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The fine genetic structure of the *paba1* region of *Aspergillus nidulans*

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1. INTRODUCTION

Intragenic recombination has been shown to occur in a number of micro-organisms. In *Drosophila*, where recombination between alleles was first detected (Lewis, 1945), a number of similar, though often variously interpreted, cases are now known. The main outcome of this development has been a considerable clarification of our ideas about the genetic material in relation to its structure and function (see review by Pontecorvo, 1958). Structurally, recombination analysis reveals the gene to be a linear array of elementary units, the mutational sites. At least in one case, that of the rII region of bacteriophage T4, where 308 sites out of an estimated total of 428 have already been mapped (Benzer, 1961), recombination analysis appears to be within reach of the attainable limits of resolution.

The present work deals with the topography of the *paba1* region and the modalities of recombination in this region. The latter is of interest as it has been maintained by several authors that allelic recombination is independent of crossing-over (Roman & Jacob, 1958; Lindegren, 1961). The present work also revealed the occurrence of strong polarity in negative interference. This has been investigated further and will be discussed elsewhere (Siddiqi & Putrament, 1961). The *paba1* region was chosen for the purposes of the present work for two reasons. Firstly, this region is situated in a favourable position on chromosome I. It is closely linked to *ad9* on the proximal side and not too far from the nearest distal marker *y*. Secondly, crosses between *paba* alleles are normally fertile (Roper, unpublished). In view of the difficulties due to sterility of crosses encountered in the mapping of *ad* loci (Pritchard, 1955; Martin-Smith, unpublished), this appeared to offer a distinct advantage.

2. MATERIAL AND PRELIMINARY EXPERIMENTS

For details of the origin of strains and the techniques of genetic analysis in *Aspergillus nidulans*, reference should be made to Pontecorvo, Roper, Hemmons, Macdonald & Bufton (1953), Pritchard (1955), Pontecorvo & Käfer (1958) and Käfer (1958).

Mutants are given allele numbers in order of isolation. The term functional region is used synonymously with gene or cistron. A region is designated by the number of the mutant first located; thus *paba1* and *paba5* are mutants belonging to the *paba1* region.

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Fifteen allelic *paba* mutants (*paba1, 2, 3, 5, 6, 9, 11, 12, 13, 15, 16, 17, 18, 19* and *20*) were chosen to investigate the fine genetic structure of the *paba1* region. All the mutants are of independent origin. Two of them, that is *paba1* and *paba5*, were produced by X-rays; the others are U.V.-induced.

All *paba* mutants respond to *p*-aminobenzoic acid (P.A.B.A.). In the absence of the required growth factor, *paba5, 16* and *19* grow slightly and, on prolonged incubation, can be distinguished from others whose requirement is total; *paba20*

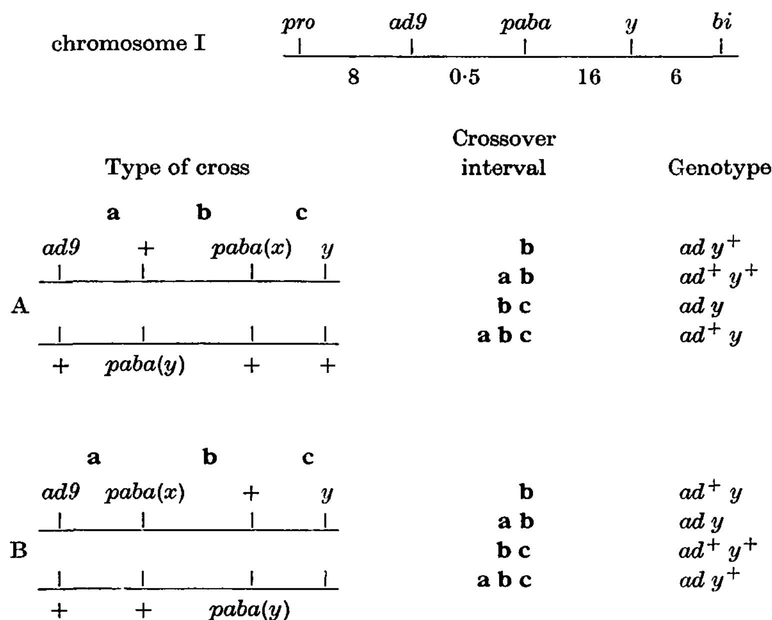

Fig. 1. Above: Map of chromosome I showing location of markers referred to in the text. Nutritional requirements are *pro*, proline; *ad*, adenine; *paba*, *p*-aminobenzoic acid; *bi*, biotin; *y* denotes yellow conidia. Below: The expected genotypes of *paba*$^+$ recombinants with respect to the outside markers in crosses between allelic *paba* mutants. A, distal *paba* allele in coupling with *ad9* and *y*; B, distal *paba* allele in repulsion with *ad9* and *y*. **a**, **b** and **c**, intervals.

is a partial mutant and grows better than *paba5, 16* and *19* in the absence of P.A.B.A. The mutants were tested against each other, in all possible pairs, for complementation, both in heterokaryons and diploids. No case of intra-cistron complementation was found. Crosses of the *paba* alleles with *pro2* and *ad9* showed that the *paba* mutants are closely linked to *ad9* and are situated between *ad9* and *y*. The recombination fraction between *ad9* and *paba6* is $0{\cdot}0051 \pm 0{\cdot}0004$. The linkage relationship of the markers used in the present work is shown in Fig. 1. Table 1 shows the reversion frequencies of the *paba* mutants, which are of the order of 10^{-9}. Since, in crosses between *paba* alleles, it was intended to plate large numbers of ascospores per dish to select P.A.B.A. independent recombinants, it was

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Table 1. *Spontaneous reversion rates of* paba *mutants.* (*Conidia of* paba bi *strain plated on M.M.+biotin*)

paba allele tested	Number of viable conidia	Number of *paba*$^+$ revertants	Reversion rate
paba1	$1{\cdot}4\times10^9$	2	$1{\cdot}4\times10^{-9}$
paba2	$7{\cdot}1\times10^8$	—	—
paba3	$1{\cdot}5\times10^9$	2	$1{\cdot}3\times10^{-9}$
paba5	$9{\cdot}4\times10^8$	—	—
paba6	$7{\cdot}0\times10^8$	1	$1{\cdot}4\times10^{-9}$
paba9	$9{\cdot}4\times10^8$	—	—
paba11	$1{\cdot}8\times10^9$	—	—
paba12	$8{\cdot}0\times10^8$	—	—
paba13	$1{\cdot}6\times10^9$	1	$6{\cdot}0\times10^{-10}$
paba15	$7{\cdot}5\times10^8$	—	—
paba16	$9{\cdot}6\times10^8$	—	—
paba17	$9{\cdot}6\times10^8$	—	—
paba18	$1{\cdot}2\times10^9$	—	—
paba19	$7{\cdot}0\times10^8$	—	—
paba20	$5{\cdot}0\times10^8$	Excessive background growth	

necessary to determine whether the viability of a *paba*$^+$ strain was affected by the density of plating (Grigg, 1952). Table 2 shows the results of a reconstruction experiment, which indicate that, in the case of conidia, the 'Grigg effect' does not occur at the concentrations used in the present work. It is assumed that this also applies to ascospores.

Table 2. *Reconstruction experiment to test for the inhibition of a* paba$^+$ *strain by the conidia of a* paba *strain*

Conidia plated on	Estimated conidia per dish from strain *paba bi*	Estimated conidia from strain *y bi*	Colonies all *y*
M.M.+biotin	$1{\cdot}5\times10^3$	250	212
	$1{\cdot}5\times10^4$	250	179
	$1{\cdot}5\times10^5$	250	173
	$1{\cdot}5\times10^6$	250	198
	$1{\cdot}5\times10^7$	250	208
		(5 dishes each)	

3. CROSSES INVOLVING THE *paba* ALLELES

For the detection and estimation of recombination fractions between allelic *paba* mutants, the method adopted by Pritchard (1955) has been followed. It will be seen that in a cross of the type *ad9 paba*(x) × *paba*(y) *bi*, if *paba*(x) is distal to *paba*(y), the *paba*$^+$ recombinants which arise by a single crossover between the *paba* mutants, will be *ad y*$^+$ with respect to the outside markers. On the other hand, if *paba*(x) is proximal, such recombinants will be *ad*$^+$ *y*. In all crosses

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paba$^+$ colonies with parental combinations of outside markers also arise. In practice this does not offer any difficulty in determining the order of the mutants, as the inequality between the two recombinant classes with respect to the outside markers is invariably very pronounced. The possible genotypes of P.A.B.A. independent recombinants in crosses between allelic *paba* mutants, and the exchange events required to produce them, are shown in Fig. 1.

For purposes of interpreting the results of these crosses and for estimating recombination fractions between *paba* alleles, it has been assumed that all *paba*$^+$ colonies arise by crossing-over. The colonies with parental combinations of outside markers are attributed to multiple crossovers within short intervals. This assumption would be unwarranted if an appreciable number of *paba*$^+$ colonies arose either by back mutation or by some other unknown process which resulted in a conversion of one of the *paba* mutants to wild type in the heterozygote, without necessarily affecting its linkage relationship. Several reasons for believing that this is not so and that the majority of the parental type recombinants are due to multiple crossing-over, have been advanced by Pritchard (1955, 1959) in connexion with the *ad8* region. These reasons are also pertinent to the present work and are corroborated by it. In addition, some further evidence in support of our assumptions has been obtained. A detailed consideration of this matter will, however, be deferred until after the presentation of data from crosses between *paba* alleles.

All *paba* mutants were, at first, crossed to *paba6*. This gave a preliminary indication of their position. Subsequent crosses were made between mutants which were near each other. In the case of adjacent mutants which usually gave few recombinants, crosses were made using both reciprocal combinations of outside markers. This permitted an unambiguous determination of the order. The cross between *paba13* and *paba18* is described as an example of the method adopted. The results of other crosses are summarized in Table 4, and only the salient features are pointed out in the text. Data from crosses between adjacent mutants with reciprocal combinations of outside markers are presented together.

Detection and estimation of recombination fraction in a cross involving paba13 *and* paba18

The cross was made in sealed Petri dishes. About a hundred perithecia were collected and washed with 1/1000 calzolene oil to remove the conidia. The washed perithecia were crushed in a test-tube and the ascospores suspended in saline. The perithecial debris was allowed to settle down and the ascospore suspension was sucked out with a Pasteur pipette. The conidial contamination in this as well as in subsequent crosses was negligible.

The ascospore suspension had a density of 6×10^6 ascospores per ml. It was distributed in 0·5 ml. aliquots over fifteen half-inch test-tubes. A small volume (5 ml.) of molten minimal medium (M.M.), supplemented with adenine and biotin, was added to each tube and this agar suspension was poured to form a thin top layer on dishes containing a bottom layer of M.M. supplemented with adenine and biotin.

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Ten dishes containing M.M. supplemented with P.A.B.A. and biotin were plated with 1 ml. of a 10^{-4} dilution of the original suspension, that is 6×10^2 ascospores per dish.

Colonies growing on the first set of plates were *paba*$^+$ recombinants. A random sample of these was classified for biotin and adenine requirement. The yellow colonies on the dishes supplemented with P.A.B.A. and biotin were *y ad*$^+$ recombinants and were used to estimate the number of ascospores derived from hybrid perithecia in the suspension. A recombination fraction of 0·16 between *ad* and *y* has been assumed for this purpose in all crosses.

The results of this cross are given in Table 3. The recombination fraction between *paba13* and *paba18* is $1{\cdot}4 \times 10^{-4} \pm 1{\cdot}4 \times 10^{-5}$. The formulae for estimating

Table 3. *Detection and estimation of recombination between* paba13 *and* paba18

Intervals:	a	b		c	
Cross:	*ad9*	*paba13*	+	*y*	+
	+	+	*paba18*	+	*bi*

Ascospores plated on M.M. + adenine + biotin: Total (m)	per dish	Colonies *paba*$^+$ ($b1$)	Ascospores plated on M.M. + P.A.B.A. + biotin (n)	Colonies *y ad*$^+$ ($a1$)
$4{\cdot}5 \times 10^7$	3×10^6	723	6×10^3	110

Classification of paba$^+$ *colonies*

	ad$^+$ *bi*$^+$	*ad*$^+$ *bi*	*ad bi*$^+$	*ad bi*
yellow	120	8	2	—
green	2	74	1	—

n = number of ascospores plated on M.M. + P.A.B.A. + biotin = 6×10^3
$a1$ = number of colonies produced by n ascospores = 110
m = number of ascospores plated on M.M. + adenine + biotin = $4{\cdot}5 \times 10^7$
$b1$ = number of colonies produced by m ascospores = 723
x = recombination fraction between *ad* and *y* = 0·16
h = fraction of viable ascospores from hybrid asci = $2a1/nx$ = 0·229
q = recombination fraction between *paba13* and *paba18* = $nb1x/ma1$ = 0·00014

$$\text{S. E. of q} = \frac{\sqrt{(qnx(2-hq)+mq(2-hx))}}{mnhx} = 0{\cdot}000014$$

recombination fractions and their standard errors are due to Dr A. Durrant and are taken from Pritchard (1955). It has been pointed out by Pritchard that this method of estimating recombination fractions is open to several sources of error such as inaccurate dilution, differential viability of genotypes or a deviation of the recombination fraction between *ad* and *y* from its standard value of 0·16. In spite of this, in our experience, although one cannot use them for very precise quantitative comparisons, the values are fairly reproducible.

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Among 207 *paba*$^+$ colonies, classified for adenine requirement, the largest number, i.e. 128, are *ad*$^+$ *y*. There is only one colony of the reciprocal genotype *ad y*$^+$. This is consistent with the order *ad9 paba13 paba18*. There are seventy-six colonies which require an additional exchange in the interval **b**, and two which require the second exchange in interval **a**. In view of the known occurrence of high negative interference in *A. nidulans*, this was expected. These colonies could also have arisen by back mutation of *paba13* or *paba18*. Plating of large numbers of conidia from the two strains, however, produced no revertants.

The rarity of *ad y*$^+$ colonies also shows that unequal crossing-over (Sturtevant, 1925) is not involved.

Crosses involving paba6 *with ten other alleles*

The cross may be represented as *ad9 paba6 y* × *paba*(x) *bi*, where *paba*(x) stands for any of the ten alleles, *2*, *3*, *5*, *9*, *11*, *12*, *15*, *16*, *18* and *19*. The results are presented in Table 4. The number of recombinants obtained in crosses with *paba12* and *paba15* is small. However, the alleles can be ordered on the basis of the single crossover class. In all other cases the order is unambiguous. Alleles *2*, *3*, *5*, *9*, *11*, *12* and *16* are proximal to *paba6* while *15*, *18* and *19* are distal to it.

In the case of *paba15* and *paba5* the number of ascospores plated was more than 10^8 and back mutation could also have occurred.

Crosses involving paba9 *with* 2, 3, 16, 18 *and* 19

In the cross with *paba16*, no recombinants were obtained. All other alleles tested are distal to *paba9*. The order of alleles in all crosses is unambiguous.

Crosses involving paba1 *with* 6, 18 *and* 19

All the three alleles tested are distal to *paba1*. The number of ascospores plated was less than 10^8 in all crosses so that the likelihood of back mutations is small.

Crosses involving paba18 *with* 2, 3, *and* 19

Alleles *2* and *3* are proximal to *paba18* while *19* is distal to it.

Crosses involving paba5 *with* 18 *and* 19

Both the alleles tested are distal to *paba5*.

Crosses involving paba11 *with* 3 *and* 5

paba3 is distal to *paba11* while *paba5* is proximal to it. In both crosses, over 10^8 ascospores were plated and back mutation may have occurred. The order of alleles, however, is unambiguous.

Cross ad9 paba16 y × paba2 bi

paba2 is distal to *paba16*. The recombination fraction between the two alleles is $1{\cdot}2 \times 10^{-5}$.

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Cross ad9 paba2 y × paba5 bi

The recombination fraction between *paba2* and *paba5* is $9{\cdot}4 \times 10^{-6}$. The order of the alleles is *ad9 paba5 paba2*.

We shall now consider crosses between adjacent pairs of mutants. The data from the two crosses between such a pair with reciprocal combination of outside markers are tabulated together. Owing to the closeness of the alleles involved, the number of recombinants in these crosses is, as a rule, small. In some crosses, none or very few recombinants were obtained. However, since in no case was there found an inconsistency between reciprocal crosses, and since the behaviour of outside markers was remarkably consistent in more than fifty different crosses, we can assign the order of the alleles with reasonable confidence, even on the basis of a few recombinants.

Since in most crosses large numbers of ascospores were plated, some back mutations may have occurred. However, as shown before, the reversion frequency of the alleles is of the order 10^{-9}.

The two reciprocal crosses are referred to as cross (a) and cross (b).

Crosses between paba13 *and* paba6

The distribution of outside markers in the two reciprocal crosses is consistent with the order *ad paba6 paba13*. The recombination fractions obtained from the two crosses are also homogeneous, the combined estimate being $5{\cdot}9 \times 10^{-7} \pm 1{\cdot}8 \times 10^{-7}$.

Crosses between paba2 *and* paba3

The order of the alleles is *ad paba3 paba2*. The results of the two crosses are qualitatively consistent and the recombination fractions are homogeneous. The combined estimate is $8{\cdot}2 \times 10^{-7} \pm 2{\cdot}9 \times 10^{-7}$.

Crosses between paba16 *and* paba3

Both crosses are consistent with the order *ad paba16 paba3*. The agreement between the two estimates of recombination fraction is very close. The combined estimate is $6 \times 10^{-6} \pm 1{\cdot}2 \times 10^{-6}$.

Crosses between paba11 *and* paba9

In cross (a), nine of the recombinants between *y* and *ad* were y^{+} *ad* and only one was *y* ad^{+}. This clearly indicates the order *ad paba9 paba11*. In the reciprocal cross (b), very few perithecia were produced. The sterility seems to be a property of the *paba11 bi* strain, as it was also encountered in other crosses where this strain was involved. From 8×10^{6} ascospores plated, of which about $5{\cdot}5 \times 10^{5}$ were viable, and from hybrid asci, no $paba^{+}$ recombinants were obtained. In view of the low recombination fraction between *paba11* and *paba9*, this was not unexpected.

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Table 4. *Detection and estimation of recombination in crosses involving* paba *alleles*

Type of cross: $\frac{ad9 \quad paba(p) \quad y \quad +}{+ \quad paba(q) \quad + \quad bi}$

Cross $p \times q$	Selection $paba^+$: Ascospores plated (in millions)	Selection $paba^+$: Colonies	Selection $ad^+ y$: Ascospores plated (in thousands)	Selection $ad^+ y$: Colonies	Recombination fraction $\times 10^6$	Classification of $paba^+$ colonies*: Colour	$ad^+ bi^+$	$ad^+ bi$	$ad\ bi^+$	$ad\ bi$	Inferred order of *paba* alleles†
6 × 2	44	64	2·2	128	4 ± 0·6	y^+	1	3	1	29	2 6
						y	1	—	29	—	
6 × 3	26	52	2·6	82	10 ± 1·8	y^+	—	5	8	24	3 6
						y	—	—	14	1	
6 × 5	210	1196	21	542	35 ± 1·8	y^+	1	17	7	100	5 6
						y	9	—	44	9	
6 × 9	15	27	2·0	20	29 ± 8·5	y^+	1	5	—	9	9 6
						y	2	—	9	1	
6 × 11	9·1	51	7·0	123	51 ± 8·5	y^+	1	8	3	22	11 6
						y	1	—	15	1	
6 × 12	40	12	6·5	80	3·9 ± 1·2	y^+	—	—	—	6	12 6
						y	—	—	6	—	
6 × 15	138	5	10·1	183	0·32 ± 0·15	y^+	—	3	—	—	6 15
						y	2	—	—	—	
6 × 16	30	67	3·0	56	19 ± 3·4	y^+	—	3	6	39	16 6
						y	—	1	15	3	
6 × 18	45	1116	5·0	147	140 ± 12	y^+	1	29	—	—	6 18
						y	69	6	1	—	
6 × 19	3·5	81	5·0	43	430 ± 81	y^+	1	14	—	—	6 19
						y	31	4	—	2	

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9 × 2	91	73	7·0	49	18 ± 3·4	y^+	2	12	—	—	9	2
						y	45	6	8	—		
9 × 3	110	54	7·0	112	4·8 ± 0·8	y^+	2	11	—	4	9	3
						y	25	2	9	1		
9 × 16	70	—	7·0	35	—	y^+	—	—	—	—	—	
						y	—	—	—	—		
9 × 18	70	1707	7·0	176	160 ± 12	y^+	11	47	—	1	9	18
						y	97	13	9	—		
9 × 19	5·6	252	35	610	410 ± 31	y^+	1	42	—	2	9	19
						y	70	5	1	—		
5 × 18	15	321	15	268	190 ± 16	y^+	2	40	—	—	5	18
						y	65	3	4	—		
5 × 19	3·75	43	5·0	18	510 ± 140	y^+	—	10	1	—	5	19
						y	26	6	—	—		
11 × 3	140	202	10	391	5·9 ± 0·51	y^+	—	34	—	7	11	3
						y	83	3	18	—		
11 × 5	180	34	16	527	0·92 ± 0·16	y^+	2	5	—	18	5	11
						y	1	1	7	—		
16 × 2	100	72	5·0	49	12 ± 2·2	y^+	3	24	—	—	16	2
						y	28	3	12	2		
2 × 5	90	266	9·0	453	9·4 ± 0·72	y^+	3	16	3	56	5	2
						y	4	1	18	1		
1 × 6	51	50	3·0	106	4·4 ± 0·75	y^+	2	10	—	—	1	6
						y	36	2	—	—		
1 × 18	10	222	10	218	160 ± 15	y^+	3	37	—	1	1	18
						y	71	6	1	—		

* Where the number of recombinants is large a random sample has been classified.
† The numbers refer to *paba* alleles. The allele on the left is nearer to *ad9*.

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TABLE 4—*continued*

Cross $p \times q$	Selection $paba^+$: Ascospores plated (in millions)	Selection $paba^+$: Colonies	Selection $ad^+ y$: Ascospores plated (in thousands)	Selection $ad^+ y$: Colonies	Recombination fraction $\times 10^6$	Colour	$ad^+ bi^+$	$ad^+ bi$	$ad\ bi^+$	$ad\ bi$	Inferred order of *paba* alleles†
1 × 19	20	251	10	50	400 ± 62	y^+	4	32	—	1	1 19
						y	89	5	—	—	
18 × 2	15	54	30	66	260 ± 15	y^+	2	1	2	31	2 18
						y	—	—	17	1	
18 × 3	10	48	1·0	4	190 ± 99	y^+	—	1	—	31	3 18
						y	1	—	15	—	
18 × 19	30·6	427	6·0	62	220 ± 29	y^+	3	30	—	—	18 19
						y	60	7	3	1	
(a) 13 × 6	240	15	12	254	0·47 ± 0·13	y^+	—	—	2	6	6 13
						y	2	—	4	1	
(b) 6 × 13	86	11	6·8	189	0·74 ± 0·23	y^+	7	—	—	—	
						y	—	4	—	—	
(a) 2 × 3	150	51	12	504	1·3 ± 0·2	y^+	1	4	15	12	3 2
						y	3	—	15	1	
(b) 3 × 2	270	9	15	125	0·64 ± 0·22	y^+	—	2	—	2	
						y	3	1	1	—	
(a) 16 × 3	120	79	8·0	141	6 ± 0·84	y^+	4	16	—	1	16 3
						y	45	2	10	1	
(b) 3 × 16	190	65	12	112	5·8 ± 0·9	y^+	—	13	3	36	
						y	—	—	13	—	
(a) 11 × 9	360	16	21	83	1·8 ± 0·49	y^+	—	5	—	9	9 11
						y	1	—	1	—	
(b) 9 × 11	8	—	10	55	—	y^+	—	—	—	—	
						y	—	—	—	—	

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(a) 12 × 2	220	39	11	159	2 ± 0·4	y^+	1	4	1	24	2 12
						y	1	—	7	1	
(b) 2 × 12	30	3	40	37	1·7 ± 0·1	y^+	1	—	—	—	
						y	—	2	—	—	
(a) 9 × 5	275	11	16·5	343	0·3 ± 0·094	y^+	—	1	—	3	?
						y	—	—	7	—	
(b) 5 × 9	200	3	10	39	0·6 ± 0·36	y^+	—	—	2	—	
						y	—	1	—	—	
(a) 1 × 2	64	8	4·0	163	0·49 ± 0·18	y^+	—	1	—	3	2 1
						y	—	—	4	—	
(b) 2 × 1	144	2	8·0	83	0·21 ± 0·15	y^+	—	1	—	—	
						y	1	—	—	—	
(a) 11 × 16	128	10	8·0	91	1·1 ± 0·37	y^+	—	3	1	—	11 16
						y	4	—	2	—	
(b) 16 × 11	16	2	10	47	4·2 ± 0·99	y^+	—	—	—	2	
						y	—	—	—	—	
(a) 17 × 6	36	6	1·8	17	2·8 ± 1·4	y^+	1	2	—	—	17 6
						y	3	—	—	—	
(b) 6 × 17	48	6	10	119	1·7 ± 0·7	y^+	—	—	—	2	
						y	—	—	4	—	
(a) 1 × 17	40	9	4·0	60	2·4 ± 0·86	y^+	—	2	—	—	1 17
						y	5	2	—	—	
(b) 17 × 1	80	2	4·0	101	0·16 ± 0·11	y^+	—	—	—	1	
						y	—	—	1	—	

* Where the number of recombinants is large a random sample has been classified.

† The numbers refer to *paba* alleles. The allele on the left is nearer to *ad9*.

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Crosses between paba12 *and* paba2

In cross (a), out of thirty-nine *paba*$^{+}$ recombinants, twenty-five are y^{+} *ad*. This clearly indicates the order *ad paba2 paba12*. In the reciprocal cross only three recombinants were obtained. All three are *ad*$^{+}$ and two are recombinants between outside markers. The two crosses are, thus, consistent. The combined estimate of the recombination fraction is $2 \times 10^{-6} \pm 1{\cdot}1 \times 10^{-6}$.

Crosses between paba9 *and* paba5

The results of the two crosses are difficult to interpret. In cross (a), eight out of eleven *paba*$^{+}$ recombinants have a parental combination of outside markers. The three y^{+} *ad* colonies would indicate the order *ad paba5 paba9*. But this cannot be confirmed by the reciprocal cross (b), since all *paba*$^{+}$ colonies in this case have parental combinations of outside markers. The number of recombinants obtained is small and it is not possible to assess the significance of this deviation from expectation. However, the fact that both crosses produced an excess of parental types may be more than a coincidence. In both crosses large numbers of ascospores were plated and back mutation may have occurred. The order of the two alleles is at present inconclusive and their relationship is being further investigated.

Crosses between paba1 *and* paba2

In the cross (a), five out of eight *paba*$^{+}$ recombinants are parental types. The other three are y^{+} *ad*. In the reciprocal cross, only two recombinants were obtained, one of which is parental type with respect to the outside markers. The other, however, is *y ad*$^{+}$. The two crosses are consistent with the order *ad paba2 paba1*.

Crosses between paba11 *and* paba16

The ten recombinants in cross (a) indicate the order *ad paba11 paba16*. Only two recombinants were obtained in cross (b). Both are y^{+} *ad* and are consistent with the result of the cross (a). The estimates of the recombination fraction, although significantly different, are reasonably close to one another in view of the large experimental errors involved.

Crosses between paba17 *and* paba6

The distribution of outside markers in the two crosses is consistent with the order *ad paba17 paba6*. The estimates of recombination fraction are also homogeneous. The combined estimate is $1{\cdot}8 \times 10^{-6} \pm 1{\cdot}5 \times 10^{-6}$.

Crosses between paba1 *and* paba17

Cross (a) indicates the order *ad paba1 paba17*. Only two recombinants were obtained in the reciprocal cross (b) but the two crosses are qualitatively consistent. The estimates of recombination fraction from the two crosses, however, differ by a factor of 15. This may have been caused by any of the sources of error pointed out previously.

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Crosses between paba13 *and* paba15

In the cross *ad9 paba13 y* × *paba15 bi*, out of 9×10^7 ascospores plated, of which $4{\cdot}8 \times 10^6$ were viable and of hybrid origin, no recombinants were obtained. In the reciprocal cross *ad9 paba15 y* × *paba13 bi*, only one *paba*$^+$ colony was obtained from $2{\cdot}8 \times 10^8$ ascospores, of which $3{\cdot}3 \times 10^7$ were viable and of hybrid origin. The genotype of this colony was y^+ *ad bi*. Since the number of ascospores plated was large, this could have arisen as a result of back mutation, together with a crossover between *ad* and *y*. It could also have been a contaminant. Mutants 13 and 15 are, therefore, considered to belong to the same site.

Crosses between paba1 *and* paba12

No *paba*$^+$ recombinant was obtained from 8×10^7 ascospores in the cross *ad9 paba12 y* × *paba1 bi*. In the reciprocal cross *ad9 paba1 y* × *paba12 bi*, from 1×10^8 ascospores plated, one *paba*$^+$ colony was obtained. This was y^+ ad^+ and could be a back mutant from *paba12* or a contaminant. Mutants 1 and 12 are, therefore, also considered to belong to the same site.

Crosses involving paba20

In the plating of ascospores from cross *y ad9 paba6* × *paba20 bi*, there was an excessive background growth due to the 'leakiness' of *paba20*. Attempts are being made to locate *paba20* by mitotic analysis, but so far diploids of *paba20* with *paba9* have failed to give *paba*$^+$ recombinants.

4. DISCUSSION

The results of fifty-two crosses between allelic mutants belonging to the *paba1* region are summarized in Fig. 2. Fourteen mutants are assigned to twelve mutational sites. The position of a mutant is considered to be conclusively established when it has been ordered in relation to the adjacent mutants on either side. The determination of the order of sites is based on the segregation of outside markers. Since crosses between adjacent pairs of mutants were carried out with reciprocal arrangements of outside markers, the ordering is qualitative and unambiguous. The only exception is the case of *paba9* and *paba5*. The order in this case is based on the genotype of only three recombinants in one cross and should be considered tentative. The figures in the map indicate recombination fractions $\times 10^5$. Where the results of reciprocal crosses are homogeneous, these represent the combined estimates; otherwise the 'best' estimate (i.e. the one with the smallest standard error) is given.

Crosses between allelic *paba* mutants were first made by Roper (unpublished). He found that the *paba* alleles recombined with low frequencies and that *paba1* and *paba5* were proximal to *paba6*. The present work has shown that *paba1* region is particularly suitable for fine genetic mapping. The high fertility of interallelic crosses and the low back-mutation rates of *paba* alleles permit detection of recombination fractions of the order of 10^{-7}. Three of the intervals in the map

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(Fig. 2) are of this order. The availability of the closely linked outside marker *ad9* makes it possible to determine the order of the mutational sites. In this respect, no inconsistency was found between any of the fifty-two crosses. There is, thus, no evidence of any deviation from a linear linkage structure. Two pairs of mutants, that is 1 and 12 and 15 and 13, failed to recombine and are assigned to identical sites. As in all recombination analysis, this conclusion is provisional.

In the detection and estimation of recombination in the crosses described, it was assumed that all *paba*$^+$ colonies arose as a result of recombination. The excess of colonies with parental combinations of outside markers was ascribed to

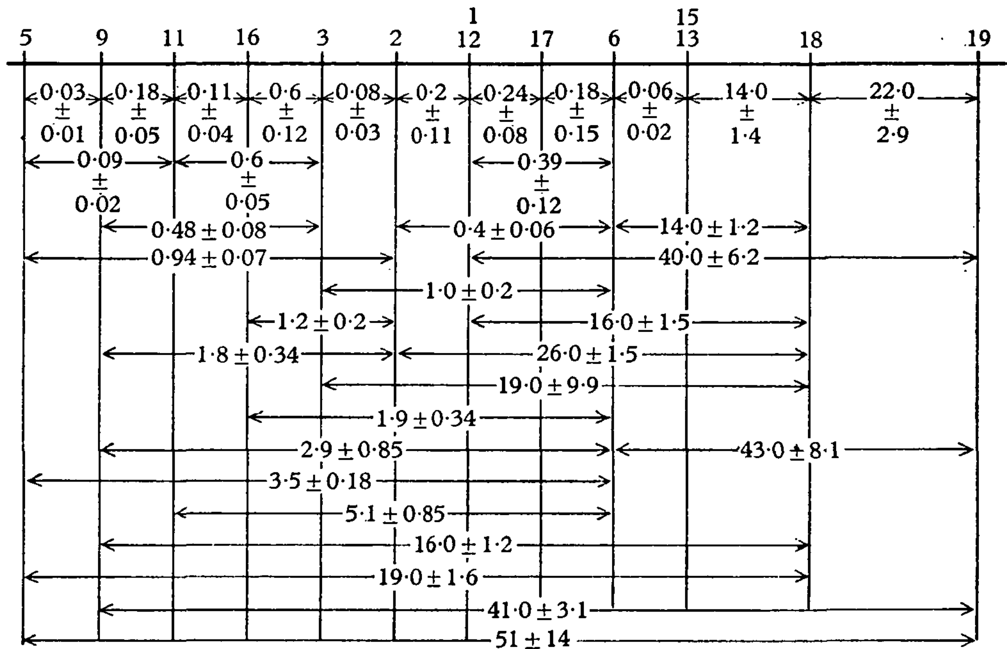

Fig. 2. Map of the *paba1* region showing recombination fraction $\times 10^5$.

the occurrence of multiple exchanges. This matter will, now, be considered further in the light of the results obtained. The distribution of the *paba*$^+$ colonies from crosses between allelic *paba* mutants, into four possible classes with respect to the outside markers (colour and adenine requirement), is shown in Table 5 and Table 6. Table 5 lists all crosses in which the proximal *paba* allele is in coupling with *ad* and *y*. Table 6 shows crosses in which the proximal *paba* allele is in repulsion with *ad* and *y*. The number and kind of exchanges that would be required to produce a particular genotype are also indicated. A single exchange between *paba* mutants in the first case (Table 5) will give rise to an *ad*$^+$ *y* colony; simultaneous exchanges in **a** and **b** or **b** and **c** will give *ad y* or *ad*$^+$ *y*$^+$ colonies respectively, while an *ad y*$^+$ colony will require three exchanges. In the second case, where the proximal *paba* allele is in repulsion with *ad* and *y*, similar exchanges will produce reciprocal genotypes with respect to the outside markers. A comparison of Tables 5 and 6 shows that the patterns of distribution of the four possible

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genotypes form mirror images of each other, and the four possible kinds of exchanges, i.e. **b, ab, bc** and **abc,** occur in the same relative order of abundance. The most numerous class is the one which needs a single exchange in **b.** The class which requires exchanges in **b** and **c** is larger than the one which needs exchanges in **a** and **b.** The least frequent class is that which requires exchanges in all the three intervals. The relative rarity of the triple crossover class also indicates that unequal crossing-over is not involved. It will be noticed that in

Table 5. *Classification of* paba$^+$ *colonies for the outside markers* ad *and* y

Interval: **a** **b** **c**

Type of cross: $\frac{ad \quad paba(p) \quad + \quad y}{+ \quad + \quad paba(q) \quad +}$

Cross	Genotypes and the required exchanges				
	$ad^+ y$	$ad\ y$	$ad^+ y^+$	$ad\ y^+$	
$p \times q$	(**b**)	(**a b**)	(**b c**)	(**a b c**)	Total
9 × 19	75	1	43	2	121
6 × 19	35	2	15	—	52
6 × 18	75	1	30	—	106
1 × 6	38	—	12	—	50
13 × 18	128	2	76	1	207
1 × 18	77	1	40	1	119
1 × 19	94	—	36	1	131
9 × 3	27	10	13	4	54
11 × 3	86	18	34	7	145
9 × 2	51	8	14	—	73
16 × 2	31	14	27	—	72
16 × 3	47	11	20	1	79
18 × 19	67	4	33	—	104
5 × 18	68	4	42	—	114
5 × 19	32	—	10	1	43
2 × 1	1	—	1	—	2
11 × 16	4	2	3	1	10
2 × 12	1	—	2	—	3
3 × 2	4	1	2	2	9
1 × 17	7	—	2	—	9
6 × 13	7	—	4	—	11
6 × 15	2	—	3	—	5
9 × 18	110	9	58	1	178

crosses where the incidence of the triple crossover class is high, it is in general correlated with a high incidence of double exchanges of both types. This pattern of distribution and the numerical order of abundance of the four possible genotypes in the two sets of crosses is consistent with a recombinational origin of most of the *paba*$^+$ colonies.

The excess of *paba*$^+$ colonies with parental combinations of outside markers cannot be accounted for by simple back mutation. Out of fifteen *paba* alleles that

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were tested, only four were found to revert. The reversion frequencies were of the order 10^{-9}. Although it is possible that in some cases a few back mutations may have occurred, the lowest frequency of parental type colonies in the crosses described is already too high (10^{-7}) to be accounted for by simple back mutation.

The parental type *paba*$^+$ colonies could, however, arise by a process variously described as 'gene conversion' (Lindegren, 1955), transmutation (Horowitz—see Beadle, 1957) or transreplication (Glass, 1957). In so far as gene conversion is assumed to be independent of recombination between alleles, it has already been

Table 6. *Classification of* paba$^+$ *colonies for the outside markers* ad *and* y

Interval: a b c

Type of cross: $\dfrac{ad \quad + \quad paba(p) \quad y}{+ \quad paba(q) \quad + \quad +}$

Cross	Genotypes and the required exchanges				
	ad y^+	ad^+ y^+	*ad y*	ad^+ *y*	
$p \times q$	(b)	(a b)	(b c)	(a b c)	Total
3 × 16	39	13	13	—	65
2 × 3	27	5	16	3	51
2 × 5	59	19	19	5	102
11 × 5	18	7	7	2	34
6 × 5	107	18	52	9	186
6 × 3	32	5	15	—	52
6 × 2	30	4	29	1	64
6 × 16	45	3	18	1	67
6 × 11	25	9	16	1	51
18 × 3	31	1	15	1	48
12 × 2	25	5	8	1	39
18 × 2	33	3	18	—	54
6 × 12	6	—	6	—	12
6 × 17	2	—	4	—	6
6 × 9	9	6	10	2	27
13 × 6	8	—	5	2	15
11 × 9	9	5	1	1	16
1 × 2	3	1	4	—	8

shown by Pritchard (1959) that the available evidence in *A. nidulans* is not compatible with this interpretation. It has been pointed out that the apparent frequencies of 'gene conversion' in different crosses between mutants belonging to the *ad8* region are correlated with the recombination fractions between alleles and can vary for the same allele, by as much as a factor of 100 between crosses. A similar correlation exists for the *paba* mutants.

If one makes the assumption that all parental type *paba*$^+$ colonies arise by conversion while those which are recombinants between outside markers arise by crossing-over, then the apparent frequencies of gene conversion and crossing-over can be calculated, provided a correction is made for crossing-over due to the

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standard distances between the *paba* mutants and the outside markers. The high and low 'conversion frequencies' of seven different *paba* alleles, calculated in the manner described by Pritchard (1959), are given in Table 7. It will be seen that the apparent conversion frequencies of one *paba* allele vary widely and are correlated with the 'crossover' frequencies between the alleles. In some cases the difference is as much as 200-fold. This would not be expected if gene conversion resulted specifically from an interaction of the mutant with its wild-type allele and was independent of recombination between the mutants.

Additional evidence against a non-recombinational origin of the parental type $paba^+$ colonies comes from a consideration of inequality between the two parental types of $paba^+$ colonies. In many crosses, one of the parental types was in large excess over the other. In terms of gene conversion this would mean that one of

Table 7. *Differences in the apparent 'conversion frequencies' of seven* paba *alleles in different crosses*

Type of cross: *ad9 paba(p) y* × *paba(q) bi*

Cross	'Crossover'	'Conversion frequency'						
$p \times q$	frequency	*paba2*	*paba3*	*paba5*	*paba6*	*paba9*	*paba11*	*paba16*
1 × 2	$1{\cdot}8 \times 10^{-7}$	6×10^{-8}						
11 × 16	4×10^{-7}						$2{\cdot}2 \times 10^{-7}$	
6 × 13	4×10^{-7}				$< 10^{-7}$			
3 × 2	$4{\cdot}2 \times 10^{-7}$		$1{\cdot}1 \times 10^{-7}$					
11 × 5	$4{\cdot}6 \times 10^{-7}$			$1{\cdot}5 \times 10^{-7}$				
11 × 9	8×10^{-7}					6×10^{-7}		
16 × 3	$3{\cdot}3 \times 10^{-6}$							$8{\cdot}4 \times 10^{-7}$
16 × 2	$5{\cdot}5 \times 10^{-6}$							$2{\cdot}3 \times 10^{-6}$
6 × 11	$2{\cdot}6 \times 10^{-5}$						$8{\cdot}8 \times 10^{-6}$	
9 × 18	8×10^{-5}					2×10^{-6}		
5 × 18	1×10^{-4}			6×10^{-6}				
18 × 3	$1{\cdot}1 \times 10^{-4}$		$3{\cdot}9 \times 10^{-6}$					
18 × 2	$1{\cdot}4 \times 10^{-4}$	$1{\cdot}4 \times 10^{-5}$						
6 × 19	3×10^{-4}				2×10^{-5}			

the mutants had a higher conversion rate than the other. Such an interpretation has been advanced to account for the observed inequality between parental type recombinants in *Neurospora* (Murray, 1960). However, it turned out that in all crosses where a marked asymmetry between the two parental types existed, the strain which carried the distal *paba* mutant was always in large excess over the parent with the proximal allele. This asymmetry has been investigated further and will be discussed elsewhere (Siddiqi & Putrament, 1961), but its bearing on the origin of the parental type $paba^+$ colonies can be seen from the following example. In the cross *ad9 paba13 y* × *paba18 bi*, out of 207 $paba^+$ colonies, 2 are *ad y* and 76 are $ad^+\ y^+$. Even after correcting for recombination between *paba* and *y*, it would appear that *paba18* has a high 'conversion frequency' ($3{\cdot}7 \times 10^{-5}$)

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while *paba13* has a low 'conversion frequency' ($1{\cdot}3 \times 10^{-6}$). In the cross *ad paba18 y* × *paba19 bi*, however, where *paba18* is proximal to *paba19*, it is *paba18* which has a low 'conversion frequency' while *paba19* has a high 'conversion frequency'. The observed asymmetry is, thus, related to the position of the mutant rather than its specific conversion rate.

The assumption that the parental type *paba*$^+$ colonies originate by recombination is also supported by the additivity of the recombination fractions. It will be noticed that, in spite of the various sources of error in the estimation of recombination fractions, their additivity on the map (Fig. 2) is remarkably good. There is, in fact, no case of any gross inconsistency. If non-recombinational processes were operating, the smaller distances, which in our case are of the order of 10^{-7}, would be specially susceptible to distortion. The fact that this is not so, indicates that mutation or any other non-recombinational process does not play an appreciable part in the origin of *paba*$^+$ colonies.

The arguments advanced so far apply to 'gene conversion' as a non-recombinational process. They are not meant to imply that non-reciprocal recombination does not occur. The problem of reciprocity in recombination is a separate one, and has been discussed recently by several authors. It appears to be generally accepted that non-reciprocal exchange or gene conversion itself is a recombinational rather than a mutational event (Roman, 1956, 1958; Freese, 1957*a*; Case & Giles, 1958; De Serres, 1958; Pritchard, 1959, 1960). Strong evidence for this belief has come from the work of Rizet and his colleagues, who have shown that, in *Ascobolus immersus*, conversion frequencies themselves are additive (Lissouba & Rizet, 1960). Models to account for 3:1 ratios in tetrads in terms of a copy choice mechanism have been suggested by Freese (1957*a*, 1957*b*), De Serres (1958) and Pritchard (1959, 1960). It is not the purpose of the present paper to enter into a discussion of these models. It may, however, be pointed out that excess of multiple crossovers (i.e. localized negative interference) and non-reciprocal recombination are not mutually exclusive. A unitary model which can account for the occurrence of both has been proposed by Pritchard (1959, 1960). On the basis of this model, a single non-synchronous switch, resulting in a 3:1 ratio for a particular allele, would still produce a genotype which is recombinant for outside markers. A parental type would require a second switch. The consequences of this model for outside marker segregation are the same whether the switches are synchronous or asynchronous.

As pointed out by Pritchard (1959), the relative frequencies of reciprocal and non-reciprocal recombination events are variable from one organism to another. In yeast (Roman, 1956, 1958*a*; Leupold, 1958) recombination seems to be predominantly non-reciprocal in two out of three cistrons analysed. In *Ascobolus* only non-reciprocal recombination occurs between mutants in one segment of the chromosome (Lissouba & Rizet, 1960). In *Neurospora* tetrads with 3:1 ratio occur with appreciable frequency (Mitchell, 1957; Giles *et al.*, 1957; Case & Giles, 1958). In *Drosophila* and *Aspergillus*, on the other hand, non-reciprocal events are very much an exception to the rule (Lewis, 1952; Green, 1954, 1955, 1960; Roper &

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Pritchard, 1955; Pritchard, 1955, 1959; for exceptions, see Chovnick, 1958; Hexter, 1958; Strickland, 1958).

Direct evidence in support of the occurrence of strong localized negative interference in *A. nidulans* has been provided by the work of Pritchard on the *ad8* region, and of Martin-Smith (unpublished) on the *ad9* region. Both these authors have recovered reciprocal products of multiple crossover events in short regions through mitotic techniques. Although mitotic analysis of the *paba1* region has not yet been attempted, it is reasonable to assume that the modalities of recombination in this region are the same as in the *ad8* and *ad9* regions.

The fine structure map of the *paba1* region also reveals some interesting topographical features. There is a marked concentration of mutational sites in the proximal region of the map extending from *paba5* to *paba13* (Fig. 3). The rest of

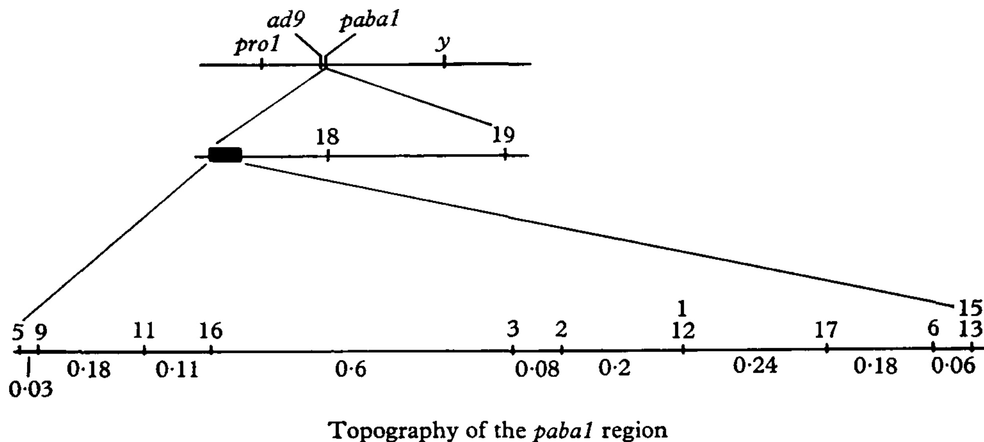

Fig. 3. Map of the *paba1* region, drawn to scale, showing recombination fraction × 10^5.

the map has only two sites. Thus twelve out of fourteen mutants fall in a limited region of the cistron which is less than 6% of its known length. A similar non-random distribution of sites has been found in the *pan-2* region of *Neurospora* (Case & Giles, 1960). It has been pointed out by Pontecorvo (1958), that in cistron maps, very closely linked sites tend to occur more often than one would expect on the basis of a random distribution of mutations within a cistron. The map of the *paba1* region illustrates this very clearly. Pontecorvo has suggested that this may be due to the existence of 'micro-regions of very high mutability'. Alternatively, it may be assumed that some of the 'mutations' in a cistron do not lead to phenotypically detectable effects and consequently are not represented on the map.

It may be pointed out that the topography of the *paba1* region is, in this respect, noticeably different from that of the rII region in the bacteriophage T4. In the case of the rII cistron, although non-random mutability at various sites is obvious, there is no evidence for any large portion of the rII region that is 'unusually crowded or roomy with respect to sites' (Benzer, 1961). Benzer has suggested that

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this might be so because 'nonsense' mutations (Crick *et al.*, 1957) which can interrupt the completion of a polypeptide chain are possible at all sites. On the other hand, it is known that a polypeptide can dispense with a considerable part of its length without losing its biological activity (Hill & Smith, 1956). If this is so, empty regions of a cistron map may correspond to the functionally less important parts of the gene and the differences between genes with respect to this feature of their topography may reflect differences in corresponding proteins.

The minimum number of sites in the *paba1* region as estimated by the method used by Pontecorvo (1958), i.e. the ratio of the smallest to the largest interval between sites, is over 1000. Three of the intervals are of the order of 10^{-7}. This is an order of magnitude less than the minimum distances encountered in the earlier work on *A. nidulans*, and on the basis of the assumptions made by Pontecorvo and Roper (1956) would correspond to one or two pairs of nucleotides.

SUMMARY

The linear relationship between fourteen allelic *p*-aminobenzoic-acid-requiring mutants of *Aspergillus nidulans* was investigated. The mutants were assigned to twelve different mutational sites within one functional region. No case of intra-cistron complementation was found. The order of sites as determined by the distribution of the outside markers in different crosses between *paba* alleles is consistent and the recombination fractions between the sites are approximately additive. Crossing-over between alleles is associated with strong localized negative interference. The topography of the *paba1* region is characterized by a marked concentration of the mutational sites in a small part of the cistron.

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