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STABILIZATION OF A DUPLICATED SEGMENT *Dp* (II-I) IN AN *uvs* MUTANT OF *Aspergillus nidulans* THROUGH GENETIC MECHANISMS

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ABSTRACT

This research presents an analysis of a mutant with a duplicated segment of chromosome II translocated to the *paba-y* interval of chromosome I. This insertion promotes alterations in the meiotic and mitotic behavior of the strain, mitotic instability, *uvs* character and deteriorated morphology. The *uvs* character is closely linked to the insertion point and was shown to be responsible for the mitotic instability. The removal of this mutation through recombination promotes the stabilization of the strain.

INTRODUCTION

Somatic instability in fungi in general, and in *Aspergillus nidulans* in particular, is commonly associated with a loss of duplicated genetic material and with alterations in DNA-repair mechanisms.

Artificial polyploid production in *A. nidulans*, based on heterokaryotic cells (Roper, 1952), and studied of mitotic recombination (Roper and Pritchard, 1955; Pontecorvo and Käfer, 1958), have provided new methods for genetic mapping and for detection of chromosomal aberrations, especially translocations (Käfer, 1958).

Bainbridge and Roper (1966) described meiotic crosses between normal *A. nidulans* strains and strains heterozygous for translocation which gave rise to

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segregants with duplicated segments, one of which was in a normal and the other in a translocated position.

These strains with duplicate chromosomal segments showed mitotic instability and occasionally exhibited addition or deletion of genetic material during vegetative growth, giving rise spontaneously to deteriorated or improved mutants, which appeared as colony sectors (Nga and Roper, 1968, 1969; Azevedo and Roper, 1970; Azevedo, 1975; Zucchi and Azevedo, 1979).

Substances such as caffeine (Roper *et al.*, 1972) and Trypan Blue (Cooke *et al.*, 1970) may alter the vegetative instability of these strains increasing the frequency of deletions for the duplicated segments. It has also been shown that instability of the duplicated strains is enhanced by incorporation of a *uvs* mutation (sensitivity to U.V. light) affecting DNA repair (Burr *et al.*, 1981, 1982), because the spontaneous lesions, frequently occurring in duplicate segments, can not be properly repaired, with a consequent increase in deletion frequency.

Here we describe the stabilization of a strain of *A. nidulans* bearing a *Dp* (II, I) that is also sensitive to U.V. light.

MATERIAL AND METHODS

Strains

Strains derived from Utrecht stocks (UT 184, UT 448) and others obtained in our laboratory (Z1, A1, B1 and B2) were used. The stocks are kept in CM at 5°.

Following Clutterbuck's (1970) proposition, the mutant alleles of the strains used were:

UT 448: *w*A2 (II white conidia; *ribo* A2, *paba* A124, *bi* A1 (I) with requirements for riboflavin, *P* aminobenzoic acid and biotin, respectively; *Acr* A1 (II) resistant to acriflavin.

UT 184: *cha* A1 (VIII) "chartreuse) conidia; *pyro* A4 (IV); *s*B3 (VI); *nic* B8 (VII); *ribo* B2 (VIII) requirements for pyridoxine, sodium thiosulphate, nicotinamide and riboflavin; *gal* A1 (III); *fac* A303 (V); *lac* A1 (VI), unable to grow in a medium containing galactose, sodium acetate and lactose, respectively, as the sole carbon source; *sul* A1 (I) and *Acr* A1 (II), resistant to sulphanylamide and acriflavin, respectively.

Z1 mutant: *w*A2 (II) white conidia; *ribo* A1, *paba* A124, *bi* A1 (I) requirements for riboflavin *p*-aminobenzoic acid and biotin, respectively; *Acr* A1 (II), acriflavin resistance; *uvs* and *det*, (I) sensitivity to UV light and deteriorated morphology, respectively. This mutant also presented a duplicate segment from chromosome II to I. (Zucchi, 1990a,b; Marin and Zucchi, in press).

Media and Solutions

MM - minimum medium, based on Van de Vate and Jansen (1978): 6.0 g $NaNO_3$; 1.52 g KH_2PO_4; 0.52 g $MgSO_4 . 7 H_2O$; 0.5 g KCl; 1.0 mg $FeSO_4 . 7 H_2O$; 1.0 mg $ZnSO_4 . 7 H_2O$; 1.0 mg $CuSO_4 . 5 H_2O$; 10.0 g glucose; 1.0 liter of demineralized water, pH 6.0. To prepare solid MM, 15.0 g of Difco bacto agar 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 biotin; 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 riboflavin; 0.5 mg aneurine; pH 6.0. The volume to 1.000 ml was completed with liquid MM.

Methods

The general methodology follows Pontecorvo *et al.* (1953). The diploids were prepared by the method of Roper (1952). The location of the mutant alleles and duplications on linkage groups was determined by haploidization of the diploids (Forbes, 1959) after *p*-fluorphenylalanine (*p*FP) (Morpurgo, 1961; Lhoas, 1961) treatment. The incubation temperature was 37°.

The mitotic instability test was performed by scoring the spontaneous sectors arising in colonies developed from a single conidium, after 6 days of incubation at 37°.

RESULTS

The UT 448 strain was treated with MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) (Zucchi, 1990a,b) and the Z mutant was isolated (first designation 118 V). This mutant was considered mitotically unstable, spontaneously giving rise to deteriorated and improved sectors. One of the deteriorated sectors, Z1 (Marin, 1983) showed meiotic and mitotic abnormalities, spontaneously giving origin to deteriorated and improved sectors. All the genetic alterations are due to the presence of a duplicate segment of chromosome II, translocated to chromosome I with the *w* marker (Castro-Prado and Zucchi, in press) and $meth^+$ (Marin and Zucchi, in press) markers inserted between the *paba* and *y* markers.

In order to determine if the $meth^+$ duplication was completely functional it was necessary to make the duplication heterozygous for its markers (*w*A2 and $meth^+$). Thus, a Z1//UT 196 diploid was prepared and after haploidization by *p*FP treatment, a haploid segregant was selected (A1) bearing chromosome I of Z1 (with the *w* $meth^+$ duplication) and chromosome II of the normal strain UT 196 (Figure 1). This A1

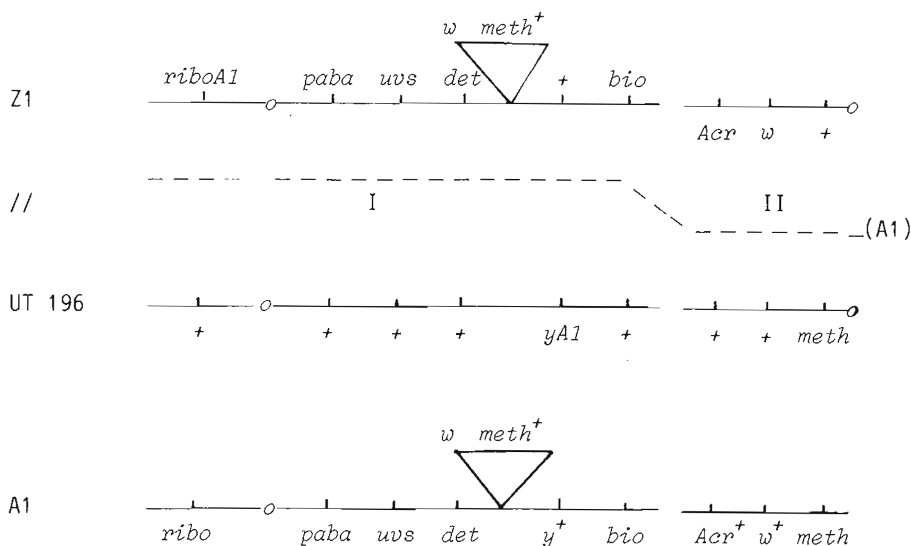

Figure 1 - Schematic representation of the Z1//UT 196 diploid and the structure of the A1 segregant.

segregant, with the *meth* A17 mutation on chromosome II, was methionine independent, thus showing that the *w* $meth^+$ duplication was functional. Analysis of this Z1//196 diploid showed several alterations, such as large numbers of $meth^+$ segregants and a strong selective pressure against the markers of chromosome I of the mutant strain (Table I). It is known that a chromosome bearing a duplicate segment is in disadvantage when haploidization is induced by *p*FP treatment (Azevedo and Roper, 1970). This could explain the results presented in Table I. Alterations were also observed in the mitotic recombination frequency between the markers of LG I and II (Table II).

The results given in Table III include only the w^+ segregants. In this cross (Z1//UT 196) w^+ is linked to *meth* (chromosome II) and is not easily separable by mitotic crossing-over. Thus, all the $meth^+$ segregants have this phenotype, owing to the $meth^+$ duplication. On the basis of these considerations, we conclude that the presence of the $meth^+$ duplication in chromosome I of the mutant promotes alterations in the recombination frequency of *paba-y* and *y-bio* and that there is an excess of double crossing-over in this region (*paba-bio* interval).

Table I - Mitotic segregation of genetic markers of the chromosomes I and II of the Z1//UT 196 diploid.

	Genetic marker	Segregants
LG I	*ribo*$^+$	79
	ribo	5
	paba$^+$	80
	paba	4
	bio$^+$	71
	bio	15
LG II	*Acr*$^+$	31
	Acr	53
	w$^+$	32
	w	52
	meth$^+$	75
	meth	9

Total number of colonies analyzed: 84 (LG = Linkage Group).

Table II - Mitotic recombination frequency between markers of chromosomes I and II in the analysis of the Z1//UT 196 diploid (LG = Linkage Group).

	Chromosomal interval	Recombination frequency (%)
LG I	*paba-y*	50.0
	paba-bio	10.7
LG II	*Acr-w*	1.2
	meth-w	27.4

In the A1 segregant, the markers involved in the duplicate segment are in a heterozygosis condition and an *uvs* mutation is also present in chromosome I. It is known that this kind of mutation is directly related to the increase of mitotic instability in strains with duplication (Bainbridge and Roper, 1966). Thus we tried to separate the *uvs* from the *meth*$^+$ duplication in order to determine its effect on the unstable behavior of this strain.

Table III - Chromosome I marker segregation related to the *meth* marker in the Z1//UT 196 diploid.

	Chromosome I			$meth^+$	*meth*	Total
(*P1*)	*paba*	∇y^+	*bio*	02	0	12
(*P2*)	$paba^+$	*y*	bio^+	03	8	11
(*R*)	*paba*	*y*	bio^+	0	0	0
	$paba^+$	y^+	*bio*	02	01	13
(*R*)	*paba*	y^+	bio^+	0	0	0
	$paba^+$	*y*	*bio*	03	0	03
(*d.c.o.*)	*paba*	*y*	*bio*	0	0	0
	$paba^+$	y^+	bio^+	13	0	13

Total number of colonies analyzed: 84. "∇" duplicate segment inserted into chromosome I. "R" recombinants class, and *d.c.o.*, double crossing-over.

We selected the B1 segregant from the progeny of the meiotic cross A1 x UT 196 (Figure 2). When tested for instability, the B1 segregant presented mitotic stability, without sectors. In this cross, the $meth^+$ meiotic segregants (Table IV) are due only to the presence of the duplicate segment w $meth^+$ in chromosome I.

Table V shows the meiotic linkage between the meth (II) and paba (I) markers.

Table IV - Meiotic segregation of genetic markers of chromosomes I and II in the A1 x UT 196 cross.

	Genetic markers	Segregants
(LG I)	$paba^+$	184
	paba	190
	uvs^+	184
	uvs	190
	det^+	182
	det	192
(LG II)	$meth^+$	183
	meth	191

Total number of colonies analyzed: 374 (LG = Linkage Group).

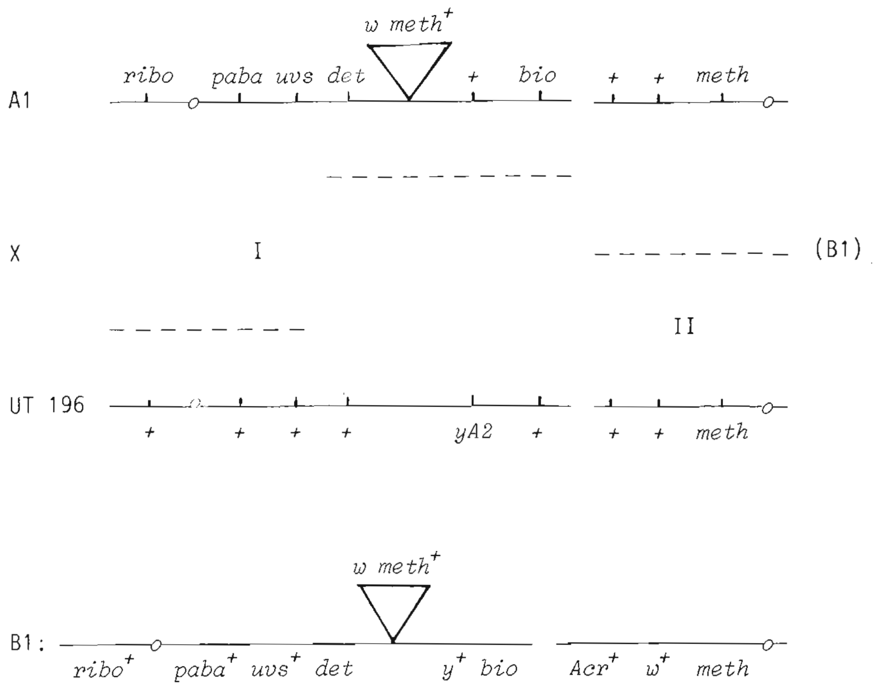

Figure 2 - Diagram of the A1 x UT 196 cross and the B1 segregant structure.

Table V - Meiotic recombination frequency of the *meth* A17 (II) marker with markers of chromosome I.

Linkage interval	Recombination frequency (%)	
	UT 448 x UT 196 (145)	A1 x UT 196 (374)
meth - ribo	46.8	37.2
meth - paba	56.5	8.3
meth - y	50.0	16.3
meth - bio	48.2	14.7
meth - uvs	-	5.1

Control cross: UT 448 x UT 196.

DISCUSSION

Strains of *A. nidulans* with duplication (one in the normal position and the other translocated from the original position) are unstable in mitosis and have reduced linear growth. Since replication errors resulted in total or partial loss of one of the duplicate segments (Nga and Roper, 1968; Azevedo and Roper, 1970; Azevedo, 1975), the strains apparently became more stable.

The effect of some chemicals upon deletion frequency in duplicate strains of *A. nidulans* suggests that the mitotic instability of these strains can be increased by inhibitors of DNA repair (Majerfeld and Roper, 1978).

The *uvs* B mutation (UV sensitivity) efficiently increases the deletion frequency and also the frequency of mitotic crossing-over between homologous segments present in double dose (Burr *et al*., 1982). In agreement with this the present work shows that the removal of the *uvs* mutation of the chromosome I restores an apparently normal behavior in mitosis. Thus the genetic instability of the Z1 mutant was partially due to spontaneous unrepaired lesions in its DNA (Roper *et al*., 1972) owing to the *uvs* mutation which interferes with the DNA repair systems.

On the other hand, the insertion of a *w meth*$^+$ duplication in the mutant chromosome I was responsible for several alterations such as:

i. High mitotic crossing-over frequency among markers of chromosome I, particularly in the interval that includes the duplicate segment (Tables II and V).

ii. Apparent increase in the mitotic crossing-over frequency among the markers of chromosome II (results not shown). It is known that the *w meth* genes (II) are closely linked, exhibiting a meiotic recombination frequency of 1% (Clutterbuck, 1981).

For these reasons the occurrence of mitotic crossing-over among these genes is practically impossible since the mitotic crossing-over frequency is 1,000 times lower than the meiotic crossing-over frequency (Pontecorvo and Käfer, 1958; Käfer, 1958).

iii. Linkage of chromosome I and II markers (*meth* and *ribo, paba, y, bio, uvs*).

iv. Alterations in meiotic segregation of the chromosome I and II markers of the B1 mutant (Castro-Prado and Zucchi, in press).

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RESUMO

Este trabalho apresenta a análise de um mutante com um segmento do cromossomo II duplicado e translocado para o intervalo *paba-y* do cromossomo I. Esta inserção promove alterações no comportamento mitótico e meiótico da linhagem. Instabilidade mitótica, caráter *uvs* e morfologia deteriorada estão relacionadas àquela mutação. O caráter *uvs* está fortemente ligado ao ponto de inserção e mostrou ser responsável pela instabilidade mitótica. A remoção desta mutação, através de recombinação, promove a estabilização da linhagem.

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