

SHORT COMMUNICATION

INACTIVATION OF DNA SEQUENCES IN *Aspergillus nidulans* THROUGH NON-LINKED DUPLICATION

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ABSTRACT

This paper describes a new system in *Aspergillus nidulans* that detects and alters duplicated DNA sequences in haploid nuclei of a premeiotic phase, before karyogamy. A similar system has previously been demonstrated in *N. crassa*, named "Rearrangements induced premeiotically" (RIP).

In a strain of *A. nidulans* bearing a large non-linked duplication (*Dp* II-I) carrying several markers, RIP acts heterogenously on the genes present in the duplication, promoting lethality and originating barren cleistothecia. Such strain have proved to be excellent system for molecular and classical genetic studies, as they are specialized in inactivating abnormally duplicated genes. In our study only classical genetic experiments were performed.

INTRODUCTION

The RIP effect was first detected in *Neurospora crassa* (Selker *et al.*, 1987) and later in *Ascobolus immersus* (Goyon and Faugeron, 1989) and in *Podospora anserina* (Debuchy *et al.*, 1988). Basically, the RIP effect is a cell strategy aiming at the inactivation of double-dose DNA segments acting only during the sexual phase (Selker *et al.*, 1987b). After fertilization, RIP can affect any cellular division before pre-meiotic synthesis and karyogamy (Selker *et al.*, 1987a). The RIP action results in point mutations (GC → AT)

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and may generate methylation sites recognizable by restriction enzymes (Selker and Stevens, 1985; Fincham, 1989). Such rearrangements may occur on linked or non-linked duplications. The latter exhibit less severe alterations and quite often they escape the RIP effect (Selker and Garret, 1988). After RIP, altered DNA sequences may continue to be sensitive to the process until divergences arise on account of mutations evoked by recurrent RIP action. At this point a resistance to the process occurs (Cambareri *et al.*, 1991).

In this paper we demonstrate, by classical genetics, the heterogeneous effects of RIP on strains which bear large non-linked duplication, and which contain three genetic markers, either in the heterozygous or the homozygous condition.

MATERIAL AND METHODS

A strain derived from Utrecht stocks (UT 196) and others obtained in our laboratory (D₁, 20² and 1⁷) were used. These were maintained on CM at 5°C.

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

UT 196: *yA*₂ (I) yellow conidia; *meth A*₁₇ (II); *pyro A*₄ (IV) with requirements for methionine and pyridoxine, respectively.

D₁: *bio A*₁ (I); *meth A*₁₇ (II), with requirements for biotin and methionine; *Dp* (II-I), duplicated segment on chromosome I (Figure 1A).

20²: *bio A*₁ (I); *meth A*₁₇ (II), with requirements for biotin and methionine, respectively (Figure 1A).

1⁷: *w A*₂ (II) white conidia; *Dp* (II-I) with *RS*⁺, duplicated segment on chromosome I with *RS*⁺ near the *Acr* marker (Figure 1B).

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

Genetic analysis was carried out according to Pontecorvo *et al.* (1953).

RESULTS AND DISCUSSION

Fruiting bodies (hybrid cleistothecia) of *A. nidulans* with a few ascospores were recovered from the cross D₁x20². D₁ is a *Dp* (II-I) strain and 20² is a genotypically identical strain, except for the duplication II-I, present in D₁ in heterozygous condition (Figure 1A).

The duplication *Dp* (II-I) is a large segment of chromosome II, including the following markers: *RS*, *Acr A*₁, *w A*₂ and *meth*⁺ inserted into chromosome I between the *paba A*₁₂₄ and *y A*₂ markers. Next to the *Acr A*₁ mutation in the segment transposed to chromosome I, there is a mutant regulatory sequence (*RS*) that inhibits the expression of

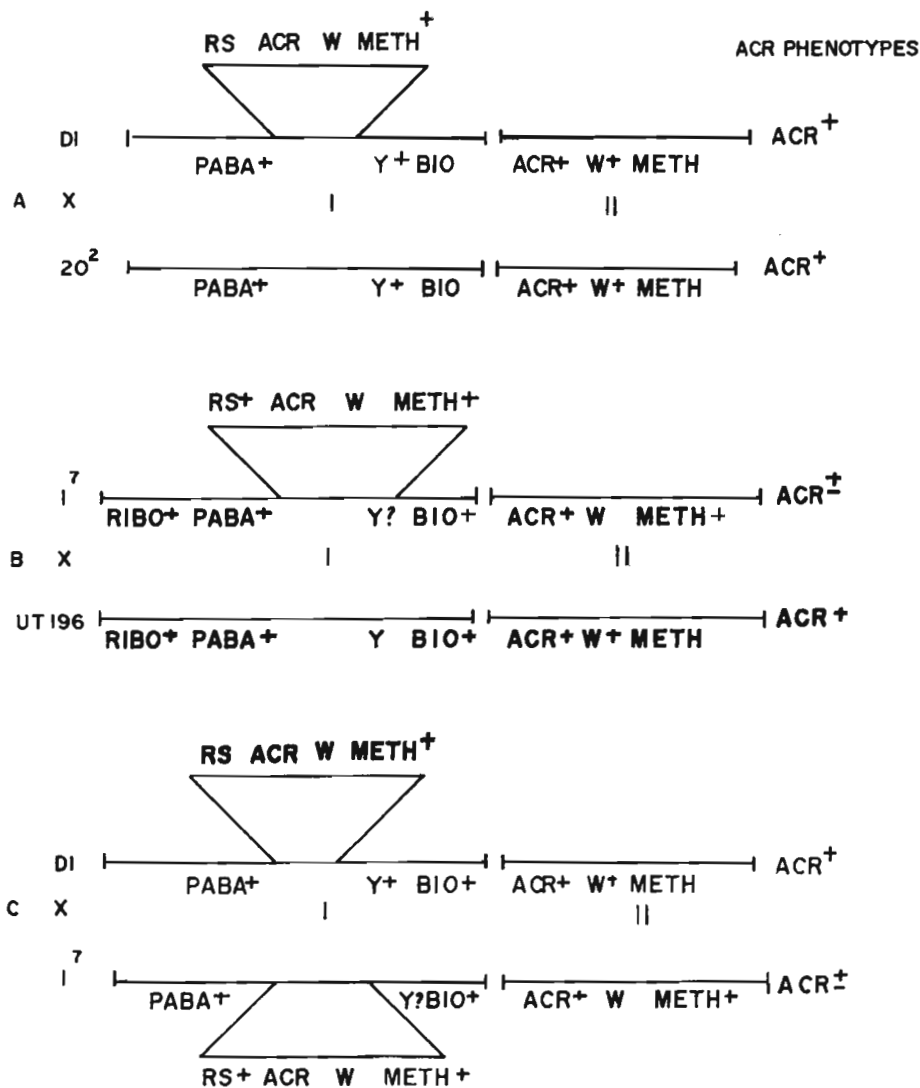


Figure 1—Partial representation of the D1x20² (1A), 1⁷xUT 196 (1B) and D1x1⁷ (1C) crosses.

acriflavine resistance (*Acr A1*). The D1 strain is phenotypically Acr^+ , but genotypically Acr/Acr^+ , as the selfed crosses (D1xD1) present *Acr* segregants among the selfed progeny as a consequence of a double crossing-over between the heterozygous duplicated segments in a quadrivalent configuration (Castro-Prado and Zucchi, 1991). This

segregation accounts for the recovery of RS^+ from chromosome II into chromosome I, close to the *Acr* A1 mutation, present in the transposed duplication. Even so, D1 being Acr A1/ Acr^+ did not present the intermediate Acr^+ phenotype. This behavior was unexpected since incomplete dominance between genes Acr^+ had been detected previously (Castro-Prado and Zucchi, unpublished results).

Only 15 ascospores were recovered from the cleistothecium of the D1x20² cross (Figure 1A) and all of them presented the following phenotype:

paba⁺, *bio* (I); *Acr*⁺, *w*, *meth*.

The extremely reduced progeny associated with the absence of *Acr*, *w* and *meth*⁺ segregants may indicate RIP inactivation of the markers of the duplication in chromosome I, in the nucleus of D1. "RIPing" affects the production of viable ascospores, leading to the absence of the parental classes $w^+ meth$ (20² strain) and $w^+ meth^+$ (D1 strain).

RIP does not produce the same pattern of alterations in non-linked as in linked duplications. Lethality (characterized by barren cleistothecia) did not always homogeneously affect all the cleistothecia products, but rather only the paternal class bearing the duplication. In addition, RIP sometimes inactivates only one of the markers present in the duplicated segment.

In 1⁷ x UT 196 a strong reduction of the parental class (1⁷, bearer of the duplication) was observed, although the total progeny was not actually reduced (Figure 1B, Table I).

The 1⁷ x D1 cross (Figure 1C), homozygous for the duplication transposed to chromosome I, produced very few progeny and inactivation of the w^+ marker from the D1 strain among the viable ascospores. The mutational alterations (methylations) caused by the RIP mechanism in the strains of this cross produced non-viable nuclei after meiosis, though it acted by independent events on both parental nuclei before karyogamy. This explains the strong reduction in progeny. All the recovered progeny were *meth*⁺ (Table I), indicating that this marker escaped from RIP inactivation in the pre-meiotic phase of the 1⁷ haploid nucleus.

The absence of Acr^+ segregants (intermediate phenotype expected in the parental 1⁷ class) also suggests the inactivation of the *Acr* marker present in the transposed duplication of the 1⁷ strain by RIP (Castro-Prado and Zucchi, 1992). Since both parental nuclei bear the Dp II-I, the apparent refractoriness of *Acr* and *meth*⁺ to the RIP effect may reflect its action on only one of the two parental nuclei. In this way, the *meth*⁺ and *Acr* segregants shown in Table I would have received these markers from the parental nucleus that was not inactivated by RIP, very probably the nucleus of the 1⁷ strain. Other evidence that relates the origin of the *meth*⁺ and *Acr* segregants to the RIP

Table I - Phenotypic frequency of the 1^7 xUT 196 and 1^7 xD1 progeny.

	Markers of chromosomes						Frequency (%)
	I			II			
	<i>ribo</i>	<i>paba</i>	<i>bio</i>	<i>Acr</i>	<i>w</i>	<i>meth</i>	
UT 196 x 1^7 cross	+	+	+	-	-	+	5.7
157 analyzed colonies	+	+	+	+	+	-	93.6
1^7 x D1 cross				-	-	+	40.0
15 analyzed colonies				+	-	+	60.0
Controls							
1^7	+	+	+	±	-	+	
D1				±	+	+	
196	+	+	+	-	+	-	

(+) and (-), wild type and mutant phenotypes, respectively; *Acr* (±) means an intermediate growth rate between resistant (-) and sensitive (+) phenotypes on CM+acriflavine.

effect is the occurrence of severe damage (gene inactivation in just one of the present copies in duplicate, since it is already known that duplicate sequences are generally submitted to equivalent but not identical damage (Grayburn and Selker, 1989; Fincham *et al.*, 1989)).

The inactivation of w^+ from the D1 strain and of *Acr* from the 1^7 strain demonstrates that RIP has heterogeneous effects on the pre-meiotic phase of both haploid nuclei and is also evidence of the independence of events. The data suggest that the alterations observed in meiotic crosses between strains of *A. nidulans* bearing the *Dp* (II-I) duplication reflect the action of RIP on non-linked duplications.

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RESUMO

Descreve-se um novo sistema em *A. nidulans* que detecta e altera seqüências de DNA em duplicata em núcleos haplóides, numa fase pré-meiótica, antes da cariogamia. Um sistema semelhante foi descrito em *N. crassa* e denominado RIP (rearrangements induced pre-meiotically).

Em uma linhagem de *A. nidulans* portadora de uma grande duplicação não ligada (Dp II-I) contendo vários marcadores, RIP age heterogeneamente sobre os genes presentes na duplicação, promovendo letalidade e originando cleistotécios estéreis. Tais linhagens têm se mostrado excelentes sistemas para estudos de genética clássica e molecular dos efeitos desse processo especializado em inativar genes anormalmente duplicados. Neste estudo, utilizaram-se somente experimentos de genética clássica.

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