diploids, only two types of haploid segregants were usually found.

We will interpret our results on the basis of the HOLLIDAY recombination model outlined in the INTRODUCTION. As a first step in our analysis we group together the various classes of recombinants indicated in Table I into some larger groups on the basis of the character and the number of the exchanges required to explain the various recombinants. As one exchange we will consider the reciprocal formation of a region of hybrid DNA in homologous chromatids, which includes one or both of the mutant sites in the *pabaA* gene, and the formation of which results in conversion of one or both of the *pabaA* alleles with or without crossing-over between the outside markers. As outside markers *y* and *proA* were chosen. The *adF* marker is less suitable because *adF* alleles have sometimes been found to simultaneously convert with *pabaA* alleles[14].

We distinguish the following three types of recombinants: (*a*) Recombinants that may have arisen from a single exchange, resulting in conversion of a *pabaA* allele without crossing-over between the outside markers (the classes 1, 3, 5, 6 and 22 of Table I; the recombinant of class 22 may have arisen by simultaneous conversion of *adF9* and *pabaA3*). (*b*) Recombinants that may have their origin in a single exchange, resulting in conversion with crossing-over (the classes 11–13 and 15–17 of Table I). (*c*) Recombinants that may have arisen from two simultaneous exchanges in which at least three strands participated, or from successive exchanges leading to second-order

TABLE II

CLASSIFICATION OF PABA-INDEPENDENT RECOMBINANTS FROM THE HETEROALLELIC DIPLOIDS uvs^{+}/uvs^{+}, *uvsD53/uvsD53* AND *uvsE82/uvsE82*

Origin of the recombinants	*Type[a] of the recombinants*	*Classes No.*	*Number of the recombinants*	*Percentage of total*
uvs^{+}/uvs^{+}	*a*	1,6	32	64
	b	11–13, 17	10	20
	c	2, 9, 14, 19, 23	8	16
Total			50	100
uvsD/uvsD	*a*	1, 5	5	13
	b	11, 16	14	36
	c	2, 4, 7, 8, 10, 18, 20, 21, 23–26	20	51
Total			39	100
uvsE/uvsE	*a*	3, 6, 22	19	58
	b	11–13, 15, 16	14	42
	c	—	—	—
Total			33	100

[a] Type *a*, recombinants arisen by conversion without crossing-over between outside markers; type *b*, recombinants arisen by conversion and/or crossing-over between outside markers; type *c*, recombinants arisen by multiple exchanges.

TABLE III

PERCENTAGES OF *paba*$^+$ STRANDS WITH PARENTAL AND RECOMBINANT OUTSIDE-MARKER COMBINATIONS FOR THE DIPLOIDS *uvs*$^+$/*uvs*$^+$, *uvsD53*/*uvsD53* AND *uvsE82*/*uvsE82*

Outside-marker class	*uvs*$^+$/*uvs*$^{+a}$ (%)	*uvsD*/*uvsD*^{a} (%)	*uvsE*/*uvsE* (with class 6) (%)	*uvsE*/*uvsE* (without class 6) (%)
pro y$^+$ (PT I)	35	20	3	6
pro$^+$ *y* (PT II)	36	30	55	6
pro y (RT I)	25	41	39	82
pro$^+$ *y*$^+$ (RT II)	4	9	3	6

[a] Both *paba*$^+$ strands of each recombinant of the classes 23–26 have been counted.

segregation (the classes 2, 4, 7–10, 14, 18–21 and 23–26 of Table I). (Class 9 may result from two exchanges in the same pair of chromatids.)

A classification of the recombinants obtained from the diploids *uvs*$^+$/*uvs*$^+$, *uvsD*/*uvsD* and *uvsE*/*uvsE* according to the three types *a*, *b* and *c* is given in Table II. Table III summarizes the outside-marker combinations found on the *paba*$^+$ strands of the various recombinants.

Recombinants from the uvs$^+$/*uvs*$^+$ *diploid*

In only 2 out of 50 PABA-independent recombinants from the *uvs*$^+$/*uvs*$^+$ diploid the nonselected strand carried the *paba* double mutant. Thus recombination in the *pabaA* gene in the *uvs*$^+$/*uvs*$^+$ diploid was usually nonreciprocal and was probably largely due to gene conversion. The latter cannot strictly be proved however, but for the recombinants of the classes 12 and 17. The latter cannot be explained by the reciprocal formation of hybrid DNA and segregation of the hybrid strands without correction of mismatched base pairs.

From Table II it may be inferred that gene conversion in most cases was not associated with crossing-over. PUTRAMENT[13] and JANSEN[10] who also studied mitotic recombination in the *pabaA* gene of *Aspergillus nidulans* have obtained similar results.

Table III shows that the frequencies of the two types of *paba*$^+$ strands with a parental arrangement of the outside markers are almost equal. This indicates that recombination between *pabaA3* and *pabaA125* in the *uvs*$^+$/*uvs*$^+$ diploid was almost nonpolarized. (This is in contrast to what PUTRAMENT[13] and JANSEN[10] found, using other *pabaA* heteroallelic combinations.)

The presence of recombinants of type *c* (Table II) shows that in our experiments some second-order segregation may have occurred in the *uvs*$^+$/*uvs*$^+$ diploid or that in some cases double exchanges occurred simultaneously. The frequency of recombinants of type *c* from our *uvs*$^+$/*uvs*$^+$ diploid is almost equal to the frequency of recombinants of type *c* among the recombinants isolated by PUTRAMENT[13] (12% in diploid D20; her classes 6, 7, 9, 23 and 25). (JANSEN[10] found only 1 recombinant of type *c*, 2%, his class 8. This may be a consequence of the fact that there was no *adF* marker in his diploids but it could also well be due to the fact that his technique of isolating recombinants was unfavourable to the occurrence of second-order segregation.)

Recombinants from the uvsD/*uvsD diploid*

First, the problem of second-order recombination has to be considered. In analysing the recombinants from the *uvsD*/*uvsD* diploid with respect to chromosome

I, haploid segregants were found that often showed more than two different genotypes although two types were always in the majority. The majority types were used to reconstruct the genetic constitution of the chromosome pair I of the original isolate, and usually the minority types were such that they could well result from second-order recombination in the isolate assumed to be the original one during the analysing procedures. For instance, in analysing class 11 recombinants sometimes a haploid "*y*" and once a haploid "*pro ad paba y*" segregant was noted (crossing-over between *proA* and *adF*); in analysing recombinants of class 1 haploid "*pro*" (crossing-over between *y* and *bi-1*), "*pro y*" (crossing-over between *pabaA* and *y*) and "*pro ad paba y*" (crossing-over between *proA* and *adF*) segregants were observed; and in analysing recombinants of class 7 haploid "*pro ad y*" (crossing-over between *proA* and *adF*) segregants were met with. In analysing the 5 terminally heterozygous recombinants from the *uvsD*/*uvsD* diploid (the classes 1 and 5), we also found diploid segregants that were "*y*" or "*bi*" and we found "*paba y*" diploid segregants in analysing class 7, 8, 10 and 11 recombinants. All these findings indicate that second-order recombination occurred in the recombinant isolates. (This was not unexpected, since the frequency of recombination in *uvsD*/*uvsD* is a good deal higher than normal[4]. The data just mentioned may indeed support an earlier assumption[4] that not only recombination in the *pabaA* gene but recombination everywhere in the genome of *uvsD*/*uvsD* is more frequent than normal.)

It must be considered that the occurrence of extra exchanges in the recombinant isolates during the analysing procedures may in some cases have led to faulty reconstructions of the genotype of the recombinant isolates. Also, the recombinant isolates themselves may already have been the product of different consecutive recombination events. This means that the pattern of recombinants isolated from the *uvsD*/*uvsD* diploid may not quite reflect the pattern of recombination in the *uvsD*/*uvsD* diploid.

Second-order mitotic crossing-over in the right arm of chromosome I, if occurring in a terminally heterozygous PABA-independent sector of a growing *uvsD*/*uvsD* colony, in half of the cases will lead to homozygosity for one of the distal markers, or for *paba* or $paba^{+}$ and one of the distal markers, and so on. Recombination between *pabaA3* and *pabaA125* in a sector that is already homozygous for *y* or *bi-1* in half of the cases will also lead to a "*y* "or "*bi*" PABA-independent recombinant. Thus a high frequency of PABA-independent recombinants homozygous for a distal marker or for $paba^{+}$ (or *paba*), and a distal marker would be indicative of a high incidence of second-order recombination in *uvsD*/*uvsD* diploids. We found indeed a much higher frequency of recombinants homozygous for *y* or *bi-1* for *uvsD*/ *uvsD* than for uvs^{+}/uvs^{+} (87% *vs.* 20%). We also obtained more recombinants that were homozygous for both $pabaA3^{+}$ and $pabaA125^{+}$ and a distal allele (the classes 23–26) from *uvsD*/*uvsD* rather than from uvs^{+}/uvs^{+} (18% *vs.* 4%). Recombinants that as a result of a second-order recombination event were homozygous for one of the *pabaA* alleles were not found, but this is small wonder since it was precluded by the procedure of selection of the recombinants.

Although the frequent occurrence of second-order recombination may have led to a disturbance of the outside-marker configuration of the PABA-independent strands, there are few reasons to expect that the occurrence of double mutant strands has been affected. From the low frequency of *paba* double mutant strands (7.7%) it may there-

fore be concluded that most of the PABA-independent recombinants from strain *uvsD/uvsD* did not arise by crossing-over between *pabaA3* and *pabaA125*. (This is in contrast to what HOLLIDAY[7,8] suggested could be found in the *uvs1/uvs1* diploid of *Ustilago maydis*. According to him intragenic recombination in the *uvs1/uvs1* strain was almost exclusively due to mitotic crossing-over.) The recombinants from our *uvsD/uvsD* diploid probably result from gene conversion, although it cannot strictly be proved for all cases that actual correction of mismatched base pairs was involved. The presence of recombinants from the *uvsD/uvsD* diploid in class 16 indicates, however, that mismatched base pairs in hybrid DNA can be corrected in *uvsD/uvsD* because this class cannot be explained without correction of mismatched base pairs. The indication that the *uvsD* mutant is capable of gene conversion, while assumedly excision-deficient[3], is of interest in the light of the finding of SPATZ AND TRAUTNER[16] that in *Bacillus subtilis* transfected with SPP1 DNA the efficiency of gene conversion is not affected by a *hcr*$^-$ mutation.

It cannot, with some certainty, be decided from the results whether conversion in the *uvsD/uvsD* diploid was polarized. Because second-order recombination may have disarranged the outside-marker configuration in the *paba*$^+$ strands, the frequencies with which *paba*$^+$ strands with PT I and PT II outside-marker configurations were found (Table III) may not reflect the frequencies with which gene conversion occurred at, respectively, the *pabaA3* and *pabaA125* sites.

Among the recombinants from *uvsD/uvsD* those of type *c* are the most frequent. These recombinants may have arisen from simultaneous exchanges (3- or 4-strand doubles) or from a few successive recombination events, *e.g.* second-order crossing-over in an already PABA-independent sector or conversion at the *pabaA* locus in a sector in which crossing-over had already taken place distal to the *pabaA* locus. It is impossible to decide which possibility holds for a given recombinant. The enhanced frequency with which recombinants of type *c* arise in *uvsD/uvsD*, if it is compared to *uvs*$^+$/*uvs*$^+$, is probably simply due to the fact that the frequency of recombination in *uvsD/uvsD* is much higher[4] (about 25 times) than in *uvs*$^+$/*uvs*$^+$. In this connection it is noteworthy that no type *c* recombinants have been found to arise from *uvsE/uvsE*, which has a very low frequency of mitotic intragenic recombination[4].

Table II shows that the *uvsD/uvsD* diploid yields more recombinants of type *b* (recombinants that may have arisen from a single exchange involving crossing-over) than of type *a* (recombinants that may have originated from a single exchange not involving crossing-over). This is the reverse of what was found for the *uvs*$^+$/*uvs*$^+$ diploid, but it may not indicate that conversion in *uvsD/uvsD* is more often than not associated with crossing-over. There may be more reason to believe that the fall in the frequency of type *a* recombinants in *uvsD/uvsD* compared to *uvs*$^+$/*uvs*$^+$, and the excess of type *b* over type *a* recombinants in *uvsD/uvsD*, is due to second-order crossing-over. If this is true, then one has also to assume that virtually all recombinants isolated from the *uvsD/uvsD* strain are a result of second-order events. For only in such a way could one explain the loss of type *a* recombinants (from 64% in *uvs*$^+$/*uvs*$^+$ to 13% in *uvsD/uvsD*) and also the rise in the frequency of terminally homozygous recombinants in *uvsD/uvsD* compared to *uvs*$^+$/*uvs*$^+$ (87% and 20%, respectively).

HOLLIDAY[8,9] has suggested that the high frequency of segregation of distal markers in the *uvs1/uvs1* diploid of *Ustilago maydis* (which has a 10 times higher frequency of allelic recombination[7]) is due to the occurrence of terminal deletions.

These deletions are, according to him, a result of breakage in one of the strands that were involved in crossing-over. We have no such evidence. If, in the case of the *uvsD/uvsD* recombinants, the finding of many "*y*" or "*bi*" recombinants were a consequence of much terminal hemizygosity, then one of the two members of the chromosome pair I could be expected to be lethally deleted and not to be recoverable in haploid segregants. Such a case was never encountered.

For the time being, we assume that the excision defect of *uvsD53* favours the occurrence of recombinagenic lesions in *uvsD/uvsD* and in that way stimulates recombination (by gene conversion) and enhances the chance of double exchanges and/or second-order segregation.

Recombinants from the uvsE/uvsE diploid

It may be recalled that the frequency of PABA-independent recombinants from the *uvsE/uvsE* diploid is so low that it is almost in the range of frequency of the reverse mutation of *pabaA3* in a *uvsE82* strain[4]. Some *uvsE/uvsE* colonies in class 6 of Table I may therefore have arisen by back-mutation of *pabaA3* rather than by recombination. The other PABA-independent colonies obtained from the *uvsE/uvsE* diploid cannot, however, be attributed to mutation and must have arisen from recombination. Apparently, there is still some recombination activity in *uvsE/uvsE*. The recombinant of class 22 has probably arisen, as was assumed, from simultaneous conversion of *pabaA3* and *adF9* (simultaneous conversion of *pabaA* and *adF* alleles has been found before by PUTRAMENT[14]). The finding of the recombinant of class 22 may therefore indicate that not all members of class 6 originate from mutation but that conversion of *pabaA3* occurs in *uvsE/uvsE*.

The frequency of recombinants from *uvsE/uvsE* with double mutant strands (9% or 19% depending on whether class 6 is included in the total of the results or not) shows that recombination between the alleles *pabaA3* and *pabaA125* in *uvsE/uvsE* is often nonreciprocal. It may therefore largely be due to gene conversion. The finding of recombinants from *uvsE/uvsE* that belong to the classes 15 and 16 indicates that the process of gene conversion was intact in *uvsE/uvsE*, for an explanation of the recombinants of the classes 15 and 16 requires actual correction of mismatched base pairs in the hybrid DNA. It is further of interest in this connection, in view of the assumed deficiency of recombination repair[4] in *uvsE82*, that SPATZ AND TRAUTNER[16], who examined gene conversion in *Bacillus subtilis* transfected with heteroduplex SPP1 DNA, found that the efficiency of conversion was not affected by rec^- mutations.

Considering the configuration of the outside markers in the $paba^+$ strands, it appears that between 42% and 88% of the $paba^+$ strands have a recombinant configuration of outside markers (Table III), depending on which part of class 6 is considered to consist of real recombinants. This could indicate that gene conversion in *uvsE/uvsE* is more frequently correlated with crossing-over than in uvs^+/uvs^+ (29%, see Table III). Table II shows the same effect.

We did not find recombinants of *uvsE/uvsE* origin of type *c*. This is interesting and may be explained as being correlated with the very low recombination frequency in this particular diploid[4].

The relative frequencies of $paba^+$ strands with PT I and PT II outside-marker arrangements (Table III) shows that, if at least part of the class 6 recombinants are real recombinants, gene conversion in *uvsE/uvsE* is polarized. In that case gene con-

version at the *pabaA3* site in *uvsE*/*uvsE* is more frequent than at the *pabaA125* site. This is in contrast to what was found for the uvs^+/uvs^+ diploid and the reverse of the results obtained by PUTRAMENT[13] and JANSEN[10].

The function of the *uvsE* gene may be the initiation or the completion of recombinant structures. This may seem to be incompatible with the finding that the pattern of recombination in *uvsE*/*uvsE* differs from that found for uvs^+/uvs^+ (gene conversion may be polarized and there seems to be a preference for conversion associated with crossing-over). It is conceivable, however, that the *uvsE* gene product, presumably a nuclease or a ligase, is part of an enzyme complex controlling recombination, and that a defective *uvsE* gene product not only decreases the activity of the whole complex but also alters the specificity of its action. Another possibility, for which recently indications have been found in *Escherichia coli*[1], is that there exists an alternative pathway for recombination in *Aspergillus nidulans* which, if followed, results in a different pattern of recombinants.

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