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ANOTHER *meth* A_{17} SUPPRESSOR OF *Aspergillus nidulans*

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ABSTRACT

Mutant 24 was selected by Zucchi (*Rev. Bras. Genet. 13*: 409-424, 1990) for its apparently high recombination frequency in the *meth-w* interval of chromosome II. It was demonstrated that some of the alterations shown by meiotic and mitotic analysis were due to a *meth* A_{17} recessive suppressor located on chromosome V, linked to the *locus* sB_3. Recombination analysis was difficult because of the presence of an anti-suppressor mutation in chromosome III of mutant 24 that acts more effectively when combined with a factor of chromosome I of the non-related strain UT 184.

INTRODUCTION

In considering the possible presence of suppressor genes that suppress total or partially one or several mutations, special attention should be paid to the presence of genes modifying the efficiency of the suppressors. Among these are the allo-suppressors and the anti-suppressors that change the number of expected recombinants of meiotic crosses and can complicate the genetic analysis and mapping of the suppressor mutation. The presence of suppressors in fungi such as *Aspergillus nidulans* and *Saccharomyces cerevisiae* (Moon and Kang, 1982; Lewis and Casselton, 1975) and in bacteria (Schwartz, 1965, 1967) confers somatic instability to the strains probably due to their association with antisuppressor genes (Gallucci and Garen, 1966; Liebman and Sherman, 1976) or to their involvement with a transposition element.

Allo-suppressors have been reported to increase the action of the suppressors in *S. cerevisiae* (Liebman *et al.*, 1976; Coppin-Raynal, 1977) and antisuppressors, that decrease the efficiency of the suppressor, have been studied by Laten *et al.*, 1978;

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Liebman and Sherman, 1979; and by Anita *et al*., 1980). Working with *Podospora anserina*, Piccard-Bennoun (1986) isolated ribosomal suppressors and detected anti-suppressor loci that were similar to the restrictive mutants of *Escherichia coli*. The author also suggested two mechanisms of antisuppression: one that may appears as a result of the antagonistic effect of two ribosomal suppressors; the other mechanism of anti-suppression may be due to a reduction of the efficiency of suppression by restricting the level of reading.

Only a few anti-suppressors have been described in fungi, and even those were not studied in depth and so, their mechanism of action and molecular nature are still unknown.

This paper deals with the location of a suppressor mutation of the *meth* A17 mutation. This suppressor is called silent because it appeared in an $meth^+$ strain and it only expresses itself in crosses with a *meth* A17 strain. The presence of this mutation is detected by the high number of the $meth^+$ w^+ recombinants and by the compact morphology it confers to the suppressed strains.

MATERIAL AND METHODS

Strains

The strains used here were the same mentioned on previous paper (Zucchi, 1990a) and the mutant 24 that was isolated after the MNNG treatment of the UT 448 strain. Same markers as in UT 448, except for the mutation which confers to it the apparent *hyper-rec* character to be described and discussed in this paper.

Media and Solutions

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 Jansen, 1970). The solid medium contained 1.5% agar.

Methods

The general methodology follows Pontecorvo *et al*. (1953). The diploids were prepared by the method of Roper (1952). Allocation of mutant alleles, duplications and suppressors to their linkage groups by mitotic haploidization (Forbes, 1959) was facilitated by the use of *p*-fluorophenulalanine (*p*FP) (Morpurgo, 1961; Lhoas, 1961). Incubation was at 37°.

RESULTS

The hyper rec 24 Character

The 24 VIII mutant selected after MNNG treatment was denoted *hyper rec* 24 because it shows an apparently high recombination frequency in the *meth-w* region of chromosome II. This frequency is determined in meiotic crosses with UT 196 (tester strain). The results of meiotic analysis are summarized in Table I.

Table I - Meiotic recombination frequency in the *meth-w* interval of chromosome II.

Crosses	RF	Paternal		Recombinant	
	meth-w (%)	*meth* w^{+}	*w* $meth^{+}$	$meth^{+}$ w^{+}	*meth w*
448 x 196	1.8	241	250	7	1
24 VIII x 196	15.0	144	110	44	1

Note: these results were obtained by random analysis of ascospores.

Complete analysis of the 24 VIII x 196 cross (data not presented) showed abnormal behavior during meiosis. Table II presents the frequency of the segregant classes of the chromosome II markers. There were 4 major classes (1), (2), (3) and (4) as distinct from the control cross, which showed only two major classes.

Table II - Segregation of the possible classes arising in the meiotic cross 24 VIII x 196.

Classes				448 x 196 (control)	24 VIII x 196
Acr	+	*w*	(1)	111	87
+	*meth*	+	(2)	92	133
Acr	*meth*	+		18	11
+	+	*w*	(3)	22	22
+	*meth*	*w*		0	0
Acr	+	+		2	6
+	+	+	(4)	3	39
Acr	*meth*	*w*		3	1

The recombination frequencies in the chromosome II markers are shown in Table III where it can be seen that the RF of *meth-w* increased and the RF of *Acr-w* was reduced.

Table III - Recombination frequency in the chromosome II markers.

Chromosome II intervals	448 x 196 (499)	24 x 196 (296)
meth - w	1.8	15.0
Acr - meth	28.8	24.0
Acr - w	27.0	13.0

Note: the number of colonies analysed is given in parentheses.

The data presented suggest that the marker order in chromosome II did not differ from the control, i.e.: *Acr-w - meth* centromere.

The results presented in Table II suggest the existence of a *meth Acr* duplication (3 and 4) that, on the basis of the order of the chromosome II markers, should include *w* in the duplication. In order to check this possibility, meiotic $meth^{+}$ w^{+} segregants from 24 VIII x 196 were crossed with another $w^{+}meth^{+}$ strain, and not even a single *w* colony emerged in the progeny (Table IV).

Table IV - Segregation of *meth* and *w* in crosses of $meth^{+}$ w^{+} strains.

Segregants $meth^{+}$ w^{+}	Genotype	Origin	Progeny			
			w	w^{+}	*meth*	$meth^{+}$
1^{9}	yA_2	24 x 196	0	45	17	28
6^{19}	yA_2; $AcrA_1$	24 x 196	0	165	57	108
2^{24}	yA_2; $AcrA_1$	24 x 196	0	156	45	111

On the basis of the results in Table IV, the suppressor hypothesis fits well with the data shown until now. Such a suppressor of *meth* A_{17} could, e.g., modify the frequency of the Acr^{+} *meth* (2) parental class in Acr^{+} $meth^{+}$ (4).

SUPPRESSOR HYPOTHESIS

A suppressor of *meth* A17 is also responsible for the increase of the $meth^+$ w^+ recombinant class by changing the phenotype of the parental *meth* w^+ in $meth^+$ w^+ pseudo-recombinants (Figure 1).

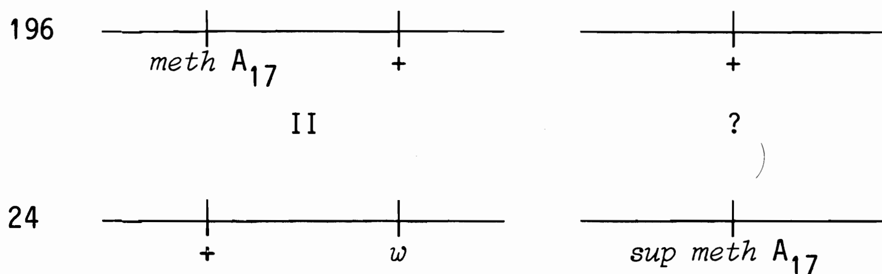

Figure 1 - Hypothesis of a *meth* A17 suppressor.

In this case, the expected frequency w^+ *meth* A17 *sup met* A17 recombinants is 25%, a value quite different from that normally found in such crosses (15.0%, Table III), and the *w meth* recombinants class should be practically absent (Table I).

A TEST FOR THE SUPPRESSOR HYPOTHESIS

Crosses of $meth^+$ w^+ segregants from 24 x 196, with a $meth^+$ w^+ strain (UT 184) showed, *meth* segregants in the progeny (Table IV). Thus, the $meth^+$ w^+ segregants are indeed w^+ *meth sup meth* or pseudo-recombinants. so, it is possible to assume that the apparent *hyper rec* 24 trait is also caused by the segregation of the *meth* A17 suppressor, not linked to the gene it suppresses. It is now denoted *sup* 24.

MORPHOLOGY OF THE SUPPRESSED STRAIN

Among the segregants of crosses with strains *sup* 24, there were several colonies with very reduced growth in medium lacking methionine. To better determine this phenomenon, several *sup* and sup^+ strains were inoculated and colony development was followed for 24 and 48 h of incubation at 37°. The results are shown in Table V.

Table V - Development of *sup*$^+$ and *sup meth* colonies in medium lacking methionine.

Strains	Genotype	Medium without methionine	
		24 h	48 h
UT 448	*meth*$^+$ *sup*$^+$	+++	+++
Mutant 30	*meth*$^+$ *sup* 30	++	+++
Mutant 29	*meth*$^+$ *sup* 29	++	++
Mutant 24	*meth*$^+$ *sup* 24	+	+
4^{12}	*meth* *sup* 24	-	+

4^{12}: Meiotic segregant from UT 448 x UT 196.

Mutants 29 and 30 were described in Zucchi, 1990a,b.

THE LINKAGE GROUP OF THE *sup* 24 MUTATION

Mitotic analysis of the 24//184 diploid showed a light selective pressure against chromosome I during the process of haploidization with *p*FP (Table VI). In addition, the chromosomes III and VIII of the mutant showed a reduction in relation to the normal chromosome.

Table VI - Haploid segregants of 448//184 and 24 VIII//184 diploids treated with *p*FP.

	Markers of 448 or Mutant							
	I	II	III	IV	V	VI	VII	VIII
Diploids	*paba*	*w*	*gal*$^+$	*pyro*$^+$	*fac*$^+$	*s*	*nic*	*ribo B*$^+$ (*paba*)
448//184	.53	.59	.40	.46	.28	.47	.48	.63
24//184	.25	.47	.11	.47	.32	.79	.08	.75

There is some evidence of two or three defects in the genome of 24 VIII involving chromosomes I, III and VII, which are responsible for slow growth rate, RF *meth w* alteration and excess of *meth*$^+$ *w*$^+$ recombinants.

These probable defects are listed in Table VII, where it is evident that the I-II and I-VII chromosome combinations are eliminated very efficiently during haploidization, indicating the existence of two defects.

Table VII - Combination of chromosomes I, II, III and VII among the haploids of 24//184.

	24-24	184-184	24-184	184-184
I - II	9	24	4	16
I - III	0	35	13	6
I - VII	1	37	12	3
II - III	3	25	22	3
II - VII	3	27	22	1
III - VII	1	42	4	3

LOCATION OF *sup* 24

In order to map *sup* 24 we prepared a strain more suitable for this objective. The strain we used was 4^{12}, which is a meiotic segregant from the 24 x 196 cross. Its complete genotype is:

4^{12}: *ribo* A_1, *paba* A_{124}, *bi* A_1 (I); *Acr* A_1, *meth* A_{17}, *w*A_2 (II).

4^{12} is a rare *meth w* recombinant but it must contain *sup* 24 because 4^{12} x 184 showed a RF *meth w* = 16.8% (Table VIII) and only one recombinant class (*w meth*$^+$).

Table VII - Meiotic analysis of 4^{12} x 184 (87 analysed colonies).

Cross	RF *meth w*	Meiotic segregants			
		w^+ *meth*$^+$	*w meth*	*w meth*$^+$	w^+ *meth*
4^{12} x 184	16.8%	43	30	13	1

Although 4^{12} has a *sup meth*, the *meth* phenotype of slow growth (Table VI; 24 h) in a medium without methionine can only be justified if 4^{12} has another defect, such as an anti-suppressor.

The results in Table IX suggest the presence of *sup* 24 in chromosome VI (4th line). If this hypothesis is valid, the *w meth*$^+$ recombinants are actually *meth sup meth*.

Table IX - Meiotic analysis of 4^{12} x 184.

Combination of the markers of chromosome II		I		III		IV		V		VI	
		pa$^+$	*pa*	*gal*	*gal*$^+$	*pyro*	*pyro*$^+$	*fac*	*fac*$^+$	*s*	*s*$^+$
meth	*w*	20	20	14	26	18	22	18	22	35	5
+	+	22	21	17	26	26	17	25	18	29	14
meth	+	0	1	0	1	1	0	0	1	1	0
+	*meth*	10	3	5	8	6	7	6	7	3	10

The reduction of *meth w s*$^+$ segregants (1st line) and the increase of *w*$^+$ *meth*$^+$ *s*$^+$ segregants (2nd line) is an indication of linkage of *sup meth* with *s*$^+$ (VI chromosome).

In order to determine the interactions between chromosomes I, II, III and VI we listed in Table X the combinations of chromosomes II, III and VI, and in Table XI the combinations of chromosomes I, II and VI. Unfortunately combinations involving chromosome VII were not possible because we did not analyse the *nic* (VII) marker among the segregants of 4^{12} x 184.

Table X - Relationships between chromosomes III and VI with *sup meth* A_{17} (II) in 4^{12} x 184 segregants.

Chromosomal combinations		Phenotype	
		meth	*meth*$^+$
III	VI		
184 (*gal*)	184 (*s*)	16	4
184 (*gal*)	4^{12} (*s*$^+$)	0	9
4^{12} (*gal*$^+$)	184 (*s*)	8	19
4^{12} (*gal*$^+$)	4^{12} (*s*$^+$)	7	10

Table XI - Relationships between chromosomes I and VI with *sup meth* A17 (II) in 4^{12} x 184 segregants).

Chromosomal combinations		Phenotype	
		meth	*meth*$^+$
I	VI		
184 (pa^+)	184 (*s*)	16	21
184 (pa^+)	24 (s^+)	6	12
24 (*pa*)	184 (*s*)	9	12
24 (*pa*)	24 (s^+)	1	13

The results in Table X show that chromosome VI (s^+) carries *sup meth* A17 but its action is efficient only in combination with chromosome III of 184 (2nd line).

In the same way, Table XI gives evidence that the *sup meth* mutation of chromosome VI is efficient when combined with chromosome I of the *hyper rec* 24 mutant (4th line).

Thus, the suppressor is present in chromosome VI linked to the s^+ marker and I_{184} and III_{184} and III_{24} may have elements related to the suppressor or a kind of anti-suppressor. Such modulators of suppressors may have independent actions.

Unfortunately we did not perform more appropriate crosses that could give a better mapping of these anti-suppressors or modulators.

Now we can better understand the results for 4^{12} x 184:4^{12}. Being a *meth* strain, it shows a residual growth in medium lacking methionine because of the presence of the *meth* suppressor which is responsible for the high number in the *meth*$^+$ w^+ class. But the suppressor is not very efficient (an efficient unlinked suppressor will show a RF of 25%) because this strain (4^{12}) has a modulator of the suppressor (*a. sup*) in chromosome III of 4^{12} which permits reduced growth after 48h of incubation (Table V). Since all of these factors are unlinked, the RF of *meth-w* in 4^{12} x·184 is not expected be as high as 25%.

We can make a scheme for 4^{12} x 184 (Figure 2). The scheme presented fits the *sup meth* hypothesis well and looks more correct when we observe in Tables I and III that the RF of *meth-w* is 15% for (24 x 196) and 16.8% (4^{12} x 184) (Tables I and VIII). These frequencies are close to the expected values, thus confirming our hypothesis. Therefore, the suppressor and anti-suppressor actions are independent and not linked. The *meth* s^+ *sup meth* segregant only appears in combination with pa^+ *a.su a* (184) and gal^+ *a.su b* (4^{12}), i.e.:

I	III	VI	II	
pa	*gal*$^+$	*s*$^+$	*meth*	= 0
pa	*gal*	*s*$^+$	*meth*	= 0
pa$^+$	*gal*	*s*$^+$	*meth*	= 0
pa$^+$	*gal*$^+$	*s*$^+$	*meth*	= 6

	I	III	VI	II
4^{12}	+ pa	a.su.b +	+ su24	meth w
184	a.su.a +	+ gal	s +	+ w^+

Figure 2 - Location of *sup* 24 and *a su* 24 (a and b).

These are the expected phenotypes among the progeny. It is evident that the anti-suppressor action is more efficient when the segregant has both elements, one in chromosome III (*gal*$^+$, of the *hyper rec* 24 mutant strain) and another in chromosome I (*paba*$^+$ of the normal strain UT 184).

DISCUSSION

Genetic analysis of the apparent *hyper-rec* 24 mutant showed several difficulties owing to the presence of suppressor and anti-suppressor genes that act through the association of two genetic elements. The presence of the suppressor of *meth* A_{17} is evident in Table I where the RF of *meth-w* is 15% in contrast to the normal value of 1% found by Clutterbuck (1981). The excess of *meth*$^+$ *w*$^+$ and the absence of the *meth-w* class indicates a rather inefficient suppressor or the presence of an anti-suppressor in the genome. This idea derives from a cross like the one shown in Table I, where the involved strains supposedly are:

w,	*meth*$^+$,	*a.su*,	*su meth*	(mutant 24)
w$^+$,	*meth*,	+,	+	(tester strain UT 196)

In such a situation the expected recombination frequency is 12.5%, which is close to the 15% value obtained.

Also the four major classes in Table II indicate that *sup meth* acts on chromosome II (parental normal). The +++ class is too large to imply a crossing-over between such close markers (*w-meth*).

The possibility of the $meth^+$ duplication being responsible for the large number of $meth^+$ w^+ recombinants (Table I) is excluded by the data given in Table IV. The suppressor hypothesis is schematically given in Figure 1 and the test for this possibility (Table IV) showed that the character of *hyper-rec* 24 is due to the *sup meth* A17 segregation, not linked to the gene it suppresses.

Mitotic analysis showed that three linkage groups are sensitive to pFP haploidization (I, III and VII, Table VI) but Table VII shows the existence of at least two defects in the genome of *hyper-rec* 24. One of them is *sup* 24 whose location was facilitated by the genetic analysis of 4^{12}, a meiotic segregant from the *hyper rec* 24 x 196 cross. The genetic analysis of 4^{12} x 184 (Tables X and XI) also revealed the action of an anti-suppressor (*a.su*), as proved by the RF of *meth-w* result.

The 16.8% RF of *meth-w* (Table IX) is very high to be merely result of an efficient, anti-suppressor action.

Besides, the absence of the w^+ *meth* class and the excess of the w^+ $meth^+$ class confirms the action of both a suppressor and an anti-suppressor.

The RF of *meth-w* expected in such case should be 25% when there is only the action of an efficient suppressor and 12.5% if there are both suppressor and anti-suppressor genes in the same strain. But the 16.8% RF confirms that the anti-suppressor is not so efficient.

Table XI shows that *sup meth* is located on chromosome VI. Table X confirms that chromosome VI bears the *sup meth* and chromosome III of the normal strain (UT 184) has an allosuppressor or chromosome III of the mutant (*hyper rec* 24) bear an anti-suppressor.

Table XI shows the possibility of an allosuppressor in chromosome I of the mutant or an anti-suppressor in chromosome I of the normal strain (UT 184).

Such factors showed independent and not linked actions. The combination I_{184} III_{24} showed and efficient anti-suppressor action. Or it may be only one anti-suppressor composed of two sub-units as shown schematically in Figure 2.

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RESUMO

O mutante 24 foi selecionado por Zucchi (*Rev. Bras. Genet. 13*: 409-424, 1990) pela sua frequência de recombinação aparentemente alta no intervalo *meth-w* do cromossomo II. Foi demonstrado que algumas das alterações mostradas pelas análises mitóticas e meióticas foram devidas a um supressor *meth* A_{17} recessivo, localizado no cromossomo V, ligado ao *locus* sB3. A análise de recombinação foi muito difícil, devido a presença de uma mutação anti-supressora no cromossomo III do mutante 24 que age mais eficientemente quando combinado com um fator do cromossomo I, da linhagem não relacionada UT 184.

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