

RECOMBINATION AND DNA MISMATCH REPAIR OF AN *Aspergillus nidulans* MUTANT

Edna Elizabeth S. Celere and Tânia M.A. Domingues Zucchi

ABSTRACT

After mutagenizing a strain of *Aspergillus nidulans* with 80 KR of γ irradiation, the surviving conidia were individually crossed to a tester strain and the meiotic recombination frequencies, in a defined chromosomal interval, were determined through spot-testing (Zucchi, Rev. Bras. Gen. 13: 409-424, 1990). The EC mutant was selected mainly through the RF *meth-w* (25%) presented in the spot-test.

In a more complete genetic analysis it was observed that the EC mutant presents several alterations that vary from cross to cross and exhibited an abnormal meiotic pattern. The mutation in EC affects only meiosis since no alteration in vegetative growth or in mitotic stability was found. Among the selfed cleistothecia products we found a wide variation in the segregation pattern of the genetic markers. Some of the observed meiotic abnormalities can be attributed to the *uvr* mutation present in the EC mutant, which is more effective when in homozygosis than in heterozygosis.

INTRODUCTION

The recombination process is extremely complex. Some of the information needed to understand it have come from studies of mutants presenting alterations in recombination frequencies. One important step of recombination in all studied organisms is the heteroduplex DNA formation, with mispaired bases on account of genetic differences between the parental strands (Whitehouse, 1963; Holliday, 1964; Meselson *et al.*, 1975). Corrections of these mismatched bases through an enzymatic mismatch repair system occurs after crossing-over, generating the parental and recombinant classes.

Correction of only a single heteroduplex molecule results in gene conversion (GC) and promotes a deviation from Mendelian segregation (Holliday, 1964; Hotchkiss, 1974; Meselson and Radding, 1975; Szostak *et al.*, 1983). Failure of correction of a mispaired base during meiotic stages results in post-meiotic segregation (PMS). If the parental alleles differ at base pairs both inside and outside of the heteroduplex, post-meiotic segregation and gene conversion may produce intragenic recombination (IGR).

Therefore, the occurrence of PMS, GC and IGR depends on DNA heteroduplex formation during recombination. There is a resultant distortion of the transmission pattern, altering the allele frequencies and generating new alleles (Leslie and Watt, 1986). This has been found in several eukaryotes, but the fungus *Aspergillus nidulans* has been little studied, mainly due to a lack of deficient mutants for mismatch DNA repair experiments.

The aim of this study was to induce mutations using gamma irradiation in an *A. nidulans* strain and to select mutants presenting altered intergenic recombination frequencies in a specific chromosomal segment, using the "spot-test" devised by Zucchi (1986). The selected mutants were tested for UV sensitivity. It is well known that most of the altered recombination mutants are also UV sensitive and DNA repair deficient e.g. in *Ustilago maydis* (Holliday, 1967) *Escherichia coli* (Howard-Flanders and Theriot, 1966) and *A. nidulans* (Jansen, 1970; Fortuin, 1971; Käfer, 1986).

Since Van de Vate and Jansen (1978) verified the occurrence of crossing-over in "selfed" cleistothecia, the selected *uvs* mutants were "self-crossed" in order to study the meiotic products.

Our method is based on the following: if crossing-over occurs in "selfed" cleistothecia and the mutation (when in homozygosis) is responsible for the altered mismatch repair, the gaps, mispaired bases, etc will remain in the DNA strands. Most of the resulting segregants will be not viable, but the viable ones will present new mutations on account of the post-meiotic segregation of mispaired bases, introduced or not removed by the altered mismatch repair system.

In this communication we present the induction and selection methodology.

MATERIAL AND METHODS

Strains

According to the proposition of Clutterbuck (1970), the mutant alleles of the strains used in the present study were:

UT 448: (Utrecht stock): *wA*₂ (II) white conidia; *ribo* A₁, *paba* A₁₂₄, *bi* A₁ (I) with requirements for riboflavine, *p*-amino-benzoic acid, and biotine, respectively; *Acr* A₁ (II) resistant to acriflavine (Figure 1A).

UT 196: Tester strain: *yA*₂ (I) yellow conidia; *meth* A₁₇ (II); *pyro* A₄ (IV) with requirements for methionine and pyridoxine, respectively (Figure 1B).

UT 184: *cha* A₁ (VIII) "chartreuse" conidia; *pyro* A₄ (IV); *sB*₃ (VI); *nic* B₈ (VII); *ribo* B₂ (VIII), with requirements for pyridoxine, sodium thiosulphate; *gal* A₁ (III); *fac* A₃₀₃ (V); *lac* A₁ (VI), with inability for galactose, acetate and lactose utilization, respectively; *sul* A₁ (I); *Acr* A₁ (II) resistant to acriflavine and sulphanilamide, respectively (Figure 1C).

3²⁵: *wA*₂ (II) white conidia; *yA*₂ (I) yellow conidia; *ribo* A₁ (I); *meth* A₁₇ (II), with requirements for riboflavine and methionine, respectively; *Acr* A₁ (II) acriflavine resistance (Figure 1D).

Media and solutions

MM - *minimum medium*, based on van de Vate and Jansen (1978); 6.0 g NaNO₃; 1.52 g KH₂PO₄; 0.52 g MgSO₄ · 7 H₂O; 0.5 g KCl; 1.0 mg FeSO₄ · 7 H₂O; 1.0 mg ZnSO₄ · 7 H₂O; 1.0 mg CuSO₄ · 5 H₂O; 10.0 g glucose; 1.0 liter of demineralized water, pH 6.0. To prepare solid MM, 15.0 g of bacto agar Difco was added.

CM - *complete medium*: 2.0 g peptone; 1.0 g casaminoacid (vitamin free); 0.25 g RNA (alkali-hydrolyzed); 0.25 g DNA (alkali-hydrolyzed); 0.02 mg biotine; 0.5 mg pyridoxine; 0.5 mg *p*-aminobenzoic acid; 0.5 mg folic acid; 1.0 mg nicotinic acid; 2.0 mg panthotenic acid; 2.0 mg choline; 4.0 mg meso-inositol; 1.0 mg riboflavine; 0.5 mg aneurine; pH 6.0. The volume is completed to 1.000 ml with liquid MM. To prepare solid complete medium, 1.5% of bacto agar Difco was added. (Jansen, 1970).

Selective medium for crosses (SMI): 200 ml of MM was supplemented with 0.2 mg of riboflavine; 0.004 mg of biotine; 200 mg of casaminoacid, and 1.0 mg of adenine. (Zucchi, 1986).

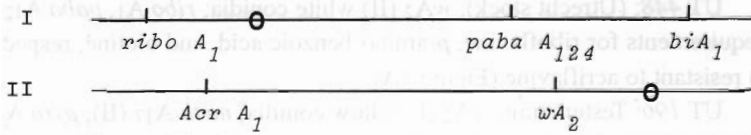
Selective medium for recombination mutant selection (SML): 200 ml of MM (liquid) supplemented with 0.2 mg of riboflavine, 0.004 mg of biotine, 0.5 µg of pyridoxine and 1.0 mg of sodium deoxycholate. (Zucchi, 1986).

Benlate solution: following Hastie, 1970.

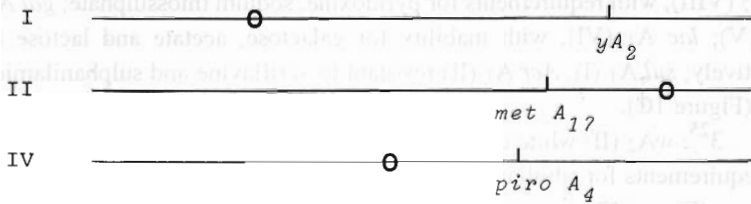
Top layer tubes: 5 ml of semi-solid MM or SM (0.7% agar) kept in a water-bath at 43-45°.

Preparation of conidial suspensions: Conidia of strains 448 and 196 were inoculated in seven positions on CM plates and incubated for four days at 37°C. The plate surface was then washed with 10.0 ml of 0.1% G 3300 and rubbed with a glass stick to liberate the conidia.

A) Strain UT448



B) Strain UT196



C) Strain UT184

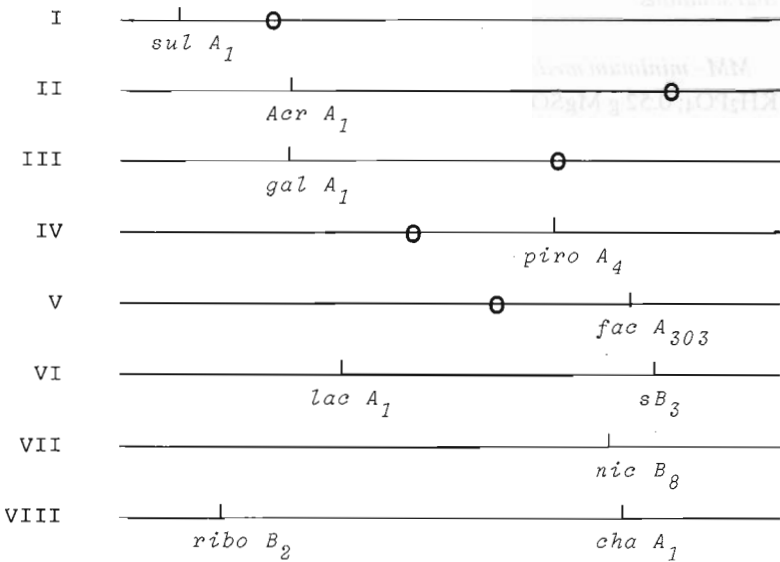
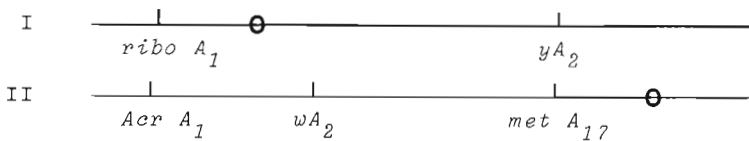
D) Strain 3²⁵

Figure 1 - Schematic representation of the used strains. Open circles figure the centromere. The distance between genetic markers are not in scale.

The suspension was filtered through cotton and the operation was repeated with 10.0 ml of saline.

The filtrate was then centrifuged for 15 min at 3000 rpm, the supernant was discarded and the precipitate was washed twice with 15.0 ml of saline. The precipitate was resuspended in 15.0 ml of saline and transferred to covered vials and homogenization was completed by sonication for 15 min.

Mutagenesis of UT448 strain: a saline suspension with 10^6 conidia/ml of the UT448 strain was distributed into 9 vials (3.0 ml each) and then γ irradiated (^{60}Co source) at increasing doses. A control was provided using non-irradiated conidia of the UT448 strain. Samples of all suspensions (control and treatment) were collected to determine the correct number of viable conidia through dilution and plating on CM and incubated for 48 h at 37°C . After this the colonies were scored and the percentage of survivors was determined in relation to the non-irradiated conidia.

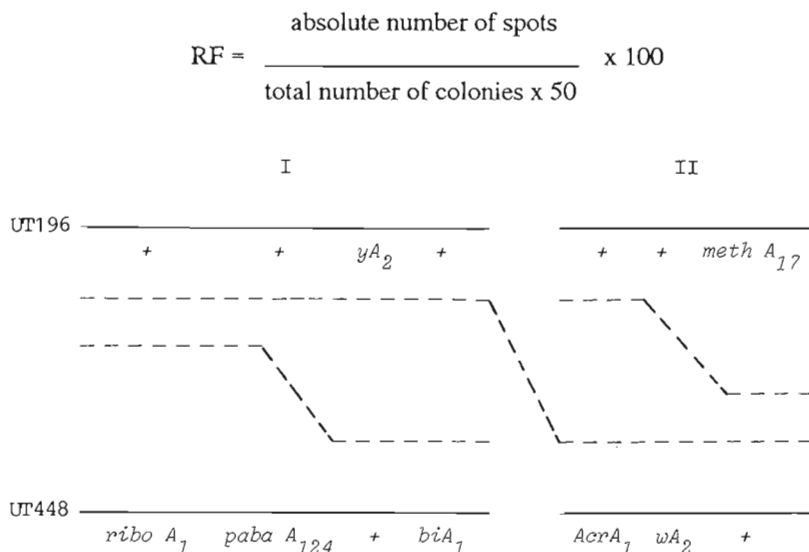
Crosses: the suspension of irradiated conidia presenting 20% survivors was diluted and seeded on CM plates in order to get 20 to 25 colonies/plate. These were incubated again for 48 h at 37°C . Conidia from the edge of each isolated colony were then individually transferred to nine defined positions (3 x 3 pattern) on CM plates. After three days of incubation at 37°C , the colonies were replicated to SM plates with a $\times 10^7$ conidia of UT 196, seeded in advance with the help of SM top-layer agar. After 48 h or incubation at 37°C the plates were sealed with adhesive tape and incubated again for 16 days.

Spot test: Fast analysis of recombination frequencies in the interval *paba-y* (I) and *meth-w* (II): After 18 days of incubation the heterokarya formed at each inoculation point, produced many cleistothecia. A large one was chosen and roughly cleaned in 3% agar. The cleistothecium was crushed against the wall of the tube containing melted MM top layer agar, in order to liberate the ascospores, and diluted successively at: 1:50 and 1:100 in melted MM top layer tubes.

The contents of each tube were then poured onto plates with SL media (SLM). After spreading, the plates were left to stand for 15 min until the top layer had settled and then incubated at 37°C for three days.

The colonies were scored on diluted plates and the spots (*i.e.* the yellow and green colonies) were scored from the undiluted plates. SLM medium selects against *meth* and *paba* and the growing *meth⁺ paba⁺* are all recombinants. The spots were mainly *y⁺* since *y⁺* is linked to *paba*. The absolute number of spots measures the *meth-w* recombination and the yellow-green ratio indicates *paba-y* recombination. Since the number of ascospores varies widely between the different crosses, the spots should be counted in relation to the total number of colonies (if the recombination is suspected to be altered). If the frequency found is different from the control (1%), further tests are needed.

The recombination frequencies were estimated, e.g., for the dilution 1:50:



Obs.: The interrupted lines indicate the more probable recombinant classes growing in the SML.

Figure 2 - Recombinants classes scored in the SML (spot-test) lacking PABA and methionine.

Figure 2 presents the possibilities of recombinant colonies growing in SML:

Genetic analysis of selected mutants: the general methodology follow Pontecorvo *et al.* (1953). Diploids were prepared through Roper's technique (1952). Haploidization was obtained after *p*-fluorohenilalanine (Morpurgo, 1961; Lhoas, 1961) or benlate (Hastie, 1970) treatment. Incubation was carried out at 37°C.

Test for UV sensitivity: colonies growing in CM plates were 10 sec. irradiated following 8 hours of pre-incubation. After irradiation the plates were again incubated in order to complete a total 48 h incubation at 37°C. Plate irradiation was made with a UV lamp General Electric G 15 T8, 15 Watts located 40 cm distant from the source. The dosage was estimated to be 26 ergs/mm²/sec.

RESULTS AND DISCUSSION

When used to induce mutations in the conidial suspension of UT448 the 60Co source emitted 15.678 KR/10cm/1 hour.

From the formulae

$$(D_1)^2/(D_2)^2 = R_2/R_1$$

the required dosis was calculated by altering the distance from the center of the source as a function of radiation dose, where:

D₁: distance of 10 cm from the center of the source

D₂: required distance

R₁: dosis per 10 cm distance

R₂: required dosis

In order to reduce the viability to 20%, about 80 KR of irradiation was needed (Table I). The suspensions of irradiated conidia were diluted and seeded on CM agar in order to score colonies and ascertain their morphologies. Conidia from the edge of each normal colony were individually crossed to UT 196 and the meiotic products obtained were submitted to the spot-test (Zucchi, 1986). Among 90 crosses, only 60 cleistothecia were fertile. The result of the spot test is in Figure 3. The mutant presenting the highest RF *meth-w* = 25.19% was selected for further and more complete analysis. This mutant was called "EC".

Table I - % of survivors of UT 448 conidia strain γ irradiated in doses varying from 0 to 100 KR. (Time of exposition: 2 hours).

Dosis KR	Distance (cm) from the center of the source	Number of colonies/plate	% of survivors
0	non irradi.	55	100
10	25.0	39	70.9
20	17.7	32	58.1
30	14.4	24	43.6
40	12.5	29	52.7
50	11.2	23	41.8
60	10.2	19	34.8
80	8.9	10	18.9
100	7.9	9	16.3

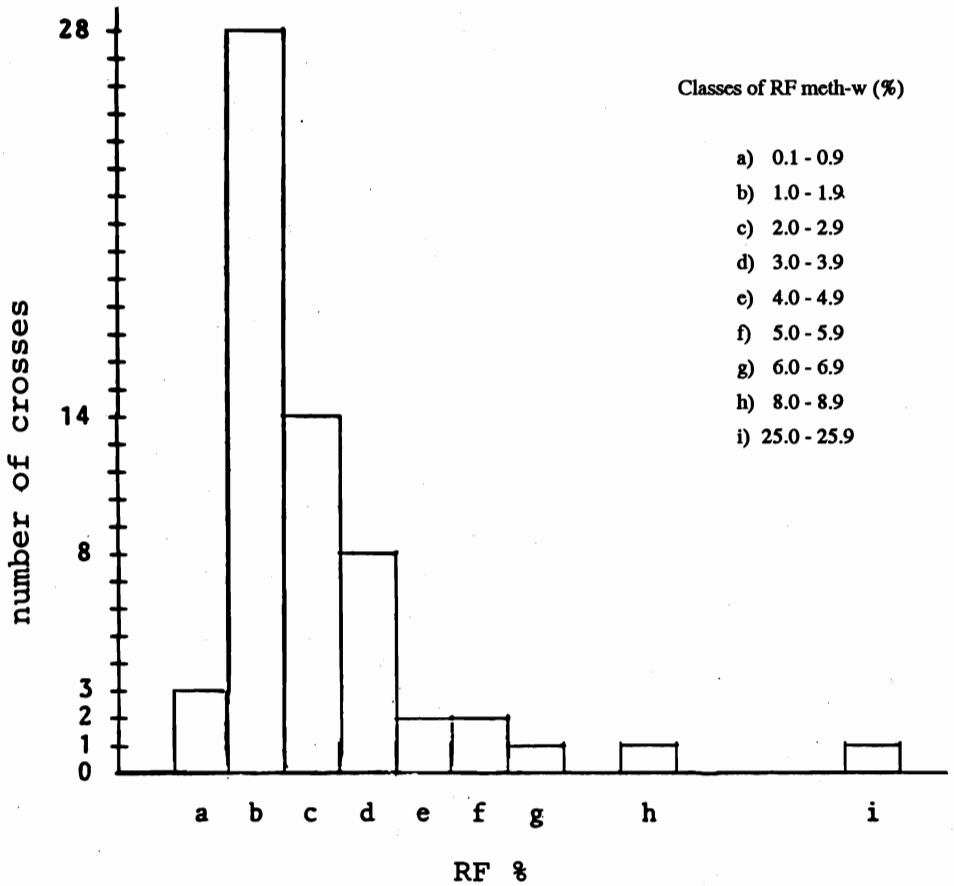


Figure 3 - RF meth-w in spot-test of the 80 KR γ irradiated UT 448 conidia.

Characterization of the EC Mutant

The EC mutant was mitotically very stable and during vegetative growth in CM no sector appeared. Seventy-five colonies derived from conidia collected from a single isolated colony were analyzed to verify their genotypes. All of them presented the same morphology and genetic markers of the normal UT 448 strain. The growth rate was also similar to that of the normal strain.

Genetic Analysis of the EC Mutant

The genetic markers of EC mutant are:

I				II		
<i>ribo A₁</i>	<i>paba A₁₂₄</i>	<i>y⁺</i>	<i>bio A₁</i>	<i>Acr A₁</i>	<i>wA₂</i>	<i>meth A₁₇</i>

Following classical methodology three additional crosses to the UT 196 testes strain were made. The meiotic analysis of the products demonstrated the meiotic behavior of all the markers of chromosomes I and II. (The control cross was UT 196 x UT 448, not irradiated). Partial results of these crosses and there between the markers are given in Tables II and III. Several alterations in the segregation and recombination frequencies of linkage group II are evident.

Table II - Recombination frequencies between the markers of chromosomes I and II.

Chromosomic intervals	UT 448 x UT 196	EC x UT 196	EC x UT 196	EC x UT 196
	control cross (275) RF (%)	cross 1 (218) RF (%)	cross 2 (589) RF (%)	cross 3 (118) RF (%)
I	<i>ribo-paba</i>	39.0	29.8	31.2
	<i>paba-y</i>	12.2	12.2	10.0
	<i>y-bio</i>	7.2	4.09	7.2
II	<i>Acr-w</i>	26.0	24.6	25.1
	<i>w-meth</i>	1.5	39.8	9.6
	<i>Acr-meth</i>	27.3	44.0	28.0

Table IV presents the relationships between markers on chromosomes I and II (*ribo-paba* and *w-meth*, respectively) in the EC x UT 196 cross (2). The main alterations were restricted to the recombinant (crossing-over classes) in chromosomes I and/or II, as follows:

Crossing-over classes	In chromosome	RF alterations
<i>ribo⁺ paba - w⁺ meth⁺</i>	I + II	14 x the control
<i>ribo paba⁺ - w meth⁺</i>	I	46 x less than the control
<i>ribo paba⁺ - w⁺ meth</i>	I	7 x less than the control

Therefore, the meiotic pattern of the classes bearing crossing-over was strongly affected.

Table III - Segregation of alleles in meiotic crosses of Ec x 196 and UT 448 x 196. Parenthesis evidence the number of analyzed colonies.

Genetical markers	448 x 196 control (275)	EC x 196 cross 1 (218)	EC x 196 cross 2 (589)	EX x 196 cross 3 (118)
<i>ribo</i>	127	85	93	58
+	128	133	496	60
<i>paba</i>	158	96	195	59
+	117	122	201	59
+	84	48	106	27
<i>y</i>	58	74	211	30
<i>bio</i>	144	79	232	62
+	131	139	357	56
<i>Acr</i>	132	159	274	52
+	143	59	315	66
<i>w</i>	133	180	299	61
+	142	38	290	57
+	133	90	317	74
<i>meth</i>	142	22	272	54

Mitotic Analysis of the EC Mutant

The analysis of the mitotic segregants of the diploid EC // UT 184 did not reveal any segregation abnormality or alteration in mitotic recombination frequencies (data not shown).

Table IV - Relationship between the *ribo-paba* (I) and *w-meth* (II) intervals (% of classes). In parenthesis is the number of analysed colonies.

EC x UT 196 cross 2 (517)			Recombinant classes (R)		Paternal classes (P)	
			<i>ribo</i> ⁺ <i>paba</i>	<i>ribo paba</i> ⁺	<i>ribo paba</i>	<i>ribo</i> ⁺ <i>paba</i> ⁺
R	<i>w meth</i>	0.6	0.2	1.3	0.8	
	<i>w</i> ⁺ <i>meth</i> ⁺	5.8	0.0	0.6	1.7	
P	<i>w meth</i> ⁺	9.2	0.2	4.4	30.0	
	<i>w</i> ⁺ <i>meth</i>	5.8	0.0	3.0	35.9	
UT 448 x UT 196 control (497)						
	<i>w meth</i>	0.0	0.0	0.0	0.2	
	<i>w</i> ⁺ <i>meth</i> ⁺	0.4	0.0	0.2	0.6	
	<i>w meth</i> ⁺	11.9	9.2	14.0	14.9	
	<i>w</i> ⁺ <i>meth</i>	11.2	7.2	16.0	14.0	

Test for Deficiency in DNA Repair

In general, mutants deficient in DNA repair are unable to remove pyrimidine dimers. Deficiency of an enzyme involved in the repair mechanism makes the mutant sensitive to low doses of UV irradiation (254 nm) and affects recombination frequencies. The EC mutant, when compared to normal strains was UV sensitive as evidenced by compact and retarded growth of the irradiated colonies after 8 h of pre-incubation. The UV dosage was 26 ergs/mm²/sec and the result was: UT 196 (*uv*⁺), UT 448 (*uv*⁺), EC (*uvs*).

Selection of Meiotic Segregants from an EC x 196 Cross

It appears the mutation in the EC mutant is present only in meiosis and after crossing-over. Some meiotic segregants derived from the EC x 196 cross were selected. These new strains (codes: 2, 6 and 1) were then crossed to normal strains and their meiotic products were analysed.

A schematic representation of their genotypes is presented in Figure 4.

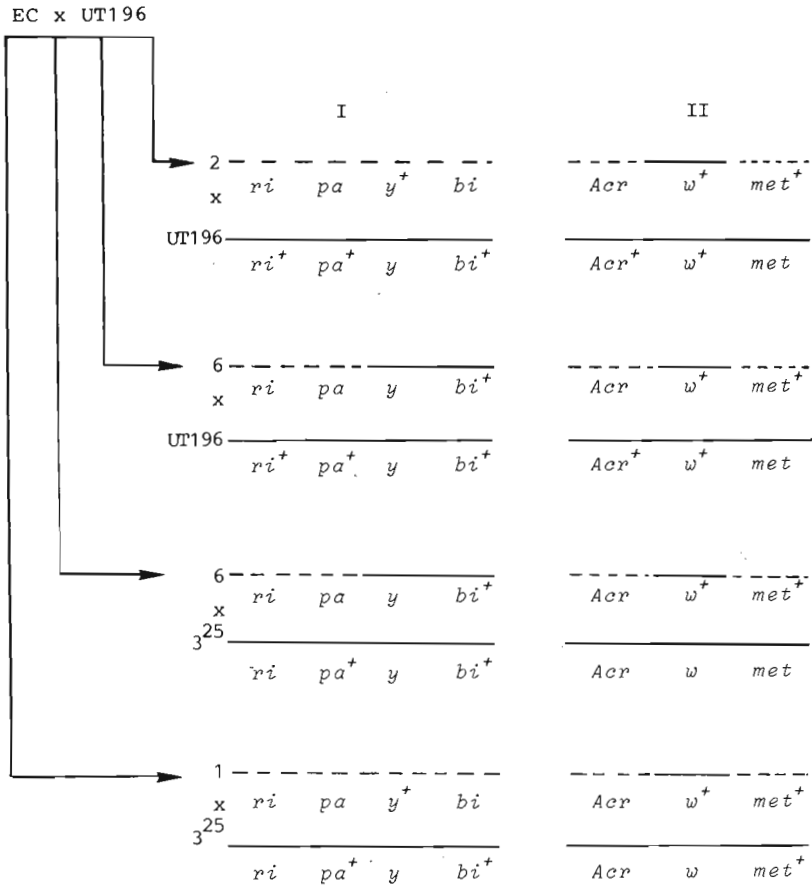


Figure 4 - Schematic representation of the meiotic segregants selected from the UT 196 x EC. - - -, Chromosomal segment from EC mutant; _____ chromosomal segment normal.

The segregation of the alleles and the recombination frequency in the several chromosomal (I and II) intervals is presented in Tables V and VI, where several alterations are evident. The unusual behavior results from the *Acr⁺/Acr* and *ribo⁺/ribo* segregation in crosses involving only *Acr* and *ribo* strains. All the cross strains (codes 2, 6 and 1) are *Acr w⁺ meth⁺* (that is, they are the result of a double crossing-over in chromosome II) and all of them have the *ribo* and *paba* markers in the chromosome I, as does the EC mutant.

Table V - Segregation of alleles in crosses between EC x EC segregants (codes 2, 6, 1) and normal strains (UT 196 and 3²⁵).

	2 x UT 196	6 x UT 196	6 x 3 ²⁵	1 x 3 ²⁵	Control UT 448 x UT 196
<i>ribo</i>	44	32	72	59	148
<i>ribo</i> ⁺	81	93	* 53	* 65	127
<i>paba</i>	52	33	70	55	117
<i>paba</i> ⁺	73	94	55	69	158
<i>y</i>	79	125	120	29	58
<i>y</i> ⁺	46	0	0	34	84
<i>bio</i>	48	-	-	61	144
<i>bio</i> ⁺	77	-	-	63	131
<i>Acr</i>	68	45	91	-	132
<i>Acr</i> ⁺	57	80	* 34	-	143
<i>meth</i>	71	77	39	49	142
<i>meth</i> ⁺	54	48	86	75	133
<i>w</i>	0	0	5	61	133
<i>w</i> ⁺	125	125	120	63	142

* Abnormal segregation.

Possibly a mutation in the *ribo-paba* interval of the EC mutant severely affects the meiotic behavior when it interacts with another element of chromosome II, close to *Acr* or to *meth*.

Anomalous segregation of the alleles could also be confirmed visually. After plating on CM the content of a crushed cleistothecia it is possible to verify the colony color variation (Table VII), which demonstrates that in the 2 x 448 cross, yellow (*y*) and "chartreuse" segregants appeared when the only colors expected would be green (*y*⁺) and white (*w*). Also in the 2 x 3²⁵ the only expected colors were yellow, green and white, but nevertheless "chartreuse" colonies also appeared. In addition it was also observed that even the *y/y*⁺ and *w/w*⁺ segregation in seven different cleistothecia of the EC x UT 196 cross presented a non-Mendelian segregation.

$$y^+/y = 199/677 \text{ and } w^+/w = 849/305.$$

Table VI - Recombination frequencies (%) in several chromosomic intervals of linkage groups I and II.

Intervals	Crosses				Control cross UT 448 x UT 196
	2 x UT 196	6 x UT 196	6 x 3 ²⁵	1 x 3 ²⁵	
<i>ribo-paba</i>	19.2	26.4	32.0	37.0	38.9
<i>ribo-y</i>	22.4	37.2	40.0	36.5	48.8
<i>ribo-bio</i>	20.8	-	-	53.9	44.0
<i>ribo-Acr</i>	26.4	38.4	39.2	-	47.6
<i>ribo-meth</i>	19.2	32.0	57.6	48.3	53.1
<i>ribo-w</i>	64.8	74.4	41.6	42.1	52.4
<i>y-Acr</i>	26.4	64.0	27.2	-	44.8
<i>y-meth</i>	40.0	61.6	30.4	46.0	55.2
<i>Acr-meth</i>	11.2	16.8	18.4	-	27.3
<i>Acr-w</i>	27.2	32.0	31.2	-	25.8
<i>w-meth</i>	28.4	30.8	33.6	61.2	1.5

Analysis of the Selfed Cleistothecia

The EC mutant and its meiotic segregants, presented clearly abnormal segregation and recombination frequencies. Such alterations varied from cross to cross, deviating from the typical meiotic pattern. The anomalous segregation of some markers such as *ribo*, *Acr* and the colors of the colonies, suggested alterations in the DNA repair system, probably acting on the heteroduplex DNA formed after crossing-over. The *uvs* character of the EC mutant supports this possibility and the final result of the altered mismatch repair should have an effect similar to that of mutator mutations. Actually, the detection of this kind of mutation is difficult because of the variation of its effects on different crosses. We therefore decided to verify the effect of this (or these) element(s) when in homozygosis. Several selfed cleistothecia of these strains were analyzed.

The data of 28 different cleistothecia originated from four EC x EC crosses are presented in Table VIII. In Table IX it is possible to verify that several other markers segregate in a more or less 1:1 fashion, except for *Acr* and *meth*. This could indicate that such markers on chromosome II do not participate in crossing-over events through meiosis and so are not submitted to the altered mismatch repair. The same is true for the *pyro* marker of chromosome IV, in cross number 3.

Table VII - Colour of the ascospore colonies segregating in different cleistothecia formed in the crosses 2 x UT 448; 2 x 3²⁵ and EC x UT 196.

Crosses	Number of cleistothecia	Colour of ascospore colonies
2 x UT 448	1	Y *
$w^+ y^+ \times w y^+$	2	Y, G *
	3	Y, G, W, cha *
	4	Y, G, W *
	5	Y, G, W *
	6	Y, G, W *
	7	Y, G, W, cha *
	8	Y, G, W, cha *
2 x 3 ²⁵	1	Y, G, W, cha *
$w^+ y^+ \times w y$	2	Y, W, cha *
	3	Y, G, W, cha *
	4	W
	5	W
	6	Y, G
	7	Y, G, W, cha *
EC x UT 196	1	no cleist
$w y^+ \times w^+ y$	2	Y, G, W
	3	Y, W
	4	Y, G, W
	5	Y, G, W
	6	Y, W
	7	Y, W
	8	no cleist
	9	Y, G, W
	10	Y, G, W
	11	Y, G, W
	12	Y, G, W
	13	Y, G, W

Obs.: Y, G, W, cha means yellow, green, white and chartreuse colonies.

* Indicates anomalous segregants.

Table VIII - Colour of the segregant colonies in several "selfed" cleistothecia of EC x EC ($w y^+ x w y^+$).

Crosses of EC x EC	Number of the selfed cleistothecia	Colour segregant
A	1, 2, 4, 6, 7, 8 5	Y, G, W Y
B	1, 3, 4, 5, 6, 7, 8, 10, 11 2, 9	Y, G, W W, Y
C	1, 2, 3, 4, 5	Y, G, W
D	1, 2, 4, 5 3	U, G, W Y, W

Obs.: Y: ($y w^+$) - yellow; G: ($y^+ w^+$) - green; W: ($? w$) - white.

Table IX - Abnormal segregation of genetic markers in "selfed" cleistothecia of EC x EC.

Genet. marker	Crosses		
	EC x EC 1	EC x EC 2	EC x EC 3
<i>ribo</i>	75	52	54
<i>ribo</i> ⁺	65	42	38
<i>paba</i>	67	46	54
<i>paba</i> ⁺	73	48	38
<i>y</i>	48	35	11
<i>y</i> ⁺	36	24	25
<i>bio</i>	65	42	41
<i>bio</i> ⁺	75	52	51
<i>Acr</i> ^R	140	90	83
<i>Acr</i> ^S	0	2	9
<i>met</i>	0	2	9
<i>met</i> ⁺	140	92	83
<i>w</i>	56	39	56
<i>w</i> ⁺	84	59	36
<i>piro</i>	not analyzed	48	9
<i>piro</i> ⁺		46	83

EC	I				II		
	<i>ribo A</i> ₁	<i>paba A</i> ₁₂₄	<i>y</i> ⁺	<i>bio A</i> ₁	<i>Acr A</i> ₁	<i>w A</i> ₂	<i>meth A</i> ₁₇

This anomalous segregation in "selfed" cleistothecia is probably related to an altered mismatch repair, acting only during meiosis and when it is in double dosage. Why *Acr* and *meth* are refractory to this effect remains to be determined whether the *uvs* character of the EC mutant is also responsible for these alterations should be clarified in a future study.

ACKNOWLEDGMENTS

Financial support by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), Brasil, is acknowledged. We are indebted to the technical assistance of Mrs. Sonia Mathias da Silva and Miss Simone Fialho da Motta.

Publication supported by FAPESP.

RESUMO

Depois da indução de mutação de uma linhagem de *Aspergillus nidulans* com 85 KR de radiação gama, os conídios sobreviventes à ação mutagênica foram cruzados individualmente com uma linhagem "testadora" e as frequências de recombinação meiótica, em um intervalo cromossômico definido, foram determinadas através do "spot-test" (Zucchi, Rev. Bras. Gen. 13: 409-424, 1990).

O mutant EC foi selecionado devido a alta RF *met-w* (25%) enquanto a RF *met-w* é 1%.

Em uma análise genética mais completa verificou-se que o mutante EC apresenta alterações que variam de cruzamento para cruzamento e apresenta um padrão meiótico anormal. A mutação em EC afeta somente a meiose desde que nenhuma alteração no crescimento vegetativo ou na estabilidade mitótica foi evidenciado. Em cleistotécios autofecundados (EC x EC) os produtos apresentaram uma grande variação no padrão de segregação dos marcadores genéticos. Algumas destas anormalidades genéticas são devido a mutação *uvs* presente no mutante EC a qual é mais eficiente quando em cruzamentos homocigotos do que em heterocigotos para esta mutação.

REFERENCES

- Clutterbuck, A.J. (1970). *Aspergillus* symbols, locus, letters and allele numbers. *Aspergillus Newsletter* 11: 25-33.
- Fortuin, J.J.H. (1971). Another two genes controlling mitotic intragenic recombination and recovery from U.V. damage in *Aspergillus nidulans* I. *Mut. Res.* 11: 149-162.
- Hastie, A.C. (1970). Benlate-induced instability of *Aspergillus* diploids. *Nature* (London) 226: 771.
- Holliday, R. (1964). A mechanism for gene conversion in fungi. *Genet. Res.* 5: 582-304.
- Holliday, R. (1967). Altered recombination frequencies in radiation sensitive strains of *Ustilago maydis*. *Mutat. Res.* 4: 275-288.
- Hotchkiss, R.D. (1974). Models of genetic recombination. *Annu. Rev. Microbiol.* 28: 445-468.

- Howard-Flanders, P. and Theriot, L. (1966). DNA repair and genetic recombination studies of mutants of *Escherichia coli* defective in these processes. *Rad. Res. (Suppl.)* 6: 154-184.
- Jansen, G.J.O. (1970). Survival of *uvr*B and *uvr*C mutants of *Aspergillus nidulans* after U.V. irradiation. *Mut. Res.* 10: 21-32.
- Kafer, E. and Mayor, O. (1986). Genetic analysis of DNA repair in *Aspergillus nidulans*: evidence for different types of MMS sensitive hyperrec mutants. *Mutat. Res.* 161: 119-134.
- Leslie, J.F. and Watt, W.B. (1986). Some evolutionary consequences of the molecular recombination process. *Trends in Genet.* 2: 288-291.
- Lhoas, P. (1961). Mitotic haploidization by treatment of *Aspergillus niger* diploids with *p*-fluorophenylalanine. *Nature* 190: 744.
- Meselson, M.S. and Radding, C.M. (1975). A general model for genetic recombination. *Proc. Natl. Acad. Sci. (USA)* 72: 358-361.
- Morpurgo, G. (1961). Somatic segregation induced by *p*-fluorophenylalanine. *Aspergillus Newsletter* 2: 10.
- Pontecorvo, G., Roper, J.A., Hemmon, L.M., McDonald, K.D. and Bufton, A.W.J. (1953). The genetics of *Aspergillus nidulans*. *Adv. Genet.* 5: 141-238.
- Roper, J.A. (1952). Production of heterozygous diploids in filamentous fungi. *Experientia* 8: 14-15.
- Szostak, J.W., Orr-Weaver, T.L., Rothstein, R.J. and Stahl, F.W. (1983). The double strand-break repair model for recombination. *Cell* 33: 25-35.
- Van de Vate, C. and Jansen, G.J.O. (1978). Meiotic recombination in a duplication strain of *Aspergillus nidulans*. *Genet. Res. Camb.* 31: 29-52.
- Whitehouse, H.L.K. (1963). A theory of crossing-over by means of hybrid deoxyribonucleic acid. *Nature* 199: 1034-1040.
- Zucchi, T.M.A.D. (1986). Estudos de fatores genéticos que alteram as frequências de recombinação em *Aspergillus nidulans*. "Livro Docência" Thesis, Faculdade de Odontologia (USP). Vols. I and II, 471 pp.
- Zucchi, T.M.A.D. (1990). Isolation of putative recombination mutants of *Aspergillus nidulans*. *Rev. Bras. Genet.* 13: 409-424. (Added after acceptance).

(Received June 16, 1989)