

LOCATION OF THE SUPPRESSOR OF THE *meth A₁₇* MUTATION IN MUTANT 29 OF *Aspergillus nidulans*

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ABSTRACT

A suppressor gene of *meth A₁₇* was located on chromosome II. This suppressor is called silent because it appears in a strain that is *meth⁺* and can only be expressed in crosses with *meth A₁₇* strains. This mutation is evidenced by an alteration in the number of *meth⁺ w⁺* recombinants and by the compact morphology it confers to the *meth* suppressed strains.

Genetic analysis of the 29 mutant was made more complex by the presence of an anti-suppressor gene that abolishes the action of the suppressor in a non related strain.

In the course of the genetic analysis the location of the anti-suppressor was determined.

INTRODUCTION

The presence of suppressor genes in fungi such as *Aspergillus nidulans* and *Saccharomyces cerevisiae* (Lewis and Casselton, 1975; Moon and Kang, 1982; Zucchi, 1990b; Zucchi, 1992) confers somatic instability to the strains, probably because of their association with anti-suppressor genes (Galluci and Garen, 1966; Liebman and Sherman, 1976a,b) or because of their involvement with a duplication or transposition element (Marin and Zucchi, 1991, 1992; Castro-Prado and Zucchi, 1991a,b).

Working with *Saccharomyces* and *Schyzosaccharomyces*, Thuriaux *et al.* (1975) showed that mutations can occur in several different genes, reducing the efficiency of some nonsense suppressors. In studies on the same organisms McReady and Cox, 1976, determined that the anti-suppressors have some specificity and act by weakening or by completely abolishing the effect of some suppressors, but not of the others (Laten *et al.*,

1978; Liebman and Sherman, 1979; Anita *et al.*, 1980). Only a few anti-suppressors have been described in filamentous fungi, and even those were not studied in depth, so that their mechanism of action and molecular nature are still unknown.

The mutant 29 found by Zucchi (1990a) was selected for study as it presented a high meiotic recombination frequency at the *meth-w* interval of chromosome II.

MATERIAL AND METHODS

Strains

Mutant 29 (Zucchi, 1990a) has the same markers as UT 448, except for a mutation which confers to it the apparent *hyper-rec* character we describe and discuss in this paper.

The other strains used, supplied by Drs. C. van de Vate and G.J.O. Jansen, from utrecht stocks, were:

UT184 (Zucchi, 1990b).

UT 450: *paba* A₁₂₄ (I); *meth* A₁₇ (II), with requirements for *p*-aminobenzoic acid and methionine, respectively.

UT 224: *w* A₂ (II), white conidia; *bi* A₁ (I); *ade* D₃ (II), with requirements for biotin and adenine, respectively.

UT 161: *w* A₂ (II), white conidia; *paba* A₁ (I); *arg* C₃ (VIII); *ribo* B₂ (VIII), with requirements for *p*-aminobenzoic acid, arginine and riboflavin, respectively; *fac* B₁₀₁ (VIII), inability to use sodium acetate as a carbon source.

Media, solutions and general methodology follow those described by Zucchi (Zucchi, 1990a,b; 1992).

RESULTS AND DISCUSSION

Mutant 29 had a considerably increased recombination frequency (RF) in the *meth-w* interval of chromosome II, when measured in crosses with the tester strain UT 196 (Table I). The increased recombination frequency only appeared with crossing-over in the "wild" class. All the markers behaved normally, except those on chromosome II (Tables II, III and IV). On the basis of the data in Table IV, the order of the markers in chromosome II is as shown in Figure 1.

After establishing the sequence of the markers, it was shown that no additional mutation occurs in this segment of the chromosome, except for the alteration in the number of recombinants present at meiosis.

The presence of a *meth* suppressor in mutant 29 is evident from the data of Table V.

Table I - Meiotic recombination frequency in the *meth-w* interval of chromosome II in crosses using the control (448) and mutant 29, with strain 196. Analysis of randomly collected ascospores.

Crosses	RF %	Paternal class		Recombinant class	
		<i>meth w</i> ⁺	<i>meth</i> ⁺ <i>w</i>	<i>meth</i> ⁺ <i>w</i> ⁺	<i>meth w</i>
448 x 196 (control)	1.8	241	250	7	1
29 x 196	19.8	52	165	43	0

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

Crosses	RF %	Paternal class		Recombinant class	
		<i>Acr meth</i> ⁺	<i>Acr</i> ⁺ <i>meth</i>	<i>Acr</i> ⁺ <i>meth</i> ⁺	<i>Acr meth</i>
448 x 196 (control)	28.8	178	177	78	6
29 x 196	40.5	49	34	69	18

Table III - Classes arising after crossing-over between the markers of chromosome II.

Classes	448 x 196 (control)	29 x 196
<i>Acr</i> + <i>w</i> (1)	111	223
+ <i>me</i> + (2)	92	104
<i>Acr me</i> +	18	3
+ + <i>w</i> (3)	22	99
+ <i>me w</i>	0	0
<i>Acr</i> + +	2	38
+ + + (4)	3	102
<i>Acr me w</i>	3	1

Table IV - Recombination frequency among the markers of chromosome II. The number of colonies analyzed is given in parentheses.

Interval	448 x 196 (499)	29 x 196 (217)
<i>meth-w</i>	1.8	19.8
<i>Acr meth</i>	28.8	40.5
<i>Acr w</i>	27.0	29.0

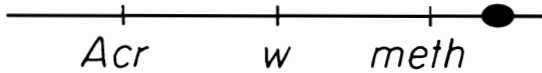


Figure 1 - Order of the markers in chromosome II: *w* lies between *meth* and *Acr*.

Table V - Crosses of meiotic *meth*⁺ *w*⁺ segregants with strain 184 (*meth*⁺ *w*⁺).

Origin of the meiotic	<i>meth</i> ⁺ <i>w</i> ⁺ segregant	Crossed with	Number of colonies	Segregants		
				<i>w</i>	<i>meth</i>	<i>Acr</i>
29 x 196	1 ⁷ (<i>y</i> ; <i>Acr</i>)	184	76	0	24	5
	3 ¹ (<i>y</i> ; <i>Acr</i>)	184	79	0	19	0

The excess of recombinants of the *w*⁺ *meth*⁺ class, supports the idea that unlinked suppressors may account for the transformation of part of the progeny of the *meth* *w*⁺ paternal class into an apparent *meth*⁺ *w*⁺ crossing-over. This seems very likely, especially on the basis of the data in Table I, in which the *meth* *w*⁺ parental class is less frequent than the other parental classes and leads us to formulate the hypothesis shown in Figure 2.

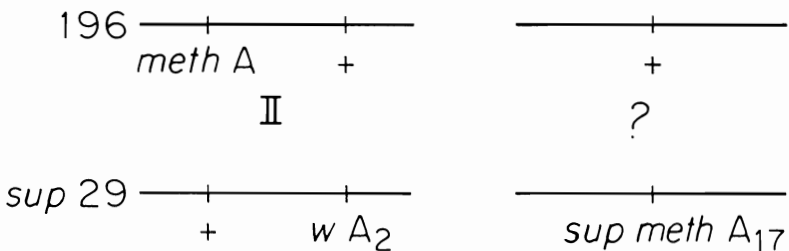


Figure 2 - Hypothesis of the *meth* A₁₇ suppressor in *sup* 29 mutant.

The expected frequency of *meth* A₁₇ segregants is 25%, very close to the results given in Table I. A tentative designation of this suppressor as *sup* 29 is proposed.

Testing the suppressor hypothesis

After crossing the apparent $w^+ meth^+$ recombinants with another true *meth*⁺ strain, the appearance of *meth* segregants in the progeny would confirm the hypothesis. Some of the apparent *meth*⁺ w^+ strains were crossed with the master strain 184 and some others with strain 602 (*paba* A₁₂₄, *bio* A₁, *arg* B₂).

Table VI shows a large number of *meth* segregants in crosses involving *meth*⁺ strains, clearly demonstrating the presence of a methionine suppressor in these segregants. Besides, the same Table VI also indicates that among the *meth*⁺ w^+ strains (C₉, D₁₆, ... 3¹) analyzed there is not a single real *meth*⁺ w^+ recombinant but only *meth sup meth w*⁺ segregants.

Table VI - *meth* segregation among the progeny of crosses of apparent crossing-over (*meth*⁺ w^+) with real *meth*⁺ w^+ strains.

Apparent crossing-over <i>meth</i> ⁺ w^+	Genotype	Crossed with	Origin	Progeny	
				<i>meth</i>	<i>meth</i> ⁺
C ₉	<i>yA; pyro</i> A ₄	602	29 x 196	21	78
D ₁₆	<i>yA; pyro</i> A ₄	602	29 x 196	10	82
B ₁₂	<i>yA; pyro</i> A ₄	602	29 x 196	17	78
K ₂₁	<i>yA; pyro</i> A ₄	602	29 x 196	21	76
L ₄	<i>yA; pyro</i> A ₄	602	29 x 196	12	86
M ₇	<i>yA; pyro</i> A ₄	602	29 x 196	15	84
1 ⁷	<i>yA; Acr</i> A ₁	184	29 x 196	25	52
3 ¹	<i>yA; Acr</i> A ₁	184	29 x 196	17	59

On the mechanism of the *meth* A₁₇ suppression

The mechanism underlying the suppression is unknown, although the results given in Table VII show that *sup* 29 is a recessive gene.

Table VII - Test for dominance or recessiveness.

Strain with suppressor	Genotype	Growth in methionine-free medium	
		Heterokaryon with 196	Diploid with 196
Q19	<i>paba</i> A ₁₂₄ ; <i>meth</i> A ₁₇ <i>sup</i> 29	no growth	growth reduced* from 4.2 to 3.6 cm
A17	<i>paba</i> A ₁₇ ; <i>meth</i> A ₁₇ <i>sup</i> 29	no growth	growth reduced* from 5.0 to 1.8 cm

* Reduction in colony diameter due to the lack of methionine.

As speculated previously, in the case of *sup* 30 (Zucchi, 1990b), the occurrence of recessive suppressors may be caused by: a) several kinds of regulatory mutations or b) an effect of gene dose. To determine which of the two hypotheses is correct, heterokaria and/or diploids were constructed, using a suppressor-bearing strain and a *meth* strain without a suppressor. Growth in methionine-free medium would be indicative of dominance and lack of growth would suggest recessivity. The growth of both strains in medium supplemented with L-methionine (0.02 mg/ml) was normal (Table VIII).

Table VIII - Mitotic analysis of the 29//184 diploid.

	Markers of the 448 or of the mutant strain							
	I	II	III	IV	V	VI	VII	VIII
Diploids	<i>paba</i> ⁺	<i>w</i>	<i>gal</i> ⁺	<i>pyro</i> ⁺	<i>fac</i> ⁺	<i>s</i> ⁺	<i>nic</i> ⁺	<i>ribo B</i> ⁺
448//184 (control)	.53	.59	.40	.46	.28	.47	.48	.63
29//184	.25	.57	.32	.27	.41	.53	.46	.46

Although more evident in the heterokarion than in the diploid *sup* 29 is a recessive suppressor.

Table VIII shows the haploid segregants from the 29//184 diploid, after pPF treatment. The combination of chromosomes I and II among these haploid segregants is observed in Table IX. The presence of chromosome I of mutant 29 promotes a reduction in the number of haploid segregants.

Table IX - Combination of chromosome I and II among the haploid segregants (in %).

Combinations		448//184	29//184
I ₂₉	II ₂₉	33	19
I ₁₈₄	II ₁₈₄	21	39
I ₂₉	II ₁₈₄	20	6.3
I ₁₈₄	II ₂₉	26	34

On the relationship between sup 29 and sup 30

The *sup 30* mutation is located on chromosome VII, linked to *nic B₈* (Zucchi, 1990b) and like *sup 29*, acts by suppressing the *meth A₁₇* mutation.

To study the relationship between the two suppressors, complementation and recombination experiments were performed using *sup 29* and *sup 30*. For this purpose, heterokaria between the following *meth A₁₇* strains containing suppressors 29 and 30 were synthesized: Q₁₉ (29) + B₁₂ (30) and C₉ (29) + I₁₈ (30).

The genotypes of these strains were:

Q₁₉: *paba A₁₂₄* (I); *meth A₁₇* (II); *sup 29*.

C₉: *yA₂* (I); *meth A₁₇* (II); *pyro A₄* (IV); *sup 29*.

B₁₂: *yA₂* (I); *meth A₁₇* (II); *pyro A₄* (IV); *sup 30* (VII).

I₁₈: *paba A₁₂₄* (I); *meth A₁₇* (II); *sup 30*.

The heterokaria did not grow in methionine-free medium, whereas good growth occurred if the medium was supplemented with methionine. Thus *sup 29* and *sup 30* complement themselves and are located on different genes. The same combinations were also meiotically crossed and the results are shown in Table X.

The large number of *meth* descendants indicates the absence of linkage between *sup 29* and *sup 30*. This not only agrees with the complementation results, but also with data to be presented further on.

Table X - Segregation of *meth*⁺ in crosses of *meth* strains.

Crosses	<i>meth</i> Descendants	<i>meth</i> ⁺ Descendants
Q ₁₉ (29) x B ₁₂ (30)	212	144
C ₉ (29) x I ₁₈ (30)	144	252

Morphology of the suppressive strain

The strain bearing suppressed *meth* A₁₇ by *sup* 29 did not return completely to the wild type morphology in methionine-free medium. The colonies were smaller, sporulated less and their centers were denser and had a transparent and irregular peripheral zone.

In the following analysis, this evidence was used to visually separate the suppressor in the strains bearing it from the *meth* A₁₇ strain (except for a few cases explained when necessary).

The linkage group of the sup 29 mutation

In an attempt to determine the linkage group of *sup* 29, analysis of the haploids of the I₂₅/UT 184 diploid was performed, where I₂₅ is a *meth* A₁₇ recombinant from the 29 x 196 cross. The full genotype of I₂₅ is: *ribo* A₁, *y*A₂; *meth* A₁₇; *sup* 29.

Whenever possible, the haploids were also counted in relation to the suppressor phenotype. The analysis of the haploid segregants suggested two chromosomes involved in the *meth* suppression: V and VI (Table XI).

Table XI - Analysis of the haploid segregants of the I₂₅/UT 184 diploid.

Combination of the chromosome II markers		I		III		IV		V		VI		VII		VIII	
		+	y	<i>gal</i>	+	<i>pyro</i>	+	<i>fac</i>	+	<i>lac</i>	+	<i>nic</i>	+	<i>cha</i>	+
<i>meth</i>	+	20	16	15	20	36	-	25	11	20	16	25	11	26	10
<i>sup</i>	+	13	7	9	11	19	1	-	20	-	20	12	8	14	6
+	+	1	1	-	2	2	-	1	1	2	-	2	1	1	1
<i>meth</i>	<i>Acr</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>sup</i>	<i>Acr</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+	<i>Acr</i>	28	-	20	20	39	1	17	23	20	20	32	8	32	8

In Table XI, the *meth*⁺ (1st line) and the *sup*⁺ (2nd line) haploids did not indicate that the *sup* 29 mutation was located on a single chromosome. The *meth*⁺ haploids plead against any location while the *sup*⁺ haploids indicate two chromosomes.

To solve this problem, all *Acr*⁺ *meth sup* haploid segregants were analyzed with respect to the different possible combinations of chromosomes V and VI, as related to the *meth* or *sup* character (Table XII).

Table XII - Determination of the chromosome bearing the *sup* 29 mutant.

Chromosomal combination		<i>Acr</i> ⁺ number (<i>meth sup</i>)	Phenotype
V	VI		
184 (<i>fac</i>)	184 (<i>lac</i>)	9	all <i>meth</i>
184 (<i>fac</i>)	I ₂₅ (+)	16	all <i>meth</i>
I ₂₅ (+)	184 (<i>lac</i>)	11	all <i>meth</i>
I ₂₅ (+)	I ₂₅ (+)	20	all with the suppressor character

The results suggest that chromosomes V and VI of I₂₅ need a kind of inter-cooperation in order to reach a real suppressive action. This explains why the *meth* progeny can carry either chromosome V or VI of I₂₅, but not both.

The anti-suppressor hypothesis

The evidence points to the presence of something unusual located on chromosomes V and VI of I₂₅. Two different hypotheses may be advanced regarding this phenomenon:

1. I₂₅ bears the suppressor in chromosomes V and VI in the form of two pieces, i.e. *sup* 29a and *sup* 29b. Both must be present in a given *meth* strain to establish the suppression (Figure 3 (1D)).

2. I₂₅ contains a "normal" suppressor gene either in chromosome V or VI and the 184 strains bears an anti-suppressor (*a. su*) in another chromosome, that can eliminate the action of the suppressor.

This hypothesis has two alternatives, *a* and *b*, depending on the location of the suppressor and anti-suppressor (Figure 3(2a, 2b)). Both hypothesis are schematically presented in Figure 3.

According to both hypotheses, only the combination of chromosomes V and VI of I₂₅ can suppress the *meth* A₁₇ mutation. The choice among the three possibilities can

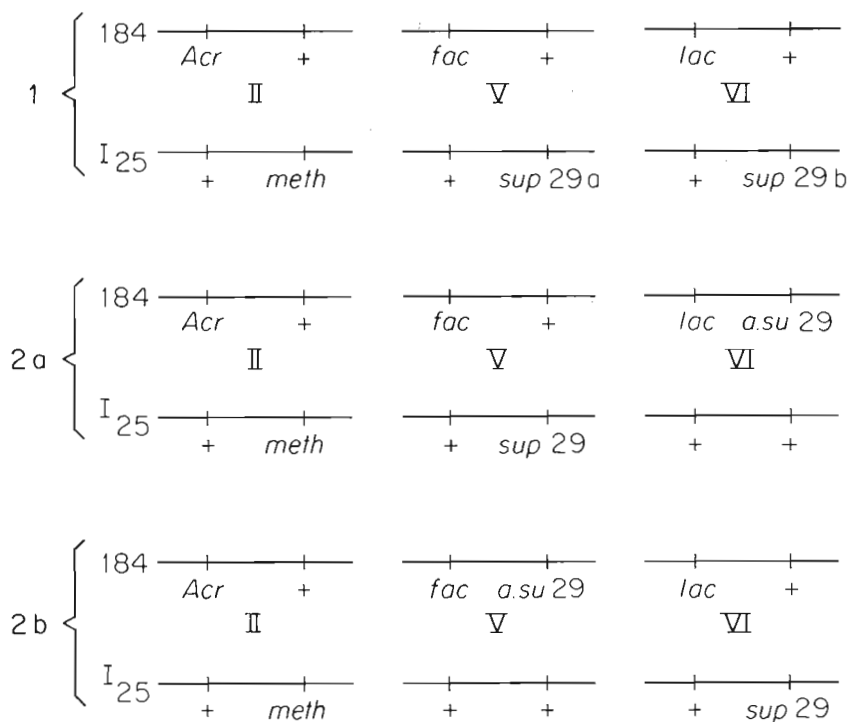


Figure 3 - The possible location of *sup 29* and *a.su 29*.

be made after observing the behavior of two I₂₅ derivatives called I_{252.21} and I_{253.24}, which are the *meth* haploids of the I₂₅//184 diploid (Table XIII). This table contains information concerning "21" and "24".

Table XIII - Genotypes and origin of the "21" and "24" haploids.

Haploid	Origin	Code	Genotype
I _{252.21}	184//I ₂₅	"21"	<i>ribo</i> Λ_1 , <i>y</i> Λ_2 , <i>bi</i> Λ_1 ; <i>meth</i> Λ_{17} <i>pyro</i> Λ_4 ; <i>lac</i> Λ_1 , <i>s</i> B ₃
I _{253.24}	184//I ₂₅	"24"	<i>ribo</i> Λ_1 ; <i>y</i> Λ_2 , <i>bi</i> Λ_1 ; <i>meth</i> Λ_{17} <i>pyro</i> Λ_4 ; <i>lac</i> Λ_1 , <i>s</i> B ₃

Since these are *meth* haploids, one may assume that the fact that they do not bear the suppressor may cause a low recombination frequency between *meth* A₁₇ and *w*A₂ in given crosses. But, when they were crossed with *UT 224: bi* A₁, *w* A₂, *ade* D₃; or with *UT 161: paba* A₁; *w* A₂, *arg* C₃; *fac* B₁₀₁, *ribo* B₂, the results presented a high RF *meth-w* (Table XIV).

Table XIV - Recombination frequencies of "21" and "24" when crossed with 224 and with 161.

Crosses	RF	Progeny			
		<i>meth-w</i> ⁺	<i>meth</i> ⁺ <i>w</i>	<i>meth</i> ⁺ <i>w</i> ⁺	<i>meth w</i>
21 x 224	14%	134	193	51	1
24 x 224	11%	150	173	58	2
21 x 161	14%	26	34	10	-
24 x 161	17%	25	34	10	-

The determinants of such a high recombination frequency are not found in normal strains 161 or 224, since these strains given normal RF (low) when crossed with the normal strain 196 (Table XV). The high RF *meth-w* in crosses including "21" and "24" with normal strains 224 or 161 should include the action of a *sup meth*, but "21" and "24" are *meth*.

Table XV - *meth-w* recombination frequency of strains 224 and 162 when crossed with 196.

Crosses	RT	Progeny			
		<i>meth w</i> ⁺	<i>meth</i> ⁺ <i>w</i>	<i>meth</i> ⁺ <i>w</i> ⁺	<i>meth w</i>
224 x 196	4.3	154	221	13	4
161 x 196	2.1	165	205	7	1

Besides, crosses of "21" and "24" strains with the UT 450 strain (all the crossed strains are *meth*) behaved unusually since these *meth* x *meth* crosses gave *meth*⁺ progeny (Table XVI).

In an attempt to map more precisely these elements the meiotic cross UT 184 x I₂₅ was undertaken. This cross is schematically given in Figure 5.

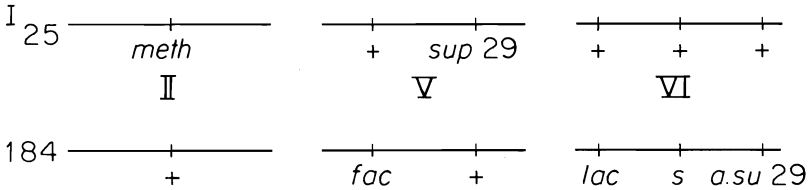


Figure 5 - Schematic representation of the UT 184 x I₂₅.

Table XVII presents the *fac*, *lac* and *s* segregation in the general progeny, in comparison to the segregation of these markers in the *sup 29* and *a.su 29* progeny respectively, scored through the suppressive phenotype of the colonies. The data are also corrected with regard to the aberrant segregation that *fac*, *lac* and *s* generate in the progeny (there is at least an evident shortage of *lac* versus *lac*⁺).

Table XVII - Segregation and RF of *fac*, *lac* and *s* in the general progeny in relation to the segregation of these markers among the *sup 29* and *a.su*⁺ progeny.

Marker	Found in the general progeny	Among descendants <i>sup 29</i> (<i>a.su</i> ⁺)	Corrected	RF
<i>fac</i>	176	16R	18.72	51% <i>fac</i> A <i>sup 29</i>
+	206	18	18.00	
In the progeny <i>sup 29</i> (<i>a.su</i>)				
<i>lac</i>	93	6R	18.75	40%
+	289	28	28.00	
<i>s</i>	107	6R	7.23	21%
+	129	28	28	

R: Recombinant class; *a.su*: anti-suppressor.

RESUMO

Este trabalho trata da localização de um gene supressor de *meth A₁₇* no cromossomo II. Este supressor é designado por "silencioso" por que ele aparece numa linhagem que é *meth⁺* e só pode se expressar em cruzamento com linhagens *meth A₁₇*. Esta mutação é evidenciada por alterar o número de recombinantes *w⁺ meth⁺* e pela morfologia compacta que ele confere às linhagens *meth* suprimidas.

A análise genética do mutante 29 se tornou mais complexa pela presença de um gene anti-supressor em uma linhagem não relacionada que abole a ação do supressor.

Durante as análises genéticas, a localização do anti-supressor foi determinada.

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