

THE EFFECTS OF A *Dp* (II-I) IN THE MEIOTIC RECOMBINATION OF *Aspergillus nidulans*

José Moacir Marin¹ and Tânia M.A. Domingues Zucchi²

ABSTRACT

This paper reports the effects of *Dp* (II-I) on the meiotic recombination of an *Aspergillus nidulans* mutant. The mutation involves a non reciprocal translocation of a segment bearing a duplication of the gene *meth*⁺ from linkage group II to I. The insertion of this segment occurs in the *paba* A₁₂₄ - *y* A₂ interval of chromosome I, promoting several genic rearrangements which alter the meiotic behavior of the genetic markers adjacent to the insertion point. The implications of the recombination when the *Dp* (II-I) is spontaneously lost by deletion or by crossing-over were also studied.

INTRODUCTION

About three decades have elapsed since studies of mitotic non-conformity in *Aspergillus nidulans* were first conducted and the occurrence of translocations in strains bearing duplicated segments was demonstrated by Baimbridge and Roper (III-VIII, 1966); Ball (III-V, 1966); Nga and Roper (I-II, 1968); Azevedo (V-VIII, 1975).

The appearance of improved and deteriorated variants in strains bearing duplicated segments was studied by Azevedo and Roper (1970). Study of mitotic instability in duplicated strains of *A. nidulans* is complicated because they are unstable (Burr *et al.*, 1982).

According to Nevers and Saedler (1977) the insertion of an exogenous element into a chromosomal region (e.g. a duplication or a transposon) generates a new genic

¹ Departamento de Ciências Morfológicas, Faculdade de Odontologia, USP, 14049 Ribeirão Preto, SP, Brasil.

² Departamento de Parasitologia, Instituto de Ciências Biomédicas, USP, Av. Prof. Lineu Prestes, 1374, 05508 São Paulo, SP, Brasil. Send correspondence to T.M.A.D.Z.

rearrangement, producing meiotic and recombination abnormalities involving that region.

Zucchi and Azevedo (1979) analysed deteriorated sectors of a strain with *Dp* (III-VIII) and observed that the duplication affects mitotic crossing-over, increasing the recombination frequency between the duplicate segments. Similarly van de Vate and Jansen (1978) verified that chromosomal duplication affects meiotic crossing-over (between duplicate segments). It seems clear that, in some cases, duplication, instability and alterations in the recombination frequency are closely related.

For the present research, several meiotic recombinants were selected. These recombinants originated from crosses of a normal strain and a duplicate one, including the spontaneous sectors of the latter. The aim was to verify the effect of duplication in meiosis and that promoted by the spontaneous loss of the duplication, either by deletion or by crossing-over.

MATERIAL AND METHODS

Strains

The strains used here were the same mentioned on previous papers (Zucchi, 1990a; Marin and Zucchi, 1991), such as: UT 448, UT 196 and the mutants Z and Z1.

Media

The minimum medium (MM) was Czapeck Dox with 1% (w/v) glucose. Complete medium (CM) contained yeast extract, hydrolysed casein, hydrolysed nucleic acids, vitamins etc. (Pontecorvo *et al.*, 1953, modified by Van de Vate and Jansen, 1978). The solid medium contained 1.5% agar.

Methods

The general methodology follows Pontecorvo *et al.* (1953). The mitotic instability was verified through the appearance of spontaneous sectors in colonies after seven days of incubation at 37°C. The test for UV sensitivity of strains and its meiotic segregants was done with UV irradiation of the colonies as follows:

1. Non-irradiated (control).
2. Without pre-incubation, 30 sec. of irradiation.
3. 8 h of pre-incubation, 30 sec. of irradiation.
4. 18 h of pre-incubation, 30 sec. of irradiation.

The four CM plates were incubated at 37°C in order to complete 48 h. Cultures were irradiated with a General Electric G 15T8, 15 Watt germicidal lamp. The plates were irradiated at 40 cm distance. The estimated dosage was 26 ergs/mm²/sec.

Selection of Meiotic Segregants

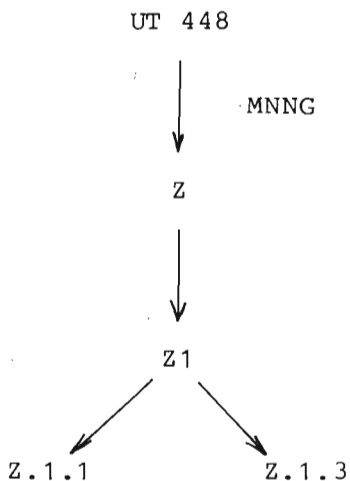
When more suitable markers in a strain were needed, recombinant (crossing-over) segregants from Z and its sectors crossed with a normal strain were selected.

RESULTS AND DISCUSSION

The UT 448 strain was mutagenized with MNNG (N-methyl-N'-nitro-N-Nitrosoguanidine) (Zucchi, 1986, 1990) and, among others, the Z mutant was selected mainly because of its mitotic instability, giving origin to deteriorated and improved sectors, spontaneously. The deteriorated sector Z1 showed mitotic instability giving rise to 2nd order improved and deteriorated sectors. Genetic analysis of 21 sectors demonstrated the presence of a duplicated segment bearing the *meth*⁺ segment transposed from chromosome II to chromosome I. The spontaneous 2nd order spontaneous deteriorated sectors result from the translocation of the extra-segment, while the improved ones appear after the spontaneous loss of the duplication. It was also verified that the insertion of the duplicated segment in linkage group I promotes phenotypic alterations of the deteriorated sectors such as the *det* character, dark color of the colony, scarce conidiation and UV sensitivity (*uvs*) (Marin, 1983; Marin and Zucchi, 1991). Figure 1 shows the main characteristics of the strains.

Through crossing-over in the *paba-y* interval of chromosomal I it was possible to substitute different chromosomal segments of the mutant with normal ones. Using these segregants in new meiotic crosses to the normal strain, it was possible to verify the consequences of the substitutions in the meiotic behavior of the *meth-w* interval of chromosome II.

The mutant strain sectors, Z1, Z1.1 and Z1.3 when crossed to UT 196 gave origin to the 10⁹, 24⁴ and 11⁸ recombinants, respectively, exhibiting the phenotypes and genotypes listed in Table I. These recombinants were crossed with UT 196 and the alterations in the RF *meth-w* were observed (Table II). In a previous paper we presented the RF *meth-w* of Z, Z1, Z1.1, Z1.3 and UT 448 when they were crossed to UT 196 and a summary of these data is given in Table III (Marin and Zucchi, 1991).



Characteristics

Strains	Morphology*	Instability sector/colony	UV sensitivity**
Z	N	0.11	+
Z.1	D	0.24	<i>ws</i>
Z.1.1	I	0.02	+
Z.1.3	D	0.14	<i>ws</i>

* N: normal; D: deteriorated; I: improved morphology.

** *ws*: higher UV sensitivity; + normal UV sensitivity.

Figure 1 - Origin of the Z mutant and its 1st and 2nd order sectors, including characteristics.

Table I - Genotypes and phenotypes of the three selected recombinants from two different meiotic crosses.

Origin	Code of selected recombinant	Genotypes				Phenotype morphology and UV sensitivity
UT 196 x Z1.1		<i>ribo</i> A ₁	<i>paba</i> ⁺	<i>y</i> A ₂	<i>bi</i> A ₁	
Z1.1: improved sector from Z1	10 ⁹	I	-----			N
		II	-----			<i>uv</i> ⁺
			<i>Acr</i> A ₁	<i>w</i> A ₂	<i>meth</i> ⁺	
UT 196 x Z1.3		<i>ribo</i> A ₁	<i>paba</i> ⁺	<i>y</i> ⁺	<i>bi</i> A ₁	
Z1.3: deteriorated sector from Z1	24 ⁴	I	-----			<i>det</i>
		II	-----			<i>uvs</i>
			<i>Acr</i> A ₁	<i>w</i> A ₂	<i>meth</i> ⁺	
UT 196 x Z1.3		<i>ribo</i> A ₁	<i>paba</i> ⁺	<i>y</i> ⁺	<i>bi</i> A ₁	
Z1.3: deteriorated sector from Z1	11 ⁸	I	-----			N
		II	-----			<i>uv</i> ⁺
			<i>Acr</i> A ₁	<i>w</i> A ₂	<i>meth</i> ⁺	

----- Indicates chromosomal segments from mutants strains.

----- Indicates chromosomal segments from the normal UT 196 strain.

Table II - *meth-w* recombination frequencies in the crosses of a normal UT 196 with the selected meiotic segregants.

Code of the selected recombinants	Phenotypes of the colonies	Crossed with	RF <i>meth-w</i> (%)
10 ⁹	N, <i>uv</i> ⁺	UT 196	4.3
24 ⁴	<i>det</i> , <i>uvs</i>	UT 196	35.8
11 ⁸	N, <i>uv</i> ⁺	UT 196	2.0

Table III - RF *meth-w* of meiotic crosses of mutant and their 1st and 2nd order sectors with UT 196. Control cross is UT 448 x UT 196. Some characteristics of the strains crossed are given.

Crosses	RF <i>meth-w</i> (%)	Characteristics
UT 448 x UT 196	1%	448: normal, <i>uv</i> ⁺ , stable (0%)
Z x UT 196	5.8%	Z: normal, <i>uv</i> ⁺ , unstable (11%)
Z1 x UT 196	23%	Z1: <i>det</i> , <i>uvs</i> , unstable (24%)
Z1.1 x UT 196	2%	Z1.1: improved, <i>uv</i> ⁺ , stable (2%)
Z1.3 x UT 196	27%	Z1.3: <i>det</i> , <i>uvs</i> , unstable (14%)

The % of sectors/colony are given in parentheses ().

When the *paba-y*⁺ of the mutant is involved in the crossing over of chromosome I alterations appear in the RF *meth-w* of chromosome II (Tables II and III). In other words, the RF *meth-w* seems dependent on the genotype of chromosome I and on the presence or not of the *meth*⁺ duplication.

The 10⁹ segregant showed typical behavior of those strains which have lost a duplication through mitotic instability. The loss, in this case, does not promote alterations in the allelic segregation.

Nevertheless the 24⁴ segregant showed an allelic segregation different from 1:1. There were alterations in the distribution of recombinant classes of chromosome I, including the appearance of a high incidence of double crossing-over, as shown in Table IV. There was an alteration in the distribution of the recombinant classes and the presence of the *uvs* element promoted alterations in the crossing-over in the regions adjacent to this *locus* also increasing the number of double crossing-overs in this interval. A similar effect was observed by Burr *et al.* (1982) in relation to the *uvs* B, which increases the mitotic intragenic crossing-over frequency, probably caused by the existence of recombinogenic lesions.

The segregant classes (double or single crossing-over) bearing *uvs* are less frequent than the reciprocal ones, perhaps due to inviability (Table IV).

Castro-Prado (1986, 1991), analysing the Z1 mutant, observed that removing the *uvs* mutation, through crossing-over, restores mitotic stability even in the presence of the *meth*⁺ duplication.

Table V also demonstrates the distortions due to the duplication in linkage group I. The effect of *uvs* and *Dp* (II-I) in the segregants is a drastic reduction of classes *c* and *d*. Additionally, the classes with double crossing-over in linkage group I (*uvs* excluded)

are much more viable (classes *a* and *b*), suggesting that *uvs* inviabilizes these classes because of the impossibility of repairing recombinogenic lesions.

Table IV - Recombination in chromosomal I of the UT 196 x 24⁴ cross.

	Crossed strains			Recombinants			
24 ⁴				<i>ribo</i>	+	-	55
				+	<i>uvs</i>	-	6
					<i>uvs</i>	<i>y</i>	5
UT 196					+	+	35
				<i>ribo</i>	+	+	14
				+	<i>uvs</i>	<i>y</i>	2

Table V - *uvs*, *Dp* (II-I) and *meth*⁺ segregation in the 24⁴ x 196 cross.

	Proposed genotype of crossed paternals				Segregants		
	I	II	I	II			
					<i>meth</i> ⁺		
						<i>yA2</i>	+ /+ <i>meth</i>
							= 26 (a)
					<i>meth</i> ⁺		
						+	<i>biA1</i> /+ <i>meth</i>
							= 28 (b)
24 ⁴	<i>uvs</i>	<i>biA1</i>	<i>wA2</i>	+			
					<i>meth</i> ⁺		
						+	+ /+ <i>meth</i>
							= 0 (c)
					<i>meth</i> ⁺		
						+	<i>biA1</i> /+ <i>meth</i>
							= 6 (d)
UT 196	+	<i>yA2</i>	+	+ <i>meth A17</i>	<i>uvs</i>		

Analysis of 11⁸ x UT 196 (11⁸ is a segregant from Z1.3 x UT 196, Table I) showed that this segregant no longer bears the *meth*⁺ duplication in linkage group I, which means that it was lost through crossing-over, during the meiotic cycle. The loss of the

duplication generates a recombinogenic site which, in another cross produces anomalous segregation of the *paba locus* (data not shown).

Strains bearing chromosomal duplications are mitotically unstable and generally the mode of instability depends on the nature of the duplicated segment and on its position in the genome.

The meiotic alterations we found in strains were due exclusively to a duplicate segment in chromosome I. The crosses of meiotic segregants with a normal strain permitted conclusions about the relationships of several alterations in meiosis, when the duplication is present or not in the crossed strain, in the translocated position.

When the duplication is maintained throughout meiosis, the result is a disorganization in the recombination of the chromosome I markers, next to the insertion place. The analysis of the $24^4 \times$ UT 196 cross showed that the UV sensitivity character (*uvs*) and not the duplication (Table V) is responsible for the low viability of the *uvs* segregants.

Borts *et al.* (1984) demonstrated through DNA recombinant technology, that in *Saccharomyces cerevisiae* the insertion of a chromosomal segment stimulates recombination at the insertion point, disorganizing the adjacent *loci*. According to McClintock (in Dellaporta *et al.*, 1984) a chromosomal insertion can stimulate a response similar to that of the SOS repair system in *E. coli*.

Majerfeld and Roper (1978) suggested that haploid duplicated strains of *A. nidulans* present two classes of spontaneous lesions in DNA: one recombinogenic and the other not. The latter, if not repaired, results in deletion of the duplication. Our data suggest that loss of the duplication can occur both two ways, promoting or not rearrangements adjacent to the insertion point of the duplication. Thus loss of the duplication through meiotic crossing-over, may originate a kind of lesion refractory to the action of a repair enzyme, resulting in abnormal segregation as a consequence of deficient repair.

ACKNOWLEDGMENTS

Financial support by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Proc. 402534/82) and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Proc. 83/0154/0), is acknowledged.

Publication supported by FAPESP.

RESUMO

Este trabalho mostra o efeito da *Dp* (II-I) na recombinação meiótica de um mutante de *Aspergillus nidulans*. Esta mutação é uma translocação não recíproca de um segmento carregando duplicação do gene *meth*⁺ do grupo de ligação II para o I. A inserção deste segmento ocorre no intervalo *paba* A₁₂₄ - y A₂ do cromossomo I, promovendo vários rearranjos gênicos que são responsáveis pelas alterações no comportamento meiótico de

marcadores genéticos adjacentes ao ponto de inserção. Foi também verificado o que ocorre na recombinação quando a *Dp* (II-I) é perdida espontaneamente por deleção ou por crossing-over.

REFERENCES

- Azevedo, J.L. (1975). Altered instability due to genetic changes in a duplication strain of *Aspergillus nidulans*. *Genet. Res.* 26: 55-61.
- Azevedo, J.L. and Roper, J.A. (1970). Mitotic non conformity in *Aspergillus*: successive and transposable genetic changes. *Genet. Res.* 16: 79-93.
- Bainbridge, B.W. and Roper, J.A. (1966). Observations on the effects of a chromosome duplication in *Aspergillus nidulans*. *J. Gen. Microbiol.* 42: 417-424.
- Ball, C. (1966). Instability associated with chromosome translocation in *Aspergillus nidulans*. *Heredity* 21: 531.
- Borts, R.H., Lichten, M., Hearn, M., Davidow, L.S. and Haber, J.E. (1984). Physical monitoring of meiotic recombination in *Saccharomyces cerevisiae*. *Cold Spring Harbor Symposia on Quantitative Biology* 49: 67-76.
- Burr, K.W., Roper, J.A. and Relton, J. (1982). Modification of chromosome instability in *Aspergillus nidulans*. *J. Gen. Microbiol.* 128: 2899-2907.
- Castro Prado, M.A.A. (1986). Estabilização de genes em dupla dose entre alguns mutantes *hiper-rec-uvs* de *Aspergillus nidulans*. Master's Thesis, USP, Ribeirão Preto.
- * Castro Prado, M.A.A. and Zucchi, T.M.A.D. (1991). Meiotic segregation of a recessive gene (*w A*₂) included in a *Dp* (II-I) of *Aspergillus nidulans*. *Rev. Bras. Genet.* 14: 249-260.
- * Castro Prado, M.A.A. and Zucchi, T.M.A.D. (1991). Stabilization of a duplicated segment *Dp* (II-I) in an *uvs* mutant of *Aspergillus nidulans* through genetic mechanisms. *Rev. Bras. Genet.* 14: 239-248.
- Dellaporta, S.L., Chamet, P.S., Mottinger, J.P., Woods, J.A., Yu, S.M. and Hicks, J.B. (1984). Endogenous transposable elements associated with virus infection in maize. *Cold Spring Harbor Symposia on Quantitative Biology* 49: 321-328.
- Majerfeld, I.H. and Roper, J.A. (1978). The effects of coumarin on the frequency of deletions in a duplication strain of *Aspergillus nidulans*. *Mol. Gen. Genet.* 159: 203-206.
- Marin, J.M. (1983). Estudo de fatores genéticos que afetam a estabilidade mitótica e recombinação em *Aspergillus nidulans*. Master's Thesis, USP, Ribeirão Preto.
- * Marin, J.M. and Zucchi, T.M.A.D. (1991). Genetic analysis of some factors affecting mitotic and meiotic behavior of a mutant of *Aspergillus nidulans*. *Rev. Bras. Genet.* 14: 9-20.
- Nevers, P. and Saedler, H. (1977). Transposable genetic elements as agents of gene instability and chromosomal rearrangements. *Nature* 268: 109-115.
- Nga, B.H. and Roper, J.A. (1968). Quantitative intrachromosomal changes arising at mitosis in *Aspergillus nidulans*. *Genetics* 58: 193-209.
- Pontecorvo, G., Roper, J.A., Hemmons, L.M., McDonald, K.D. and Bufton, A.W.J. (1953). The genetics of *Aspergillus nidulans*. *Adv. Genet.* 5: 141-238.

- Van de Vate, C. and Jansen, G.J.O. (1978). Meiotic recombination in a duplication strain of *Aspergillus nidulans*. *Genet. Res.* 31: 29-52.
- Zucchi, T.M.A.D. (1986). Estudos de fatores genéticos que afetam as frequências de recombinação em *Aspergillus nidulans* (EIDAM) WINTER. Associate Professor's Thesis, USP, Ribeirão Preto.
- * Zucchi, T.M.A.D. (1990). Isolation of putative recombination mutants of *Aspergillus nidulans*. *Rev. Bras. Genet.* 13: 409-424.
- Zucchi, T.M.A.D. and Azevedo, J.L. (1979). Mitotic instability in a III-VIII duplications strain of *Aspergillus nidulans*. *Rev. Bras. Genet.* 2: 93-108.

(Received June 16, 1989)