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The highly polarized recombination pattern within the methA gene of *Aspergillus nidulans*

Recombination within the methA cistron of *A. nidulans*

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SUMMARY

The recombination between the methA alleles of *Aspergillus nidulans* is very strongly polarized. The mutants can be mapped in respect to each other and in respect to the flanking markers, both on the basis of the relative frequency of one of the P classes and one of the R classes. In all crosses it is the distal mutant which converts predominantly.

In one combination of the mutants crossed the low temperature at which meiosis proceeded increased recombination frequency and modified the recombination pattern.

1. INTRODUCTION

In *Aspergillus nidulans* extensive studies on intragenic recombination were so far carried out on four genes, all situated on the left arm of chromosome I: adE (Pritchard, 1955, 1960), pabaA (Siddiqi, 1962; Siddiqi & Putrament, 1963; Putrament, 1964), adF (Calef, 1957; Martin-Smith, 1961), and lys-51 (Pees, 1965, 1967). In all cases more or less marked polarity in the recombination pattern was observed, but the most frequent class of wild-type recombinants always showed recombinant arrangement of flanking markers. Strikingly different results, however, will be presented here.

2. MATERIAL AND METHODS

The methA mutants studied in the present work were kindly offered us by Professor W. Gajewski and Mrs J. Litwinska. The mutants, isolated following ultraviolet irradiation (Gajewski & Litwinska, 1968), are designated methA17, methA32, methA33, methA34, methA40 and methA59. They will be further referred to by their numbers only: 17, 32, etc. It was found in the present work that mutant 40 showed partial complementation with 32 and 33.

The methA cistron is mapped in the left arm of chromosome II, in the order: AcrA1-methA-w-2-centromere. The distances AcrA1-methA and methA-w-2 obtained in the present work were higher than those reported earlier (Gajewski &

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Litwinska, 1968), and were 25 and 8 meiotic map units respectively. Since it was found in several tests in the course of the present work that the recombination fraction in the AcrA1-w-2 interval was 0·25 (cf. Dorn, 1967), it can be assumed that in this interval the distribution of 2- and 4-strand double exchanges is non-random, 2-strand double exchanges prevailing over 4-strand doubles. This results in an apparent shortening of the interval; in the presence of an additional marker, methA, more two-strand double exchanges are scored, this leading to an apparent lengthening of the interval.

The methA mutants revert spontaneously with a frequency of 1–10 per 10^6 conidia (Gajewski & Litwinska, 1968). To avoid as much as possible scoring of mutational revertants together with the true intragenic recombinants, doubly selective media were used in analyses of all but two crosses (59×17 and AcrA1 40×17 w-2). For instance, the ascospores from a cross

$$\frac{\text{adF9} \quad \text{y} \quad +}{+ \quad + \quad \text{bi-1}} \qquad \frac{+ \quad 17 \quad +}{\text{AcrA1} \quad 40 \quad \text{w-2}} \qquad \frac{+}{\text{nicB8}}$$

were plated on minimal medium (MM) supplemented with biotin, so that only 1/4 of the actual number of the meth$^+$ intragenic recombinants could grow into colonies, while the mutational revertants from conidia and selfed ascospores were eliminated. To estimate the number of viable ascospores of crossed origin in selective platings, the appropriately diluted samples of ascospore suspensions were plated on MM so supplemented that 1/4 of the viable ascospores of crossed origin could grow into colonies. (In the cross presented above MM supplemented with biotin and methionine was used.)

Several crosses were incubated not only at the standard temperature, i.e. 37 °C, but also at 26 or 23 °C to see if temperature at which meiosis takes place influences intragenic recombination. To test the possible influence of increased osmotic pressure on recombination, a number of crosses were carried out on MM supplemented with KCl (a final concentration 0·5 M) at 37 °C. In these conditions the majority of crosses failed to produce sufficient amounts of perithecia, so that only a few could be analysed.

The standard media and techniques used were as described previously (Pontecorvo *et al.* 1953).

3. RESULTS AND DISCUSSION

The results of analyses of crosses involving six methA mutants are presented in Table 1. In all crosses the most numerous was one class with parental arrangement of the flanking markers, Acr$^+$ w-2 in crosses 1 and 4, AcrA1 w$^+$ in crosses 2, 3 and 5, AcrA1 w-2 in cross 6, and Acr$^+$ w$^+$ in the remaining three crosses. Second-frequent was in all crosses one of the classes with recombinant marker arrangement: AcrA1 w-2 in crosses 1 and 4, Acr$^+$ w$^+$ in crosses 2, 3 and 5, Acr$^+$ w-2 in cross 6, and AcrA1 w$^+$ in the remaining three crosses. On the basis of these data and interallelic recombination frequencies it was possible to establish the most likely sequence of all the mutants except 33. An additional cross which was not

included in Table 1, AcrA1 59 × 33, permitted the tentative placement of 33 to the left from 59. The map of the mutants is presented in Fig. 1.

The AcrA1–methA interval is rather long, and additional exchanges, independent of the intragenic recombinational events must have occurred there (for detailed discussion see Whitehouse & Hastings, 1965). They changed the proportions of P^D and R^1 classes: a number of intragenic recombinational events in which no

Table 1. *Frequencies and pattern of recombination between the MethA alleles*

Cross	Methionine prototrophs: Number	Recombinants per 10^5 ascospores	Classification of methionine prototrophs (per cent of total no. of recombinants) P^D	P^P	R^1	R^2
1. Acr 59 × 34w	120	0·2 ± 0·1	70·0	4·2	25·0	0·8
2. Acr 59 × 17w	261	17·0 ± 0·8	75·5	2·5	21·5	0·5
3. Acr 32 × 17w	245	11·8 ± 0·7	70·2	4·9	22·4	2·5
4. Acr 32 × 34w	22	0·04 ± 0·2	63·7	9·0	27·3	0·0
5. Acr 40 × 17w	387	1·6 ± 0·9	71·8	3·1	24·3	0·8
6. Acr 40w × 17	763	3·4 ± 0·2	72·1	3·4	23·5	1·0
7. Acr 40w × 32	137	4·6 ± 0·3	69·3	6·6	23·4	0·7
8. Acr 40w × 33	577	5·2 ± 0·2	64·3	5·4	28·4	1·9
9. Acr 40w × 34	287	4·0 ± 0·2	66·2	4·9	25·8	3·1

P^D, parental combination of flanking markers with distal methA allele; P^P, parental combination of flanking markers with proximal methA allele; R^1, majority non-parental combination of flanking markers indicating the order of mutants shown on Fig. 1; R^2, minority non-parental combination of flanking markers.

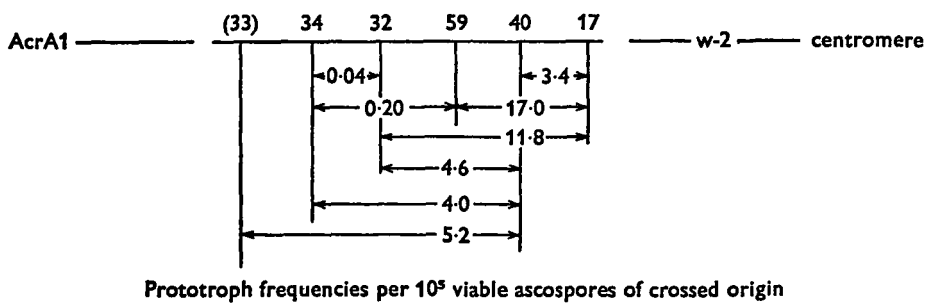

Fig. 1. The map of the methA alleles.

exchanges of flanking markers took place and which should be classified as P^D were actually classified as R^1 owing to independent exchanges in the AcrA1–methA interval. Since the R^1 class is three times less frequent than the P^D class, the additional exchanges which re-established parental marker arrangements in the actual R^1 recombinants must have been less numerous. Thus the estimated proportion of the P^D class of the recombinants must be considered as lower than the actual frequency of the intragenic recombinational events not accompanied by exchanges of the flanking markers.

Studies on intragenic recombination in the presence of flanking markers, by means of half-tetrad analysis, carried out on *Aspergillus* (Putrament, 1964), and tetrad analysis carried out on yeast (Fogel & Hurst, 1967), *Podospora anserina* (Marcou, 1969) and *Ascobolus* (Baranowska, 1970) show that recombinant marker arrangement in wild-type strands does not mean that the intragenic event was reciprocal. In fact most of such recombinants are due to the events described as inexactly reciprocal exchanges or conversions accompanied by crossing over. In yeast the intragenic recombination was distinctly polarized, the distal mutant converting more frequently than the proximal one. The overwhelming majority of convertants with recombinant marker arrangements were due to conversions of the distal mutants.

The data obtained on *Podospora* and *Ascobolus* show also that the wild-type recombinant strand with parental marker arrangement may result not only from simple conversion, but also from the events described as double crossing over or double conversion since in some tetrads there is a wild-type recombinant with marker arrangement like that of one of the parental strains, and a double mutant with marker arrangement such as that of the other parental strain. Such events, however, seem to be infrequent.

Thus, in single-strand analysis the overwhelming majority of selected intragenic recombinants having parental marker arrangements can be considered as being due to single conversion of the mutant introduced into a cross with these markers. On the other hand, the frequencies of the P classes indicate underestimated values of the true frequencies of non-reciprocal exchanges which took place within the gene, since some of such events gave rise to wild-type strands with recombinant marker arrangements and accordingly were classified as R classes.

In the present work, therefore, the actual proportion of the meth$^+$ recombinants which resulted from conversion of a distal or left-hand mutant was most probably still higher than the per cent of the P^D classes actually found (Table 1). The polarization found here is, thus, extremely strong, comparable to that observed in the 46 gene of *Ascobolus* (Rizet, Lissouba & Mousseau, 1960; Lissouba, 1960; Lissouba *et al.* 1962; Rossignol, 1964).

For the reasons discussed above it seems possible that, in the four other *Aspergillus* genes studied in detail, non-reciprocal intragenic exchanges were in fact much more frequent than can be judged by the proportions of the R^1 and two P classes of selected recombinants. But then the obvious question arises, why within some genes the simplest events, i.e. conversion without additional marker exchange, are most frequent, while in other genes more complex events predominate. These differences cannot be attributed entirely to mutant specificity because almost all *Aspergillus* mutants used for fine gene structure analysis were induced with ultraviolet and thus cannot differ strikingly in character. Yet the behaviour of random samples of adE, pabaA, adF and lys-51 alleles was similar, while a random sample of the methA alleles behaved quite differently.

Murray (1968, 1969) found in *Neurospora* that neither translocation nor gross inversion changed the intensity and direction of polarity (with respect to flanking

markers) of recombination within the me-2 and me-7 genes. She suggests that polarity is intrinsic to the region in which recombinants are being selected. The results presented here are not in disagreement with this suggestion.

Table 2. *The influence of temperature and increased osmotic pressure at which meiosis proceeded on recombination within the methA gene*

Cross		Methionine prototrophs: Number	Methionine prototrophs: Recombinants per 10^5 ascospores	Classification of methionine prototrophs (per cent of total no. of recombinants): P^D	P^P	R^1	R^2
Acr 32 × 17w:	control	245	11·8 ± 0·7	70·2	2·5	22·4	4·9
	26 °C	615	13·7 ± 0·5	70·0	2·1	25·9	2·0
	KCl	123	18·6 ± 0·2	58·5	6·5	22·0	13·0
Acr 40w × 33:	control	577	5·2 ± 0·2	64·3	5·4	28·4	1·9
	23 °C	247	8·2 ± 0·2	63·2	4·9	28·3	3·6
Acr 40w × 34:	control	287	4·0 ± 0·2	66·2	4·9	25·8	3·1
	23 °C	189	3·5 ± 0·3	65·7	7·9	25·4	1·2
Acr 40w × 17:	control	763	3·4 ± 0·2	72·1	3·4	23·5	1·0
	23 °C	564	8·3 ± 0·4	59·0	7·1	32·3	1·6
	KCl	364	5·9 ± 0·8	68·9	3·8	26·0	1·3

The attempts to modify the intragenic recombination pattern are presented in Table 2. In the cross 32 × 17 both the frequency and pattern of recombination were identical, irrespective of the temperature at which meiosis proceeded. Increased osmotic pressure of the crossing medium led to a slight increase of the total recombination frequency, and decrease of the P^D class of recombinants (on the borderline of statistical significance). But the cross was partly sterile, the sample analysed was small, so that the result does not seem to be reliable.

Crosses 33 × 40 and 34 × 40 gave practically identical results, irrespective of the temperature at which meiosis proceeded.

In the cross 40 × 17 low temperature led to an increased recombination frequency, and statistically significant modifications in the proportions of the P and R classes of recombinants. The same tendency, although much less pronounced, was observed in this cross performed on KCl-containing medium.

The increased proportion of the R^1 class in the cross 40 × 17 incubated at 23 °C could be due either to modifications of the intragenic recombinational events, or to increased frequencies of independent exchanges in the AcrA1–methA interval. The second assumption was checked by testing the recombination frequency in the AcrA1-w-2 interval in a random sample of ascospores. It turned out that at lower temperature the recombination in this interval was 20–24 % in different samples tested, i.e. it was within the limits of the standard value. Thus it seems justified to assume that the temperature-dependent differences in the distribution of spanning markers in the 40 × 17 cross were due to modifications in the intragenic recombinational events. Crosses 33 × 40, 34 × 40 and 40 × 17 presented in

Table 2 were isogenic, mutants 17, 33 and 34 being isolated from the same adF9y strain (Gajewski & Litwinska, 1968). Thus one can further assume that it was the particular configuration of the 40 and 17 mutants which was sensitive to the lowered temperature at which meiosis proceeded.

The influence of temperature on the frequency and pattern of intragenic recombination has been reported previously (Stadler, 1959; Lissouba, 1960; Boucharenc, Mousseau & Rossignol, 1966; Lamb, 1968). The possible implications of this phenomenon will be discussed elsewhere (A. Putrament, in preparation).

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