

## CHARACTERIZATION OF THE Dp (II,I) DUPLICATION IN *Aspergillus nidulans*: PRESENCE OF THE *Acr A<sub>1</sub>* GENE AND ITS REGULATORY TRANSCRIPTION SEQUENCE IN THE TRANSPOSED SEGMENT

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### ABSTRACT

Segregation of *w* and *Acr* markers was demonstrated in self-crossed strains of *Aspergillus nidulans* that possess the Dp (II,I) duplication, and sensitivity to acriflavin.

Segregation of gene *w* confirms heterozygosity of this gene in strains that bear *w*<sup>+</sup> on chromosome I (duplication) and *w* on chromosome II. On the other hand, the segregation of gene *Acr* suggests the presence of this gene in the Dp (II,I) duplication, making the strains heterozygous for the *Acr* gene: *Acr*<sup>+</sup> (chromosome II) and *Acr* (duplication, chromosome I). This result is only possible if a regulatory mutant sequence is present in the duplicated segment, repressing the expression of *Acr*, since *Acr/Acr*<sup>+</sup> strains present an intermediate phenotype *Acr*<sup>±</sup> between *Acr*<sup>+</sup> (sensitive to acriflavin) and *Acr* (resistant to acriflavin) strains.

This hypothesis was confirmed by the detection of *Acr*<sup>+</sup> segregants in heterozygous and homozygous crosses for the Dp (II,I) duplication.

### INTRODUCTION

In eukaryotic systems, RNA polymerase II requires two regulatory sequences to initiate the transcription of structural genes: the promoter site (Hahn *et al.*, 1985; Struhl, 1986) and the "enhancer" elements, as has been found in mammalian cells (Ephrussi *et al.*, 1985; Croce and Klein, 1985), and the UAS sequences (upstream activator sequences)

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in yeasts (West *et al.*, 1984; Struhl, 1984). Such sequences, when recognized by transdiffusible factors, can control transcription.

The integrity of such sequences is directly related to their activities since mutations in specific regions of these regulators can completely hinder genic expression (Jarren and Meselson, 1991; Xia *et al.*, 1991).

In *Saccharomyces cerevisiae* the efficient transcription of gene ARG 4 requires several regulatory sites: two upstream activator sequences (UAS) and the TATA site of the promoter ARG 4. Such sequences can interact specifically with the GCN<sub>4</sub> protein (Hinnebush, 1988), which regulates the transcription of several genes related to amino acid biosynthesis. Deficiency mutations of two upstream activator sequences (UAS) have proven to be unable to derepress the ARG 4 gene, under conditions of amino acid starvation (Thiry-Blaise and Lopes, 1990).

Activation of the *arg B* gene of *Aspergillus nidulans* in *Escherichia coli* can occur through a DNA rearrangement, e.g. insertion of the IS2 element next to the 5' terminus of the gene (Dmochowska *et al.*, 1986). Many bacterial genes present transcription activation after insertion of IS2, which is probably the donor of the -35 element of the prokaryotic promoter (Jaurin and Nomark, 1983).

In prokaryotic systems, mutations involving the promoter site can either stimulate or block transcription (Reiter *et al.*, 1990; Lucht and Bremer, 1991).

Base substitution inside the -35 region of the promoter *Iac UV5* of *E. coli* affects both the affinity of RNA-polymerases for the promoter and the frequency of formation of the open complex (Kobayashi *et al.*, 1990).

The Dp (II,I) duplication of *A. nidulans* includes the *w* A<sub>2</sub> and *meth*<sup>+</sup> markers, transposed from chromosome II to chromosome I and inserted between the *pabaA*<sub>124</sub> and *yA*<sub>2</sub> genes (Castro-Prado and Zucchi, 1991). The B<sub>1</sub> strain, bearing the duplication Dp (II,I), is heterozygous for the markers present in double dose. This strain, which is sensitive to acriflavin in selfed crosses, shows segregation of genes *w* and *Acr* (Castro-Prado and Zucchi, 1991). The segregation of *w* reveals the heterozygosity of this marker in strain B<sub>1</sub>, but the segregation of *Acr* suggests the presence of this gene in the Dp (II,I) duplication, turning B<sub>1</sub> heterozygous for the three markers, i.e., *Acr*, *w*, *meth*<sup>+</sup> (chromosome I, duplication transposed) and *Acr*<sup>+</sup>, *w*<sup>+</sup>, *meth* (chromosome II, normal position). Although heterozygous for gene *Acr*, strain B<sub>1</sub> is phenotypically sensitive to acriflavin.

## MATERIALS AND METHODS

### *Strains and Derivatives*

Strains derived from Utrecht stocks (UT 448, UT 196) and others maintained in our laboratory (D<sub>1</sub>, B<sub>1</sub> 20<sup>2</sup>, NM<sub>1</sub>, 1<sup>21</sup>, 2<sup>22</sup> and 1<sup>7</sup>) were used. The stocks are kept in CM at 5°C.

Following Clutterbuck (1970) the mutant alleles of the strains used were:

UT 448: *w* A<sub>2</sub> (II) white conidia; *ribo* A<sub>1</sub>, *paba* A<sub>124</sub>, *bio* A<sub>1</sub> (I) with requirements for riboflavin, *p*-aminobenzoic acid and biotin, respectively; *Acr* A<sub>1</sub> (II) resistant to acriflavin.

UT 196: *y* A<sub>2</sub> (I) yellow conidia; *meth* A<sub>17</sub> (II); *pyro* A<sub>4</sub> (IV) with requirements for methionine and pyridoxine.

D<sub>1</sub>: *bio* A<sub>1</sub> (I); *meth* A<sub>17</sub> (II), with requirements for biotin and methionine; Dp (II,I) duplicated segment on chromosome I.

B<sub>1</sub>: *bio* A<sub>1</sub> (I); *meth* A<sub>17</sub> (II), with requirements for biotin and methionine; Dp (II,I), duplicated segment on chromosome I.

20<sup>2</sup>: *bio* A<sub>1</sub> (I); *meth* A<sub>17</sub> (II), with requirement for biotin and methionine.

NM<sub>1</sub>: *y* A<sub>2</sub> (I), yellow conidia, *meth* A<sub>17</sub> (II), *pyro* A<sub>4</sub> (IV); *nic* B<sub>2</sub> (VII), with requirement for methionine, pyridoxine and nicotinamide, *fac* A 303 (V), unable to grow on a medium containing sodium acetate; *Acr* A<sub>1</sub> (II), resistant to acriflavin.

1<sup>7</sup>: *w* A<sub>2</sub> (II) white conidia; Dp (II,I) with *RS*<sup>+</sup>, duplicated segment on chromosome I with *RS*<sup>+</sup> near the *Acr* marker.

1<sup>21</sup>: *w* A<sub>2</sub> (II) white conidia; Dp (II,I) with *RS*<sup>+</sup>, duplicated segment on chromosome I with *RS*<sup>+</sup> near the *Acr* marker.

2<sup>22</sup>: *w* A<sub>2</sub> (II) white conidia; *paba* A<sub>124</sub>, *bio* A<sub>1</sub> (I) with requirement for *p*-aminobenzoic acid and biotin; Dp (II,I) with *RS*<sup>+</sup>, duplicated segment on chromosome I with *RS*<sup>+</sup> near to *Acr* marker.

### Media and Solutions

Complete (CM) and minimum (MM) media were those described by Van de Vate and Jansen, 1978. For solid medium, 1.5% Difco Bacto Agar was added.

### Methods

Genetic analysis was carried out according to Pontecorvo *et al.* (1953) and the diploid strains were prepared by the method of Roper (1952). Haplodization was performed using a germicidal GE UV lamp, 15 watts G 1578, located 20 cm from the cultures for 15 sec.

The estimated dose of optic energy used was  $3.2 \times 10^{-4}$  watts/cm<sup>2</sup>. The incubation temperature was 37°C.

## RESULTS

The D<sub>1</sub> strain recovered from the progeny of the B<sub>1</sub> x 20<sup>2</sup> cross (Figure 1) was mitotically stable despite the Dp (II,I) duplication. Self-crosses (D<sub>1</sub> x D<sub>1</sub>) yielded a very

reduced number of a single class of phenotypic progeny which differed from the parental class, i.e., *bio*<sup>+</sup> (I); *Acr*, *w*, *meth*<sup>+</sup> (II). A previous analysis (Castro-Prado and Zucchi, unpublished results) provided strong evidence for the action of RIP (rearrangements induced pre-meiotically; Selker, 1990) on the *w* gene which could explain the absence of *w*<sup>+</sup> segregants and the reduced number of viable progeny in all cleistothecia analyzed. The segregation of the *Acr* gene in these crosses suggested the presence of this gene in the duplicate segment, which turned the D<sub>1</sub> strain heterozygous for three markers of the Dp (II,I) duplication: *Acr*, *w*, *meth*<sup>+</sup> (chromosome I, duplication)/*Acr*<sup>+</sup>, *w*<sup>+</sup>, *meth*<sup>+</sup> (chromosome II, normal position). Thus, the expressed phenotype was acriflavin sensitive (*Acr*<sup>+</sup>), had green conidia and was methionine independent. Evidently, due to the D<sub>1</sub> heterozygosity for the *Acr* marker (*Acr*/*Acr*<sup>+</sup>) and the semidominance relationship between the *Acr*<sup>+</sup> and *Acr* alleles (Kafer and Mayor, 1986), an intermediate *Acr*<sup>±</sup> phenotype was expected (Figure 2). Since D<sub>1</sub> was sensitive to acriflavin (*Acr*<sup>+</sup>), the presence of a mutant regulatory site (*RS*) of *Acr* expression seemed possible. Such a *RS* site, close to the *Acr* marker (I), could affect *Acr* gene transcription and the mutant site could be altered by the insertion of the duplication into chromosome I. In addition, *RS* may be one of the sequences recognized by the RNA-polymerase to initiate the transcription of eukaryotic structural genes such as the promoter, UAS or enhancer, the integrity of which acts decisively in the positive control of transcription.

During prophase I of meiosis, the *Acr* segregants from the selfed cross D<sub>1</sub> x D<sub>1</sub> may be those recovering the wild *RS*<sup>+</sup> sequence by crossing over (Figure 3) through the pairing of duplicate segments in non-homologous chromosomes I and II, in the quadrivalent configuration (Castro-Prado and Zucchi, 1991). Confirmation of the hypothesis that *RS*<sup>+</sup> can be recovered by crossing-over, was provided by the analysis of haploid segregants of the D<sub>1</sub>//NM<sub>1</sub> diploid, haploidized with a low UV dose. This is how

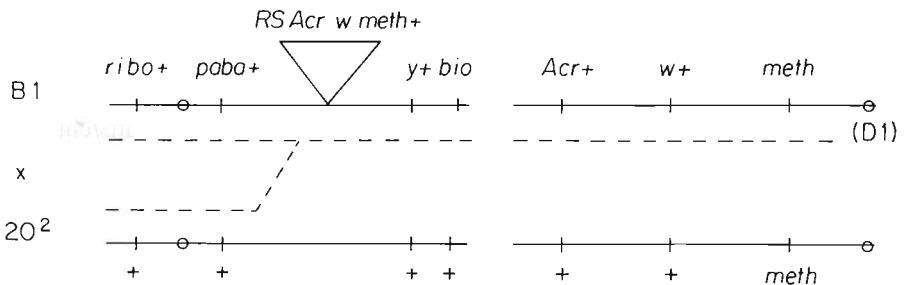


Figure 1 - Diagram of the B<sub>1</sub> x 20<sup>2</sup> cross and the D<sub>1</sub> segregant structure.

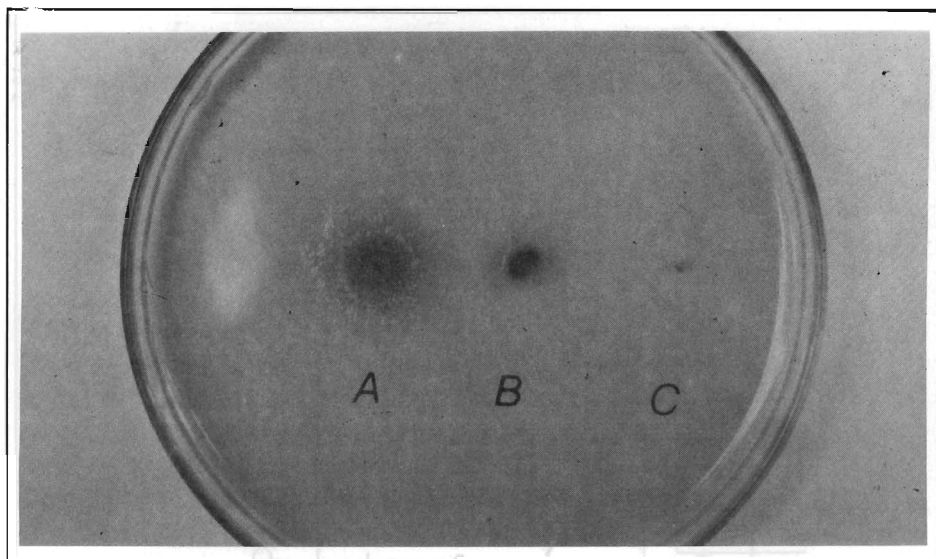


Figure 2 - Morphological characteristics of *Acr* (A), *Acr<sup>+</sup>/Acr* (*Acr<sup>±</sup>*) (B) and *Acr<sup>+</sup>* (C) strains.

strains with the *Acr<sup>±</sup>* phenotypes were recovered (Figure 4). Segregation of the *w* marker was observed among the haploids, giving a frequency of  $2 w^+ : 1 w$ , indicating mitotic pairing between homologous segments of chromosomes I and II.

Among the haploids, a segregant ( $1^7$ ; Figure 6) with phenotype *Acr<sup>±</sup>*, *w*, *meth<sup>+</sup>*, was selected. This segregant probably originated from pairing of chromosome I (D<sub>1</sub>) and II (D<sub>1</sub>) during mitosis before haploidization (Figure 5). Under such conditions,  $1^7$  was a partial diploid for the *Acr* marker. The proposed crossing-over model is shown in Figure 5, on the basis of evidence showing that UV irradiation increases the mitotic recombination frequency in diploids since only one of the members possesses a duplication (Pires, 1988).

In order to determine whether the genotype strain  $1^7$  really was:

(I)  $RS^+ Acr$  / (II)  $RS^+ Acr^+$

this strain was crossed with the Master UT 448 ( $RS^+ Acr$ ). The presence of *Acr<sup>±</sup>* segregants in the meiotic progeny would prove the heterozygosity of *Acr* in the  $1^7$  strain. The phenotypic classes *Acr<sup>+</sup>*, *Acr<sup>±</sup>* and *Acr* were recovered (Table I). The presence of *Acr<sup>+</sup>* among the segregants confirmed the proposed genotype for  $1^7$ .

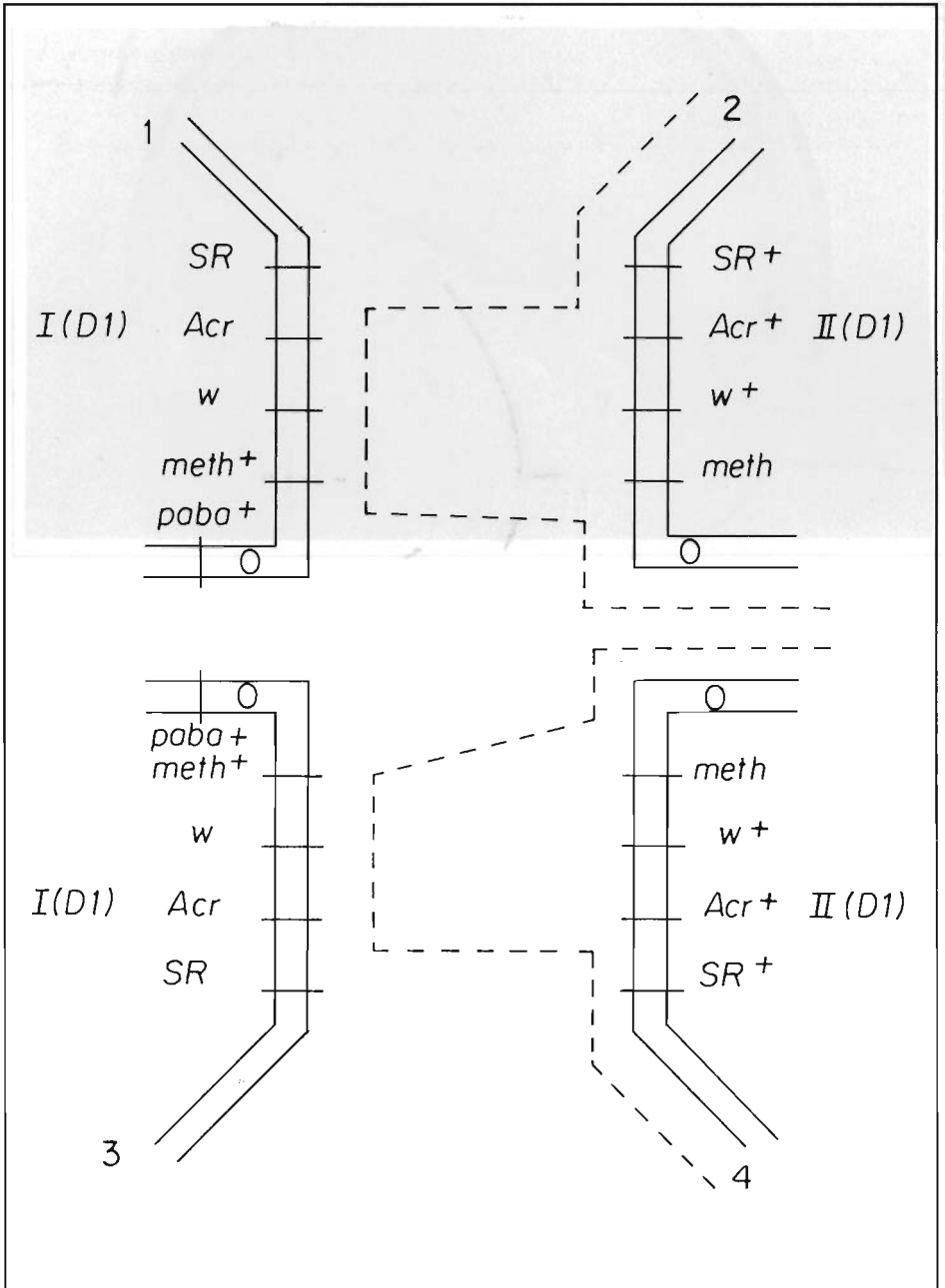
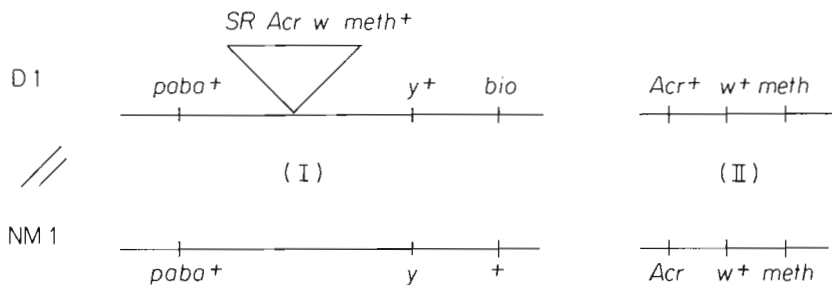
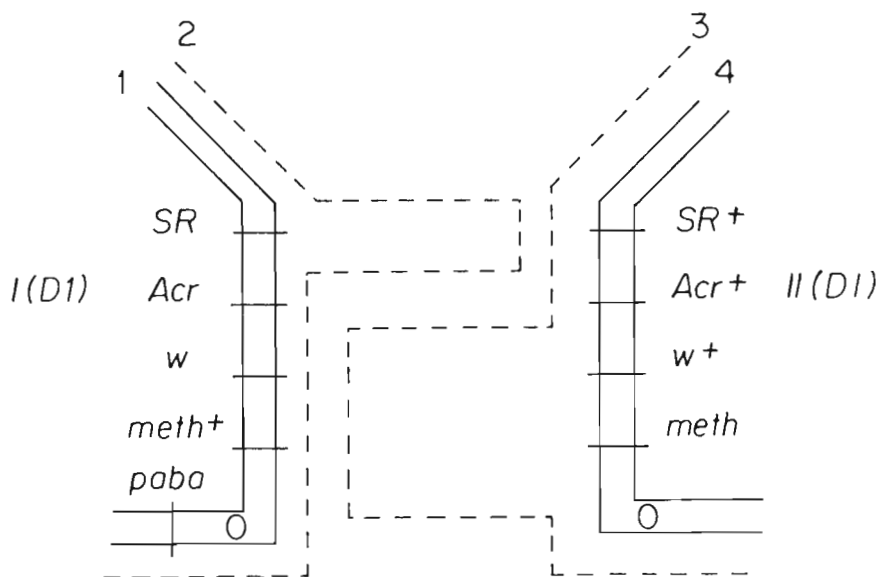
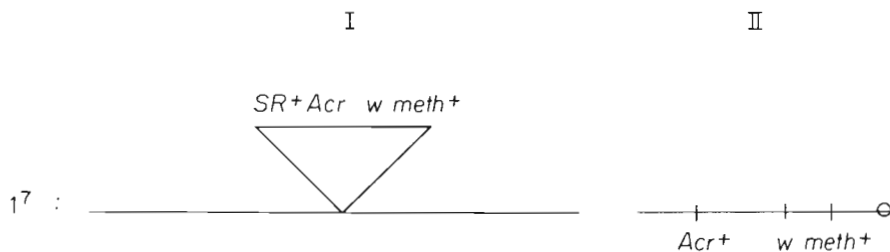


Figure 3 - Origin of *Acr w meth<sup>+</sup>* segregants from self-cross  $D_1 \times D_1$ , through normal segregation of one and two or three and four chromatids migrating to the same pole, in meiosis I.

Figure 4 - Partial representation of the  $D_1//NM_1$  diploid.Figure 5 - Partial representation of the origin of  $Acr^+$ ,  $w$ ,  $meth^+$  haploids derived from  $D_1//NM_1$ . The segregation of the mitotic recombinant chromatid to the same pole occurs before haploidization. Chromosomes I and II from the  $NM_1$  strain are not given.

Figure 6 - Representation of strain  $1^7$ .Table I - Meiotic segregation for *Acr* A<sub>1</sub> marker in the  $1^7$  x UT 448 cross. (83 colonics analyzed).

Phenotypes	Frequencies (%)
<i>Acr</i> <sup>±</sup>	24.1
<i>Acr</i>	36.1
<i>Acr</i> <sup>+</sup>	39.8

Two strains with the *Acr*<sup>±</sup> phenotype ( $1^{21}$  and  $2^{22}$ ) were isolated among the progeny of the  $1^7$  x UT 448 cross in a further test:

$1^{21}$  (*Acr*<sup>±</sup>) was backcrossed to UT 448 (*Acr*)

and

$2^{22}$  (*Acr*<sup>±</sup>) was self-crossed ( $2^{22}$  x  $2^{22}$ ).

The *Acr*<sup>+</sup> segregation among the progeny of  $1^{21}$  x UT 448 cross, and both *Acr*<sup>+</sup> and *Acr* segregation in the  $2^{22}$  x  $2^{22}$  selfed cross (Table II) fully confirmed our hypothesis, i.e., they showed that the *Acr* mutation was present in the duplicated segment (*Dp* II,I) next to a regulatory mutant sequence (*RS*), which could be substituted only by recombination mechanisms such as mitotic or meiotic crossing-over.

Table II - Meiotic segregation of *Acr* in  $1^{21} \times \text{UT 448}$  and  $2^{22} \times 2^{22}$  crosses. (150 colonies analyzed for each cross).

Phenotypes	Frequencies (%)	
	$1^{21} \times \text{UT 448}$	$2^{22} \times 2^{22}$
<i>Acr</i> <sup>±</sup>	20	67
<i>Acr</i> <sup>+</sup>	44	27
<i>Acr</i>	36	6

## DISCUSSION

*Acr* segregation in selfed crosses of *Acr*<sup>+</sup> strains such as B<sub>1</sub> × B<sub>1</sub> (Castro-Prado and Zucchi, 1991) and D<sub>1</sub> × D<sub>1</sub> suggests the presence of the *Acr* marker in the transposed *Dp* (II,I) duplication, but the expression of acriflavine resistance was not observed in these strains.

Considering the importance of the transcription control elements in prokaryotes and eukaryotes, such as promoters, enhancers and UAS, and especially the crucial role of their integrity (Giniger *et al.*, 1985) the inactivation of the *Acr* gene in the *Dp* (II,I) duplication, by mutation of a regulatory sequence of this gene (*RS*) is likely.

Zucchi (1990) mutagenized with MNNG the UT 448 strain and obtained results that support the hypothesis that the promoter or an "UAS - like" element functionally linked to the *Acr* marker, was altered after the insertion of the *Dp* (II,I) duplication into chromosome I.

The appearance of *Acr* segregants in crosses homozygous for the *Dp* (II,I) duplication could only be possible if the wild type regulatory sequence (*RS*<sup>+</sup>) was recovered by a meiotic crossing-over near the *Acr* gene on chromosome I (Figure 3).

The isolation of  $1^7$  segregants, exhibiting *Acr*<sup>±</sup> phenotype, showed that the *RS*<sup>+</sup> could also be recovered by mitotic crossing-over near the *Acr* marker on chromosome I (Figure 5).

The recovery of *Acr*<sup>+</sup> segregants in crosses heterozygous for the duplication, such as  $1^7 \times \text{UT 448}$  and  $1^{21} \times \text{UT 448}$ , and the recovery of both *Acr*<sup>+</sup> and *Acr* phenotypes, in crosses homozygous for the *Dp* (II,I) duplication, such as  $2^{22} \times 2^{22}$ , confirmed not only the genotype of the strains of *Acr*<sup>±</sup> phenotype, but also the recovery of *RS*<sup>+</sup> by meiotic crossing-over.

Presently it is not possible to determine the recovery frequencies of the *Acr*<sup>±</sup>, *Acr*<sup>+</sup> and *Acr* phenotypes in these crosses since several factors are involved, such as,

pairing between homologous segments of chromosomes I and II, the RIP effect and/or pre-meiotic recombination (Castro-Prado and Zucchi, unpublished results). All of these factors may induce heterogeneous effects, depending on the type of cross.

However, the results suggest that *RS* has an important function in the transcription of the *Acr* gene and its behavior may be related to a recognition site for transdiffusible factors with which RNA-polymerase II may be associated. Further studies are needed to test this hypothesis.

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## RESUMO

Linhagens de *Aspergillus nidulans* portadoras da duplicação dp (II,I) e sensíveis à Acriflavina, quando submetidas à análise de cleistócios autofecundados apresentaram segregação dos genes *w* e *Acr*.

A segregação do gene *w* é decorrente de sua heterozigiosidade em linhagens que apresentam *w* no cromossomo I (duplicação) e *w*<sup>+</sup> no cromossomo II. Por outro lado, a segregação do gene *Acr* sugere sua presença na duplicação Dp (II,I) o que torna tais linhagens heterozigotas também para o marcador *Acr*: *Acr* (cromossomo I, duplicação) e *Acr*<sup>+</sup> (cromossomo II).

Isto somente será possível se uma seqüência regulatória mutante estiver também presente na duplicação Dp (II,I), impedindo a expressão do gene *Acr*, pois linhagens heterozigotas *Acr/Acr*<sup>+</sup> têm fenótipo intermediário (*Acr*<sup>±</sup>) entre linhagens sensíveis (*Acr*<sup>+</sup>) e resistentes (*Acr*) à Acriflavina.

Confirmação destas hipóteses, bem como a recuperação da seqüência regulatória selvagem por crossing-over próximo à *Acr*, vieram através da obtenção de segregantes *Acr*<sup>+</sup> em cruzamentos homozigotos e heterozigotos para a duplicação Dp (II,I).

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