THE NATURE OF MITOTIC INTRAGENIC RECOMBINATION IN *ASPERGILLUS NIDULANS*

GIORGIO MORPURGO AND LAURA VOLTERRA

*Laboratori di Chimica Biologica, Centro Internazionale di Chimica Microbiologica,
Istituto Superiore di Sanità, Roma, Italy*

Received May 8, 1967

IN practically all systems so far investigated (for review see HOLLIDAY 1964; WESTERGAARD 1964; WHITEHOUSE and HASTINGS 1965; HOLLIDAY 1966) intragenic and intergenic crossing over are characterized by widely different properties. The situation can be summarized as follows: intergenic crossing over is generally a reciprocal event, often associated with positive interference. Some evidence is available in phages and bacteria (MESELSON and WEIGLE 1961; KELLENBERGER, ZICHICHI and WEIGLE 1961; MESELSON 1964; OPPENHEIM and RILEY 1966) indicating that breakage and reunion is the operative mechanism. Intragenic crossing over is essentially a nonreciprocal event (CASE and GILES 1958; LISSOUBA, MOUSSEAU, RIZET and ROSSIGNOL 1962; KRUSZEWSKA and GAJEWSKI 1967) but it may yield a mixture of reciprocal and nonreciprocal products. It is often polarized and associated with negative interference (SIDDIQI 1962). Various hypotheses have been advanced to explain these intriguing results (TAYLOR 1963; HOLLIDAY 1964; WHITEHOUSE and HASTINGS 1965; HOLLIDAY 1966; WHITEHOUSE 1966) but in the main the problem remains unsolved.

Discussion is still open on the following points: Can a single mechanism explain the different types of intragenic recombination and can a single mechanism leading to intergenic recombination also account for intragenic recombination? We may also inquire whether intragenic recombination is an event that occurs at some fixed point in the chromosomes. A second question, related to the first, concerns the level of chromosomal organization involved in intragenic recombination. Does physical exchange of parts of chromosomes occur at the level of the chromatid, for example a minimum of two double-stranded DNA molecules, or does it derive from a process involving two single-stranded DNA molecules?

Mitotic crossing over in molds does not seem to be essentially different from the meiotic process, except for its low frequencies. An advantage of mitotic crossing over is that selective methods can be developed for a rare process such as intragenic recombination, permitting recovery of more than one strand, thus avoiding the laborious examination of some thousands of asci. Another advantage is that the diploid state may maintain in the same cell the two strands deriving from a single exchange. The method has, however, some disadvantages: (1) In mitotic crossing over we do not have the four products of the tetrad, and only half-tetrad analysis is possible. (2) We cannot study the direct products of recombination but analysis is done on a population of cells derived from the original one in which

recombination occurred. This fact can be important because post-recombinational segregation could occur.

The present analysis of diploids resulting from intragenic recombination has been facilitated by selective techniques which have been developed in our laboratory (CALVORI and MORPURGO 1966).

MATERIALS AND METHODS

Media: A complete medium (CM) and a minimal medium (MM) described previously have been used (FRATELLO, MORPURGO and SERMONTI 1960).

Strain: A diploid strain (P-2) heteroallelic for two recessive mutations, *pfp16* and *pfp24* that determine para-fluorophenylalanine (PFP) resistance, has been used. The genetic constitution of the first chromosome of the diploid (other chromosomes are not relevant for the present analysis) is shown in Figure 1. *pfp16* was induced by ultraviolet light (UV); *pfp24* by nitrous acid (N).

Symbols for mutant alleles used by PONTECORVO and KÄFER (1958) have been adopted. Numbers refer to meiotic distances measured by KÄFER (1958).

The spontaneous frequency of back-mutants among plated conidia is less than 1×10^{-8} for both *pfp* alleles (CALVORI and MORPURGO 1966). The relative position of the two markers has been established mitotically (MORPURGO unpublished), since the meiotic crosses were sterile, not producing ascospores.

Selection of PFP-sensitive recombinants: Recombinants resulting in PFP-sensitivity can be selected easily on agar-solidified minimal medium to which L-3-aminotyrosine and phenylanthranilic acid (AT+PA Medium) are added to a final concentration of 0.01 and 0.047 (w/v), respectively, (CALVORI and MORPURGO 1966). The pH of the medium must be accurately adjusted to 4.5. On this medium phenotypically wild-type diploids, homozygous (*pfp*⁺/*pfp*⁺) or heterozygous (*pfp*⁺/*pfp*) for the *pfp*⁺ gene, are selected, since PFP-resistant colonies (*pfp/pfp*) are incapable of growth. With this method about 10×10^6 conidia per dish can be screened. The conidia of strain P-2 (Figure 1) used for these experiments were harvested from dishes of CM supplemented with 1mm PFP in order to avoid formation of large clones of revertants.

Analysis of the recombinant diploids: The analysis of intragenic recombination clearly requires complete determination of the diploid recombinant genotypes. The genotypes of the diploid recombinant colonies have been analyzed by selecting mitotic second-order haploids: in all cases we have analyzed the resistant strand; in some cases both strands from the first-order diploid recombinants were analyzed. About 40 conidia harvested from the sporulated surface of each recombinant diploid were seeded in 12 cm dishes, containing 10 ml CM. After 24 hours a second layer of 10 ml CM, plus 1.5 mm PFP, was poured into the dishes (sandwich plates). PFP stops the growth of the microcolonies which have arisen in the previous 24 hours, but after three or four days vigorous resistant sectors appear. These are typically haploid and derive from the PFP-resistant strand. Diploid sectors, which originate from an exchange between the centromere and the *pfp* gene, also appear.

It is known (MORPURGO 1961; LHOAS 1961) that PFP has a strong haploidizing action, but

FIGURE 1.—Genotype of diploid strain P-2. Only markers in linkage group I are shown.

with this method only sectors with the resistant strand appear, because PFP, present in the dishes, does not permit growth of colonies having the sensitive strand.

In some cases we also determined the genotypic constitution of the PFP-sensitive strand. In these cases the diploid was haploidized using the "formalin technique" (FRATELLO, MORPURGO and SERMONTI 1960).

RESULTS

The expected genotypes of para-fluorophenylalanine-sensitive heteroallelic diploids are shown in Table 1, according to the classical scheme which assumes that recombination takes place at the level of whole chromatids. In Table 2 are shown the second-order segregants which should be recovered on sandwich plates, selecting for PFP-resistant haploids or mitotic crossover diploids, on the basis of this scheme.

Ninety-two first-order recombinant colonies have been analyzed, and the results are summarized in Table 3. It can be seen that more types can be recovered than expected on the basis of the classical scheme. (Classes A and B are almost symmetrical and are due to some nonreciprocal process, while classes C,D,E,F, seem to be due to a reciprocal exchange in a limited region.)

Unequal crossing over: If we examine the genotypes of the first order segregants listed in Table 3 an important difference can be observed: disregarding the diploid sectors, some colonies (Table 3 classes A,B,C,E,H,L) always give one type of haploid sector, either yellow or dark green, fully resistant to PFP. A second type of colony (Table 3 classes D and F) produces two types of haploid sectors: the dark green fully resistant, and a yellow sector which is semilethal on the PFP medium. This sector is neither fully resistant nor completely sensitive to PFP,

TABLE 1

Expected genotypes of PFP-sensitive diploid segregants from a PFP-resistant heteroallelic diploid of Aspergillus nidulans

Parental diploid P-2:	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16+</i>	<i>pfp24-</i>	+	<i>pro1</i>	+	+	<i>ad20</i>	<i>bi1</i>
	+	+	<i>pfp16-</i>	<i>pfp24+</i>	<i>an1</i>	+	<i>paba1</i>	<i>y</i>	+	+
Assumed mechanism of segregation	Genotype of segregants*									
Gene conversion of the distal allele <i>pfp16</i> to wild type	(1)	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16+</i>	<i>pfp24-</i>	+	+	<i>ad20</i>		
		+	+	<i>pfp16+</i>	<i>pfp24+</i>	<i>an1</i>	<i>y</i>	+		
Gene conversion of the proximal allele <i>pfp24</i> to wild type	(2)	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16+</i>	<i>pfp24+</i>	+	+	<i>ad20</i>		
		+	+	<i>pfp16-</i>	<i>pfp24+</i>	<i>an1</i>	<i>y</i>	+		
Mitotic recombination and segregation with a parental strand	(3)	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16+</i>	<i>pfp24-</i>	+	+	<i>ad20</i>		
		<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16+</i>	<i>pfp24+</i>	<i>an1</i>	<i>y</i>	+		
Mitotic recombination and segregation with the reciprocal strand	(4)	+	+	<i>pfp16-</i>	<i>pfp24-</i>	+	+	<i>ad20</i>		
		<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16+</i>	<i>pfp24+</i>	<i>an1</i>	<i>y</i>	+		

* Markers useless for the present analysis were not considered in the table.

TABLE 2

Expected PFP-resistant second-order segregants from PFP-sensitive first order segregants shown in Table 1

Assumed mechanism of segregation		Expected genotype of the second-order segregants*						
1. Gene conversion of the distal allele <i>pfp16</i> to wild type	(1a) diploid	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16</i> ⁺	<i>pfp24</i> ⁻	+	+	<i>ad20</i>
		<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16</i> ⁺	<i>pfp24</i> ⁻	+	γ	+
	(1b) haploid	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16</i> ⁺	<i>pfp24</i> ⁻	+	+	<i>ad20</i>
2. Gene conversion to the proximal allele <i>pfp24</i> to wild type	(2a) diploid	+	+	<i>pfp16</i> ⁻	<i>pfp24</i> ⁺	<i>an1</i>	+	<i>ad20</i>
		+	+	<i>pfp16</i> ⁻	<i>pfp24</i> ⁺	<i>an1</i>	γ	+
	(2b) haploid	+	+	<i>pfp16</i> ⁻	<i>pfp24</i> ⁺	<i>an1</i>	γ	+
3. Mitotic recombination and segregation with a parental strand	(3a) diploid	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16</i> ⁺	<i>pfp24</i> ⁻	+	+	<i>ad20</i>
		<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16</i> ⁺	<i>pfp24</i> ⁻	+	γ	+
	(3b) haploid	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16</i> ⁺	<i>pfp24</i> ⁻	+	+	<i>ad20</i>
4. Mitotic recombination and segregation with the reciprocal strand	(4a) diploid	+	+	<i>pfp16</i> ⁻	<i>pfp24</i> ⁻	+	+	<i>ad20</i>
		+	+	<i>pfp16</i> ⁻	<i>pfp24</i> ⁻	+	γ	+
	(4b) haploid	+	+	<i>pfp16</i> ⁻	<i>pfp24</i> ⁻	+	+	<i>ad20</i>

* Markers useless for the present analysis were not considered in the table.

Homozygosis of *an1* marker is expected only when the second-order diploid segregants derive from a crossing over in the region centromere—*an1*.

though it is fully viable on CM. It must be noticed that the requirement for *ribo1* in the semiresistant strand shows that the colony was derived by reciprocal crossing over proximal to *ribo1*, very probably in the *pfp* gene, which would be modified by it. This fact could be explained by a very high frequency of unequal crossing over in mitosis.

Segregation of the distal marker su1ad20: In 20 cases when the diploid is derived from a mitotic recombination and segregation with the reciprocal strand (classes E and F of Table 3), it is possible to determine whether, in the haploid second-order segregants, the distal marker *su1ad20* accompanies *ribo1*. According to the scheme shown in Table 2 (4b) the PFP-resistant haploid second-order segregants all should require adenine. On the contrary, in all cases analyzed, haploid PFP-resistant segregants are green and presumably *ad20*, but do not require adenine; they all appear to carry *su1ad20*. This fact shows that only a portion of the chromosome participates in intragenic recombination. Another explanation could be that the genetic constitution indicated for chromosome I is wrong. However, in the abnormal case (No. 12) summarized in Table 4, haploid second-order segregants that were adenine requiring were found following intragenic recombination in the *pfp* region. This fact indicated that *su1ad20* is in coupling with *ad20*, as indicated in Figure 1. The same fact has been tested by crossing ten independent colonies of the strain which in the diploid P-2 carries the *pfp16* mutant, with a

TABLE 3

*The genetic constitution of chromosome I of 92 PFP-sensitive colonies obtained on AT+PA medium**

Parental diploid P-2:		<i>su1ad20</i>	<i>ribo1</i>	<i>pfp16+</i>	<i>pfp24-</i>	+	+	<i>ad20</i>	
		+	+	<i>pfp16-</i>	<i>pfp24+</i>	<i>an1</i>	<i>γ</i>	+	
Class		Genotype†							No. of colonies
A		<i>su1ad20</i>	<i>ribo1</i>	<i>pfp-r</i>	+	+	+	<i>ad20</i>	16
		+?‡	+	<i>pfp-s</i>	<i>an1?</i>	<i>γ</i>	+		
B		<i>su1ad20?</i>	<i>ribo1?</i>	<i>pfp-s</i>	+	+	+	<i>ad20</i>	21
		+?	+	<i>pfp-r</i>	<i>an1</i>	<i>γ</i>	+		
C		<i>su1ad20</i>	<i>ribo1</i>	<i>pfp-r</i>	+	+	+	<i>ad20</i>	6
		+?	<i>ribo1</i>	<i>pfp-s</i>	<i>an1</i>	<i>γ</i>	+		
D		<i>su1ad20</i>	<i>ribo1</i>	<i>pfp-r</i>	+	+	+	<i>ad20</i>	13
		+?	<i>ribo1</i>	<i>pfp-sr</i>	<i>an1</i>	<i>γ</i>	+		
E		<i>su1ad20</i>	+	<i>pfp-r</i>	+	+	+	<i>ad20</i>	4
		+?	<i>ribo1</i>	<i>pfp-s</i>	<i>an1</i>	<i>γ</i>	+		
F		<i>su1ad20</i>	+	<i>pfp-r</i>	+	+	+	<i>ad20</i>	16
		+?	<i>ribo1</i>	<i>pfp-sr</i>	<i>an1</i>	<i>γ</i>	+		
G		<i>su1ad20?</i>	+	<i>pfp-s</i>	+	+	+	<i>ad20</i>	2
		+?	<i>ribo1</i>	<i>pfp-sr</i>	<i>an1</i>	<i>γ</i>	+		
H		<i>su1ad20?</i>	+	<i>pfp-s</i>	+	+	+	<i>ad20</i>	6
		+?	<i>ribo1</i>	<i>pfp-r</i>	<i>an1</i> ‡	<i>γ</i>	+		
I		?	?	<i>pfp-s</i>	?	+	+	<i>ad20</i>	2
		?	?	<i>pfp-s</i>	?	<i>γ</i>	+		
L		<i>su1ad20?</i>	<i>ribo1</i>	<i>pfp-s</i>	+	+	+	<i>ad20</i>	2
		+?	<i>ribo1</i>	<i>pfp-r</i>	<i>an1</i>	<i>γ</i>	+		
M		heterogeneous colonies§							4

92

* Markers useless for the present analysis were not considered in the table. The analysis does not permit a distinction between *pfp16* and *pfp24*; *pfp-r* and *pfp-s* mean respectively resistance and sensitivity to PFP; *pfp-sr* indicates semiresistant strands.

† *su1ad20* has not been tested in cases where a question mark appears.

‡ In one case *an1* is in nonparental arrangement.

§ The colonies from which two or more genotypically different types are recovered are considered heterogeneous. A test for heterogeneity of the colonies has been done on 24 colonies.

PFP-sensitive strain: in all cases adenine-requiring strains appeared in the expected frequency, showing that the position of the marker is not altered.

Segregation of the proximal marker an1: In 21 cases which belong to classes C,D,E and F of Table 3, segregant sectors have been analyzed for genotypic consti-

TABLE 4

Genetic constitution of the types originated from the nonhomogeneous colonies

Segregant No.	Genotypic constitution of the types							Possible explanation	
9	a)	<i>su1ad20</i>	+	<i>pfp-r</i>	+	+	<i>ad20</i>	Independent crossing over at the level of the two single DNA strands nonhomologous in one case.	
		+	?	<i>ribo1?</i>	<i>pfp-s</i>	<i>an1?</i>	γ		
	b)	<i>su1ad20</i>	+	<i>pfp-r</i>	+	+	<i>ad20</i>		
		+	?	<i>ribo1</i>	<i>pfp-sr</i>	<i>an1</i>	γ		
12	a)	+	+	<i>pfp-r</i>	+	+	<i>ad20</i>	No simple model to explain genetic instability is available.	
		<i>su1ad20?</i>	<i>ribo1</i>	<i>pfp-s</i>	<i>an1</i>	γ	+		
	b)	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp-s</i>	+	+	<i>ad20</i>		
		+	?	+	<i>pfp-r</i>	<i>an1</i>	γ		
	c)	it produces again both <i>a</i> and <i>b</i> and the unstable type <i>c</i>							
15	a)	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp-r</i>	+	+	<i>ad20</i>	Double gene conversion and segregation of the reciprocal strand.	
		+	?	+	<i>pfp-s</i>	<i>an1?</i>	γ		
	b)	<i>su1ad20?</i>	<i>ribo1?</i>	<i>pfp-s</i>	+	+	<i>ad20</i>		
		+	?	+	<i>pfp-r</i>	<i>an1</i>	γ		
19	a)	<i>su1ad20?</i>	<i>ribo1?</i>	<i>pfp-s</i>	+	+	<i>ad20</i>	Intragenic crossing over and simultaneous gene conversion.	
		+	?	+	<i>pfp-r</i>	<i>an1</i>	γ		
	b)	<i>su1ad20</i>	+	<i>pfp-r</i>	+	+	<i>ad20</i>		
		+	?	<i>ribo1</i>	<i>pfp-sr</i>	<i>an1</i>	γ		

Symbols as in Table 3.

tution with respect to the proximal marker *an1*. The following generalization obtains: yellow sectors always require aneurin (thiamine) while dark green sectors never require it. This is as expected in the absence of an exchange between *pfp* and the centromere.

In seven cases which belong to classes H and L of Table 3 the same analysis has shown that the *an1* marker is in nonparental combination with *γ* in only one case.

Selection on AT+PA medium supplemented with aneurin: 14×10^8 conidia, harvested from the strain P-2, were seeded on AT+PA medium supplemented with aneurin in order to test the possibility that an exchange could occur proximal to *pfp* in the region centromere-*an1*. Seven aneurin requiring colonies out of 90 were recovered. The conidia of the seven first-order segregants were seeded on sandwich plates to recover haploid second-order segregants. In four of them, also, the sensitive strand was determined using the formalin technique. Genotypes of the colonies are shown in Table 5, where it can be seen that only one colony

TABLE 5

Genetic constitution of seven aneurin requiring colonies obtained on AT+PA medium supplemented with aneurin

Class No.	Genotype						No. of colonies
1	<i>su1ad20</i>	+	<i>pfp-r</i>	<i>an1</i>	+	<i>ad20</i>	1
	+?	+	<i>pfp-s</i>	<i>an1</i>	γ	+	
2	<i>su1ad20?</i>	+	<i>pfp-s</i>	<i>an1</i>	+	<i>ad20</i>	3
	+?	+	<i>pfp-r</i>	<i>an1</i>	γ	+	
3	<i>su1ad20</i>	+	<i>pfp-s</i>	<i>an1</i>	+	<i>ad20</i>	1
	+?	<i>ribo1</i>	<i>pfp-r</i>	<i>an1</i>	γ	+	
4	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp-s</i>	<i>an1</i>	+	<i>ad20</i>	1
	+?	<i>ribo1</i>	<i>pfp-sr</i>	<i>an1</i>	γ	+	
5	<i>su1ad20</i>	<i>ribo1</i>	<i>pfp-r</i>	<i>an1</i>	+	<i>ad20</i>	1
	+?	+	<i>pfp-s</i>	<i>an1</i>	γ	+	7

Symbols as in Table 3.

produced a semiresistant sector; this colony was simultaneously homozygous for *ribo1* and *an1*.

Homogeneity of 92 selected PFP-sensitive segregants: The colonies which grow on AT+PA medium are composed of a population of conidia derived from the original cell in which the recombinational process has occurred; if the process of recombination is such (see DISCUSSION) that post-recombinational segregation can occur, two genetically different types of conidia may be recovered in the same colony.

A sample of conidia was harvested from 24 recombinant colonies and plated on CM—(Table 3, 3 from class A, 4 from class B, 1 from class C, 4 from class D, 1 from class E, 4 from class F, 2 from class H, 1 from class L, and 4 from class M). The colonies which arose from the plating presumably derived from single uninucleated conidia. From each colony a new plating was made, using the sandwich technique, to obtain sectoring of the PFP-resistant strand. Sectors of the different clones were isolated and analyzed for color, and nutritional markers and sectors from different isolates derived from the same clone were tested for homogeneity. Twenty-five colonies deriving from each of the 24 recombinants were examined. Of these, four turned out to be nonhomogeneous, producing colonies heterozygous for PFP-resistance, with different genetic constitutions. The analysis of these four colonies is summarized in Table 4.

Colonies 9, 15, and 19 gave two different diploid genotypes which in successive experiments turned out to be stable. Colony 12 segregated three types of colonies, as shown in Table 4: one of these gives a pattern identical to that presented by the colony originally selected on AT+PA medium, and is genetically unstable.

DISCUSSION

The data presented clearly show that intragenic recombination produces colonies that can be basically classified into two types: (1) nonreciprocal recombinants (Table 3 classes A, B, H, L), and (2) reciprocal recombinants having an outside-marker configuration consistent with that expected from a single physical exchange in a limited region of the chromosome (Table 3 classes C, D, E, F). These data are in agreement with those obtained by PUTRAMENT (1964) and JANSEN (1966) for the *pab1* gene, and by PETRELLI and RICCI (1966) for the *pfp* gene of *Aspergillus nidulans*.

Two novel features have been noted in the analysis of the recombinant types: (1) Frequently the recombinational event produces a new type of sector partially resistant to PFP. This sector type is present only when the outside marker *rib1* is in a nonparental configuration with respect to the color marker, in the haploid second-order segregants. The simplest interpretation for this result is that it is caused by a modification of the *pfp* gene due to unequal crossing over, as described by MAGNI (1963) in yeast and by STRIGINI (1965) in phage. Since sectors of this type never appear in the classes carrying the parental arrangement for *rib1* (Table 3 A, B), the idea that the two types of recombinants are due to basically different mechanisms is strongly suggested. (2) In all cases (except the unstable case No. 12; see Table 4) crossing over in the *pfp* gene is never followed by the segregation of *su1ad20*; this result is certainly not due to the occurrence of a second exchange, since the spontaneous frequency of mitotic recombination in the region centromere-*rib1*, which is about 40 meiotic map units long, is about 1×10^{-4} (MORPURGO 1963).

This result is similar to what LISSOUBA, MOUSSEAU, RIZET, and ROSSIGNOL (1962) found in their meiotic analysis in *Ascobolus immersus*, and establishes quite clearly the presence of a point of discontinuity, or "linker", distal to *rib1*.

An interesting point is that the region defined by the linker is not limited to one gene, as *rib1* is obviously a different gene from *pfp*. Moreover, we are forced to conclude that both types of intragenic recombinants arise by a mechanism different from that leading to intergenic recombination, because intragenic recombination never produces segregation of the distal markers.

This view is further supported by the fact (BECCARI, MODIGLIANI and MORPURGO 1967) that fluorouracil and fluorodeoxyuridine greatly increase intergenic crossing over without affecting intragenic recombination. However, ROMAN and JACOB (1958) in yeast, observed exactly the opposite phenomenon: ultraviolet treatment greatly enhanced intragenic recombination. FOGEL and HURST (1963) observed an increase of both reciprocal and nonreciprocal recombination in a diploid strain of *Saccharomyces* after UV irradiation. JANSEN (1966) and KWIATKOWSKI and GRAD (1965), in *Aspergillus nidulans*, have also shown a great increase in UV-induced intragenic mitotic recombination; KÄFER and CHEN (1964) and KWIATKOWSKI (1962) observed an effect at the intergenic level, but unfortunately the data are not comparable, as these authors used nonselective

systems. It is further pertinent that JESSOP and CATCHESIDE (1965) have described a gene in *Neurospora crassa* that affects intragenic recombination without altering the frequencies of intergenic recombination.

The scheme outlined in Tables 1 and 2 is based on the assumption that recombination takes place, as in the classical theories, at the chromatid level. The data do not confirm this simple assumption but can be reconciled well with the hypothesis of HOLLIDAY (1964). According to HOLLIDAY's model both gene conversion and crossing over occur between single DNA strands; in a first stage there is a physical exchange in a segment limited by linkers, followed in a second stage by a repair process involving substitution of the mismatched bases. The model explains the frequent coincidence of conversion and crossing over shown by MITCHELL (1955), and also why postmeiotic segregation sometimes occurs, depending on the efficiency of the repair process, as observed in *Sordaria fimicola* by KITANI, OLIVE and EL-ANI (1962), in *Ascobolus immersus* by LISSOUBA, MOUSSEAU, RIZET and ROSSIGNOL (1962), and in *Neurospora* by STADLER and TOWE (1963). Following this model, in our material two possibilities can be

FIGURE 2.—Intracistronic crossing over between single DNA strands, eventually followed by the repair of mismatched bases. Intragenic crossing over between single DNA strands is shown in (a). The polarity of the strands is indicated by the arrows. In (b) the vertical arrow indicates the direction of the postulated repair process. In (c) the genetic constitution of the diploids that can be selected on AT+PA medium in the absence of the repair process is shown. In (d) are shown the two possible diploid genotypes that can be recovered when the repair process operates. Possible assortment of the diploids is indicated by the arrows. The segregation of the strands is shown by Arabic numerals. The scheme is highly simplified, as only four strands out of eight were considered. Different heterogeneous colonies can be recovered if all the eight strands are considered. The linker distal to *ribo1* is not shown in the figure because its exact position is unknown (see text).

envisaged: (A) Recombination takes place inside *pfp*; (B) Recombination takes place in some part of the chromosome proximal to *pfp*. Case A can be further subdivided: (1) Recombination between single DNA strands of the same polarity is not followed by a repair mechanism. In this case we would obtain only homogeneous colonies on AT+PA medium. This case is outlined in Figure 2(c). (2) Recombination between single DNA strands is followed by repair; in this case one chromatid (of the four) will be composed of two single DNA strands which are both PFP sensitive. Replication of the strands accompanied by segregation with homologous chromatids in a normal mitotic division would produce non-homogeneous colonies, as outlined in Figure 2(d). In both cases 1 and 2, when recombination occurred in *pfp*, *an1* would remain in the parental combination with the color marker, as we have in fact observed in 21 cases. The scheme of Figure 2 is highly simplified, as only four strands out of eight are taken into consideration. Case B (Figure 3) does not produce colonies capable of growth on

FIGURE 3.—Crossing over proximal to *pfp* between single DNA strands, and repair of mismatched bases. Crossing over between single DNA strands of two chromatids is shown in (a). The polarity of the strands is indicated by the arrows. In (b) vertical arrows, marked by Roman numerals, indicate the direction of the postulated repair process. Only repairs which can produce a PFP-sensitive colony have been considered in the figure. In (c) the genetic constitution of the diploids that can be selected on AT+PA medium is shown; Roman numerals refer to the direction of the repair mechanism as indicated in (b). Each process would produce two diploids, depending on whether post-recombinational segregation occurs with the crossover chromatid or with the parental chromatid; segregation of the strand is indicated by Arabic numerals. The scheme is highly simplified as only four strands out of eight were considered. Double repair would produce a heterogeneous colony.

AT+PA medium unless a repair mechanism changes the mismatched bases. All the possibilities deriving from case B are outlined in Figure 3, where the postulated crossing over is indicated in the region *an1*—*pfp*. In this case *an1* would remain in parental arrangement with γ ; if crossing over occurred between the centromere and *an1*, *an1* should be in repulsion to γ in the resistant strand. Experimentally, in seven cases analyzed for *an1* (Table 3 classes H and L) only one *an1* strand has been found. However, when first-order segregants were selected on AT+PA medium supplemented with aneurin, first-order diploid segregants requiring aneurin (thiamine) were found. This fact shows that, at least in some cases, when there is recombination in the *pfp* gene, a crossing over proximal to it occurs (there is one exception, which represents a very peculiar case, being also homozygous for *ribo1*). None of the aneurin requiring colonies produces a semi-resistant sector; this shows that when the crossing over is proximal to *pfp*, the mechanism of recombination does not alter the *pfp* gene. Possibilities which arise from case B cover all the cases we have experimentally found. However, we cannot accept the idea that all the colonies we have selected derive from a recombinational event proximal to *pfp*. In effect only when there is a nonparental arrangement of the outside marker *ribo1* do semiresistant sectors appear; in this case it is a reciprocal recombination which accompanies modifications of the *pfp* gene, suggesting that in these cases the crossing over is intragenic and possibly unequal. A very high frequency of mutation associated with mitotic recombination has been found by MAGNI (1963) in yeast.

In conclusion, the data support the idea that the PFP-sensitive diploid colonies derive from a recombinational event between single strands, either in *pfp* or in a region proximal to it. If proximal, recombination must be followed by repair of the mismatched bases. Heterogeneous colonies might be derived either from an exchange in *pfp*, followed by repair, or from an exchange proximal to *pfp*, followed by a double repair. A tentative explanation of the mechanism which may produce heterogeneous colonies can be found in Figures 2 and 3. The genotype of the colonies observed does not correspond exactly to the scheme outlined in Figures 2 and 3. These schemes are, however, extremely simplified and are useful only to show what kinds of possibilities may arise if the exchange takes place at the level of single DNA strands. Many different types of mixed colonies can be expected with the same basic mechanism. It is important to stress that mixed colonies may arise only if recombination occurs at the level of some subchromosomal element, possibly a single strand of DNA. Unless the chromosome was polytenic, recombination involving whole chromatids could not produce mixed colonies.

Thanks are due to MR. G. CONTI and to MR. G. OBERHOLTZER for able technical assistance.

SUMMARY

A technique is described that permits the analysis of mitotic intragenic recombination within a gene (*pfp*) determining para-fluorophenylalanine resistance. The following results have been obtained: (1) Intragenic recombination is due

to both reciprocal and nonreciprocal processes. In the case studied, nonreciprocal recombination produces two symmetrical classes. (2) The reciprocal event is followed by segregation of the immediately distal marker *ribo1* close to *ppf*, but it is never followed by the segregation of *su1ad20*, a more distantly removed distal marker in the same chromosome arm. This result implies the existence of a point of discontinuity in the chromosome. (3) When intragenic recombination is reciprocal, a new type of sector appears, possibly due to unequal crossing over. (4) Intragenic recombination may produce mosaic colonies, suggesting that it occurs at some subchromosomal level. (5) All the results are explainable by assuming that intragenic recombination is due to an exchange between single DNA strands either in the *ppf* gene or in the region proximal to it, eventually followed by a repair process, as postulated by HOLLIDAY.

LITERATURE CITED

- BECCARI, E., MODIGLIANI, and G. MORPURGO, 1967 Induction of inter- and intragenic mitotic recombination by fluorodeoxyuridine and fluorouracil in *Aspergillus nidulans*. *Genetics* **56**: 7-12.
- CALVORI, C., and G. MORPURGO, 1966 Analysis of induced mutations in *Aspergillus nidulans*. I. UV- and HNO₂-induced mutations. *Mutation Res.* **3**: 145-151.
- CASE, M. E., and N. H. GILES, 1958 Evidence from tetrad analysis for both normal and aberrant recombination between allelic mutants in *Neurospora crassa*. *Proc. Natl. Acad. Sci. U.S.* **44**: 378-390.
- FOGEL, S., and D. D. HURST, 1963 Coincidence relations between gene conversion and mitotic recombination in *Saccharomyces*. *Genetics* **48**: 321-328.
- FRATELLO, B., G. MORPURGO, and G. SERMONTI, 1960 Induced somatic segregation in *Aspergillus nidulans*. *Genetics* **45**: 785-800.
- HOLLIDAY, R., 1964 A mechanism for gene conversion in fungi. *Genet. Res.* **5**: 282-304. ——— 1966 Studies on mitotic gene conversion in fungi. *Genet. Res.* **8**: 323-337.
- JANSEN, G. J. O., 1966 UV-induced mitotic recombination in the *paba₁* cistron of *Aspergillus nidulans*. Doctoral thesis. Drukkerij Elinkwijk, Utrecht.
- JESSOP, P. A., and D. G. CATCHESIDE, 1965 Interallelic recombination at the *his-1* locus in *Neurospora crassa* and its genetic control. *Heredity* **20**: 237-256.
- KÄFER, E., 1958 An 8-chromosome map of *Aspergillus nidulans*. *Advan. Genet.* **9**: 105-145.
- KÄFER, E., and A. CHEN, 1964 UV-induced mutations and mitotic crossing-over in dormant and germinating conidia of *Aspergillus nidulans*. *Microbial. Genet. Bull.* **20**: 8-9.
- KELLENBERGER, G., M. L. ZICHICHI, and J. J. WEIGLE, 1961 Exchange of DNA in the recombination of bacteriophage. *Proc. Natl. Acad. Sci. U.S.* **47**: 869-878.
- KITANI, Y., L. S. OLIVE, and A. S. EL-ANI, 1962 Genetics of *Sordaria fimicola*. V. Aberrant segregation at the *g* locus. *Am. J. Botany* **49**: 697-706.
- KRUSZEWSKA, A., and W. GAJEWSKI, 1967 Recombination within the γ locus in *Ascobolus immersus*. *Genet. Res.* **9**: 159-177.
- KWIATKOWSKI, Z., 1962 Radiation action on the mitotic crossing-over in *Aspergillus nidulans*. II. *Acta Microbiol. Polon.* **11**: 3.
- KWIATKOWSKI, Z., and K. GRAD, 1965 A comparison of the ultraviolet effect on the mitotic recombination in two different cistrons of *Aspergillus nidulans*. *Acta Microbiol. Polon.* **14**: 15-18.

- LISSOUBA, P., J. MOUSSEAU, G. RIZET, and J. L. ROSSIGNOL, 1962 Fine structure of genes in the ascomycete *Ascobolus immersus*. *Advan. Genet.* **11**: 343-380.
- LHOAS, P., 1961 Mitotic haploidization by treatment of *Aspergillus niger* diploids with para-fluorophenylalanine. *Nature* **190**: 744.
- MAGNI, G. E., 1963 The origin of spontaneous mutations during meiosis. *Proc. Natl. Acad. Sci. U.S.* **50**: 975-980.
- MESELSON, M., 1964 On the mechanism of genetic recombination between DNA molecules. *J. Mol. Biol.* **9**: 734-735.
- MESELSON, M., and J. J. WEIGLE, 1961 Chromosome breakage accompanying genetic recombination in bacteriophage. *Proc. Natl. Acad. Sci. U.S.* **47**: 857-868.
- MITCHELL, M. B., 1955 Aberrant recombination of pyridoxine mutants of *Neurospora*. *Proc. Natl. Acad. Sci. U.S.* **41**: 215-220.
- MORPURGO, G., 1961 Somatic segregation induced by *p*-fluorophenylalanine. *Aspergillus News Letter* **2**: 10. — 1963 Induction of mitotic crossing over in *Aspergillus nidulans* by bifunctional alkylating agents. *Genetics* **48**: 1259-1263.
- OPPENHEIM, A. B., and M. RILEY, 1966 Recombination following conjunction in *Escherichia coli*. *J. Mol. Biol.* **20**: 331-357.
- PETRELLI, M. G., and R. RICCI, 1966 Fine structure analysis of the gene for *p*-fluorophenylalanine resistance in *Aspergillus nidulans*. *Ann. Ist. Super. Sanità* **2**: 379-392.
- PONTECORVO, G., and E. KÄFER, 1958 Genetic analysis based on mitotic recombination. *Advan. Genet.* **9**: 71-104.
- PUTRAMENT, A., 1964 Mitotic recombination in the *paba₁* cistron of *Aspergillus nidulans*. *Genet. Res.* **5**: 316-327.
- ROMAN, H., and F. JACOB, 1958 A comparison of spontaneous and ultraviolet-induced allelic recombination with reference to the recombination of outside markers. *Cold Spring Harbor Symp. Quant. Biol.* **23**: 155-160.
- ROPER, J. A., and E. KÄFER, 1957 Acriflavin-resistant mutants of *Aspergillus nidulans*. *J. Gen. Microbiol.* **16**: 660-667.
- SIDDIQI, O. H., 1962 The fine genetic structure of the *paba₁* region of *Aspergillus nidulans*. *Genet. Res.* **3**: 69-89.
- STADLER, D. R., and A. M. TOWE, 1963 Recombination of allelic cysteine mutants in *Neurospora*. *Genetics* **46**: 1323-1344.
- STRIGINI, P., 1965 On the mechanism of spontaneous reversion and genetic recombination in bacteriophage T4. *Genetics* **52**: 759-776.
- TAYLOR, J. H., 1958 The organisation and duplication of genetic material. *Proc. 10th Intern. Congr. Genet.* **1**: 63-78.
- WESTERGAARD, M., 1964 Studies on mechanism of crossing over. I. Theoretical considerations. *Compt. Rend. Trav. Lab. Carlsberg* **34**: 350-405.
- WHITEHOUSE, H. L. K., 1966 An operator model of crossing-over. *Nature* **211**: 708-713.
- WHITEHOUSE, H. L. K., and P. J. HASTINGS, 1965 The analysis of genetic recombination on the polaron hybrid DNA model. *Genet. Res.* **6**: 27-92.