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Mitotic and meiotic instability produced by an informational suppressor in *Aspergillus nidulans*

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ABSTRACT. The genetic instability induced by the informational suppressor *suO1* in *Aspergillus nidulans* is described. *suO1* was selected through the properties of coreversion of several physiologically unrelated mutations. This mutation decreased the conidia and ascospores viability of the suppressor strains. These strains were unstable at mitosis and meiosis, producing improved derivatives, phenotypically different from the original strains. Meiotic crosses, heterozygous for the suppressor mutation, produced barren cleistothecia. *suO1* was unstable in the restoration of the genetic alterations present in the genome of M-15 mutant, producing more than 25.0% unsuppressed progeny in heterozygous crosses for auxotrophyc mutations (for instance, *su*, *ribo* x su^+, $ribo^+$) . Our results suggested that *suO1* altered the fidelity of the translation process, inducing mistranslation of normal and mutant mRNAs.

Key words: *Aspergillus nidulans*, barren cleistothecia, gene suppressor, conidial viability.

RESUMO. **Instabilidade mitótica e meiótica produzida por um supressor informacional em *Aspergillus nidulans*.** A instabilidade genética de linhagens de *Aspergillus nidulans*, portadoras de um supressor informacional (*suO1*), foi estudada no presente trabalho. *suO1* foi selecionado através de sua capacidade de suprimir várias mutações auxotróficas e de coloração de conídios, presentes no genoma do mutante M-15. Este supressor mostrou-se também capaz de reduzir a fertilidade e a viabilidade conidial das linhagens suprimidas. Estas linhagens mostraram-se instáveis na mitose, produzindo espontaneamente setores mitóticos fenotipicamente distintos da linhagem original, e na meiose, produzindo cleistotécios contendo reduzido número de ascósporos viáveis. Em cruzamentos heterozigotos (*su*, *ribo* x su^+, $ribo^+$) mais de 25.0% da progênie exibiu o fenótipo mutante *ribo*, destacando a baixa eficiência da mutação *suO1*. Nossos resultados sugerem que *suO1* induz erros na tradução de mRNAs selvagens e mutantes.

Palavras-chave: *Aspergillus nidulans,* instabilidade mitótica, instabilidade meiótica, gene supressor

The gene suppression phenomenon was extensively studied after it became evident that lesions in DNA molecules might be corrected during protein synthesis, restoring the normal phenotype (Gorini 1970, Dequard-Chablat *et al.* 1986, Haggerty and Lobett 1997). When gene suppression occurs because of mutations in structural genes of macromolecules involved in the translation process, the suppressor gene is called informational. A result of suppression is the production of a protein with aminoacid sequence distinct from the mutant protein. In this context, efficiency of suppressor mutation is generally evaluated in terms of functionality of restored protein (Gorini 1970, Martinelli *et al.* 1992).

Ribosomes guarantee the selection of specific tRNAs that translate mRNA. Modifications in this complex organelle would allow the occurrence of translation errors. Mistranslation would be caused by impairing access of the tRNA to the ribosome or by changes in codon-anticodon pairing. The formation of proteins with incorrect aminoacid sequence would be the consequence of translation errors (Stansfield *et al.* 1998).

Two kinds of suppressor mutations have been described in ascomycetes. They were mapped to transfer RNA genes or genes that code for proteins involved in translation. The effects of suppressor mutations affecting ribosomal components were very similar to those caused by paromomycin in

auxotrophic strains (Picard 1973, Martinelli 1984, Mironova *et al.* 1995). Paromomycin is an aminoglycoside antibiotic that binds to ribosomal RNA in the aminoacyl-tRNA site causing misreading of the genetic code (Fourmy *et al.* 1996, Moore 1997, Blanchard *et al.* 1998). Hypersensitivity to paromomycin showed by suppressor strains was an evidence of the additive effects of suppressing mutations and the antibiotic (Roberts *et al.* 1979, Coppin-Raynal 1981, Fourmy *et al.* 1998). Mutations in ribosomal proteins S28 and S4 and changes in 18S rRNA of *Saccharomyces cerevisiae* showed that they affected translation fidelity and ribosomal sensitivity to aminoglycosides (Chernoff 1994, Synetos *et al.* 1995).

Changes in protein synthesis induced by informational suppressors was shown to impair the sexual cycle of some ascomycetes. Cellular differentiation during the sexual reproduction of *Podospora anserina* was blocked by high rates of misreading induced by very efficient suppressors (Picard-Bennoun 1982, Gagny and Silar 1998). Decrease in fertility was also registered in *Aspergillus nidulans* strains bearing informational suppressors (Roberts *et al.* 1979, Bratt and Martinelli 1988). These strains produced cleistothecia containing reduced number of viable ascospores. Mutations in genes coding for ribosomal components were associated with low fertility of these strains (Bratt and Martinelli 1988).

In a previous work we characterized an informational suppressor in *A. nidulans*, named *suO1* (Franzoni and Castro-Prado, 2000). It was recessive both in the diploid and in the heterokaryon formed by the mutant M-15 and the *su+* master strain. *suO1* is an allele-specific, gene-unspecific suppressor mutation, that suppresses several physiologically unrelated mutations. The suppressor strains were sensitive to low temperatures and paromomycin. Characteristics of *suO1* showed that it controls the fidelity of translation and marked it as a ribosomal suppressor (Franzoni and Castro-Prado 1997, 1998).

The present work showed mitotic instability of suppressor strains which spontaneously give rise to mitotic derivatives phenotypically different from the original strains. Meiotic instability of these strains was also shown in hybrid crossings, heterozygous for *suO1.* The suppressor gene was unstable with regard to frequency of co-reversion of the mutations under analysis. Results were correlated to high frequency of translation errors induced by suppressing mutation.

Materials and methods

Strains. The *A. nidulans* strains were derived from FGSC (A288), Utrecht (UT448, UT196) and Glasgow stocks (G351, G159). The M-15 mutant strain was derived from A288 master strain after N-methyl-N'-nitro-N- nitrosoguanidine (NTG) treatment (1.0 μg/mL, pH 6.0). The *methA* strain is a mitotic segregant derived from the UT448//UT196 diploid strain and the 520 strain is a meiotic segregant derived from UT448 x UT196 cross.

M-15 and Y-15: *yA2* (I); *wA3* (II), white conidia; *adE20* (I), *pyroA4* (IV*); nicB8* (VII); *riboB2* (VIII), with requirements for adenine, pyridoxine, nicotinamide and riboflavine, respectively; *sB3* (VI) with sulphate transport impairment; *galA1* (III), *facA303* (V*)*, unable to grow on galactose and ammonium acetate as the sole carbon source; *suA1adE20* (I), suppressor of *adE20* (specific for this *ad* allele). *suO1*(VIII), informational suppressor. Y-15 is a mitotic segregant derived from M-15 mutant.

G351: *fwA1* (VIII), fawn conidia, *pabaA1* (I), with requirements for *p*-aminobenzoic acid; *alx4* (III), non-utilization of allantoin as nitrogen source; *suaA101* (III) allele specific suppressor; *sB43* (VI), sulphate transport impairment; *alcR125* (VII) non-utilization of ethanol as the sole carbon source.

UT196: *y*A1 (I) yellow conidia; *methA17* (II), *pyroA4* (IV), with requirements for methionine and pyridoxine, respectively.

UT448: *w*A2 (II) white conidia; *riboA1*, *pabaA124*, *biA1* (I), with requirements for riboflavin, *p*-aminobenzoic acid and biotin, respectively; *AcrA1* (II) resistant to acriflavin.

520: *pabaA124* (I), *biA1* (I), *methA17* (II), with requirements for *p*-aminobenzoic acid, biotin and methionine, respectively.

methA: *yA2* (I) yellow conidia; *methA17* (II), with requirement for methionine.

G159: *pabaA1, biA1* (I), with requirements for *p*-aminobenzoic acid and biotin, respectively.

A288: *wA3* (II), white conidia; *adE20* (I), *yA2* (I); *pyroA4* (IV*); nicB8* (VII); *riboB2* (VIII), with requirements for adenine, pyridoxine, nicotinamide and riboflavine, respectively; *sB3* (VI) with sulphate transport impairment; *galA1* (III), *facA303* (V*)*, unable to grow on galactose and ammonium acetate as the sole carbon source; *suA1adE20* (I), suppressor of *adE20* (specific for this *ad* allele).

Culture media. Complete medium (CM) and minimal medium (MM) were employed as described by Van de Vate and Jansen (1978). Selective medium (SM) was supplemented MM, according to the requirements of each strain. Solid medium contained 1.5% agar. Incubation was at 37º C.

Genetic techniques. The general methodology

followed Pontecorvo *et al.* (1953). Diploid strains were prepared by Roper's method (1952). Mitotic haploidization was carried out spontaneously by growing the diploid strains in plates containing CM during seven days. Heterokaryons were prepared in liquid MM plus 2.0% CM. Cleistothecia were obtained from the heterokaryons after 21 days of incubation in sealed Petri dishes containing solid supplemented MM according to the requirements of the crossed strains.

Ascospore and conidia viabilities. The number of ascospores per cleistothecium or conidia per mL was determined by counting in a haemocytometer. Samples of ascospore or conidia suspensions at appropriate dilutions were seeded in CM and the developed colonies were observed in comparison with the number determined in the haemocytometer, after 48 h of incubation at 37°C.

Results

Mitotic instability induced by a suppressor mutation. The M-15 mutant was obtained by treatment of the A288 master strain with N-methyl-N'-nitro-N-nitrosoguanidine (NTG). The mutant strain was selected in Minimal Medium supplemented with pyridoxine only. The suppressor gene was named *suO1*. This mutation was mapped on chromosome VIII. The susceptibility of M-15 toward aminoglycoside antibiotics and cold temperature suggests that *suO1* is an informational suppressor (Franzoni and Castro-Prado, 2000). *suO1* produces a mutant with reduced conidial viability and suppresses all mutations tested in its genome . The suppressor mutation is unstable with regard to frequency of co-reversion of auxotrophyc and conidial color mutations of M-15. This characteristic of *suO1* causes great mitotic instability in M-15 and suggests the induction of high frequency of translation errors by the suppressor gene (Table 1, Figure 1).

M-15 has white conidia (*w*) but prototrophic mitotic sectors *w*+ spontaneously appear. These segregants have greater conidial viability than M-15 and show phenotypic segregation of the *w* gene and of their auxotrophic mutations during mitotic growth (Table 1, Figures 1 and 2). These observations are consistent with Gorini's (1970) who suggested that ribosomal suppressors should mistranslate a great number of codons.

Meiotic instability induced by *suO1*. Instability of the suppressor mutant was more evident during the sexual cycle. Production of cleistothecia was recorded with reduced number of viable ascospores and variation in suppression of certain mutations present in the genome of suppressor strains (Tables 2 and 3).

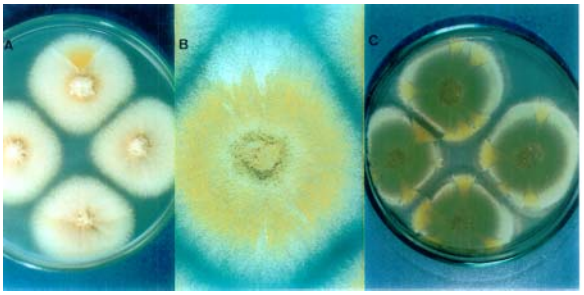

Figure 1. Mitotic instability of the suppressor strains: (A) M-15, (B) Y-15 and (C) 2.25 (meiotic segregant of M-15 x UT448 cross)

Table 1. Phenotypic analysis of spontaneous mitotic segregants derived from M-15 (1,2) and Y-15 mutant strains (3-6)

Mitotic Segregants	SM	*ribo*	*sB3*	*pyro*	*gal*	*fac*	*nic*	*w*
M-15	+	+	+	-	+	+	+	-
1	-	-	-	-	-	-	-	-
2	+	+	+	+	+	+	+	+
Y-15	+	+	+	+	+	+	+	+
3	+	-	+	-	+	+	+	-
4	+	+	+	-	+	+	+	-
5	-	-	-	-	+	-	-	-
6	+	-	+	-	+	+	+	-

SM = MM supplemented with riboflavine (1.0 µg/ml), pyridoxine (1.0 µ/ml), nicotinamide (0.05 µg/ml) and sodium thiosulfate (0.25µg/ml)

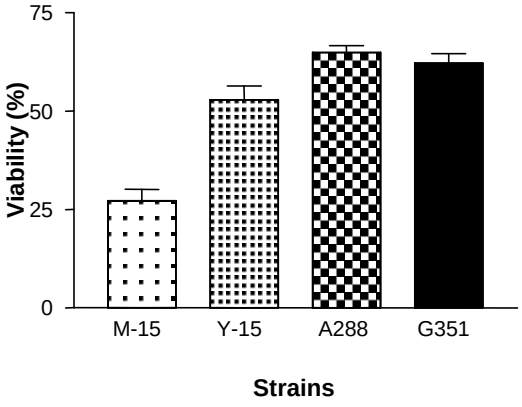

Figure 2. Conidial viability of M-15, Y-15 and G351 mutant strains, and the A288 control strain.Column represents mean ± SDM of six experiments

In the M-15 x *methA* cross changes in the segregation of *paba*, *bi*, *w*, *pyro*, *fac*, *sB3* and *ribo* markers have been observed. Only *pyro* and *fac* genes showed the expected meiotic segregation (3+ : 1-) which characterizes the presence of a suppressor gene in the M-15 mutant. For the remaining mutations (*w*, *sB3* and *ribo*), predominance in the

segregation of non-suppressed alleles has been observed. Results suggest that *suO1* caused high levels of reading errors during the translation process and changed the meiotic segregation of markers under analysis. Origin of *paba* and *bi* segregants in this cross indicated that changes in translation fidelity may also alter the expression of normal genes (Table 2).

Table 2. Meiotic segregations of markers of chromosomes I, II, IV, V, VI, VIII obtained from M-15 x *methA* cross

Chromosomes													
I		I		II		IV		V		VI		VIII	
paba		*bi*		*w*		*pyro*		*fac*		*sB3*		*ribo*	
-	+	-	+	-	+	-	+	-	+	-	+	-	+
36	64	35	65	73	27	26	74	17	83	53	48	68	32

Table 3. Number of total viable ascospores obtained from M-15 x M-15, Y-15 x Y-15 e 520 x 520 (control) selfed crosses

Number of viable ascospores per cleistothecium			
Nº of Cleistothecia	M-15 x M-15	Y-15 x Y-15	520 x 520
1	20	50	28000
2	7	Zero	8000
3	10	Zero	6250
4	10	82	6800
5	50	50	26000

The passage of M-15 and Y-15 suppressor strains through the sexual cycle resulted in the production of *barren* cleistothecia, containing reduced number of viable ascospores. These results were seen in hybrid crossings and self-crossed ones. They may be associated with reading errors induced by suppressor mutation during the synthesis of proteins essential to the sexual cycle. The reduction of viable ascospores per cleistothecia was obtained in crosses homozygous and heterozygous for *suO1* (Tables 3 and 4).

Discussion

The informational suppressor *suO1* was selected through the properties of coreversion of physiologically unrelated mutations. It had a profound effect on the phenotype of suppressor strains, decreasing conidial and ascospores viability (Figure 2, Tables 3 and 4).

Strains containing this suppressor mutation were unstable at mitosis and more so at meiosis, producing faster growing derivatives, phenotypically different from the original strains. Improved sectors, with several auxotrophyc markers could also be originated from the suppressor strains (Table 1, Figure 1). Assuming that the restoration of the functional protein depends on the incorporation of the correct aminoacids into peptide chains, the absence of suppression might be the result of the synthesis of incorrect proteins during translation. In this way, the mitotic instability and the reduction of ascospore and conidial viability shown by the suppressor strains is an indication of translation ambiguity induced by *suO1.* The suppression induced by suO1 conferred the suppressor strains the wild phenotype (Tables 1 and 2).

The translation errors induced by *suO1* might be also responsible for the different phenotypic classes obtained among the progeny of the M-15 x G159 and Y-15 x G159 crosses, in which the parental genotypes were the same (Table 4, Figure 2).

Table 4. Meiotic segregation of markers of chromosomes I, II, IV, VI and VIII in M-15 x G159 (A) and Y-15 x G159 (B) crosses

	Chromosomes											
Crosses	I		I		II		IV		VI		VIII	
	y		*paba*		*w*		*pyro*		*sB3*		*ribo*	
	-	+	-	+	-	+	-	+	-	+	-	+
A (Cleistothecium 1)	30	43	84	85	96	73	88	81	86	83	61	108
B (Cleistothecium 1)	12	32	31	13	1	43	0	44	0	44	1	43
B (Cleistothecium 2)	1	2	2	3	2	3	0	5	1	4	0	4
B (Cleistothecium 3)	1	10	10	1	0	11	0	11	0	11	0	11

⋆ 1, 2, 3, Total viable progenies of these cleitothecia were analyzed

In crosses with *methA* strain (*su*+), the M-15 suppressor strain produced more than 25.0% unsuppressed progeny for the *w*, *sB3* and *ribo* markers. This could be explained by the low efficiency of the suppressor mutation. On the assumption that ribosomal suppressors should lack codon-specificity and mistranslate a great number of codons, a viable ribosomal suppressor should have a very low efficiency (Gorini, 1970; Picard, 1973).

The production of more than 25.0% auxotrophic progeny for the *paba* and *bi* genes in the M-15 x *methA* cross, a homozygous *paba*+,*bi*+ x *paba*+,*bi*+ cross, lends considerable weight to our premise that *suO1* may impair the accuracy of protein synthesis. Mistranslation of normal mRNAs might lead to the synthesis of defective proteins, in spite of the presence of the wild-type genes in the genome (Table 2). In this way, alterations in the synthesis of the proteins involved in the control of gene expression or in cellular differentiation, would also explain the decrease in conidia and ascospore viability of the suppressor strains. The production of barren cleistothecia was observed in hybrid and selfed crosses (Figure 2, Tables 3 and 4).

In many multicellular organisms, early development depends on translation of maternally inherited mRNAs, transcribed during oogenesis. The molecular mechanisms which mediate translation control of maternal mRNA are therefore critical for the synthesis of essential factors for

growth and cell viability. In this way, mutations in genes coding for ribosomal proteins or factors which become attached to the ribosome during translation could change the synthesis of regulatory proteins and, as a result, give rise to intense alterations in the cell differentiation pattern. Translation fidelity and cell viability are closely related processes (Seydoux, 1996; Gagny and Silar, 1998). Therefore, studies of mutations that disturb the level of translation accuracy are important to identify new components of the translation machinery, or new functions for the already known translation factors (Martinelli *et al.,* 1992; Kuchino, 1996; Le Goff *et al.,* 1997).

The present work emphasized the alterations induced by *suO1*, an assumed ribosomal suppressor, during mitosis and meiosis of suppressor strains. In order to characterize this suppressor mutation, further studies with a system of *in vitro* protein synthesis will be necessary.

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