

## LOCATION OF THE SUPPRESSOR OF *meth A<sub>17</sub>* MUTATION IN THE 30 MUTANT OF *Aspergillus nidulans*

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### ABSTRACT

Mutant 30 was studied because of its peculiar meiotic and mitotic behavior. The high frequency of recombination in the *meth-w* interval of chromosome II is due to a silent and recessive *meth A<sub>17</sub>* suppressor. Mitotic analysis revealed strong selective pressure against chromosome I and VII. The morphology of the suppressed strain in a medium lacking methionine permitted the determination of the location of *sup 30* in chromosome VII linked to the *nic B8 locus* (26.8%).

### INTRODUCTION

In the course of the recombinational process several genetic or environmental factors can affect the final result of the recombination frequencies.

When the aim is the genetic study of induced factors promoting changes in the meiotic or mitotic recombination frequencies in a defined genetic interval, it is important to consider several genetic factors affecting recombination in a localized fashion such as translocation, duplication, transposon, suppressor, etc. Some complex interactions that can possibly occur among the new genic products and caused by mutation should also be considered.

An interesting kind of interaction is the one occurring between the product of a suppressor mutation and the mutation it suppresses. One of the mechanisms postulated for the suppressive effect is the opening of alternative metabolic pathways. Although there is not a general theory for explaining suppressor action, several types of evidence show that metabolic lesions can be overcome through the action of suppressor mutations that bypass the defective component.

Among the *meth* suppressors studied in several lower eukaryotes, new alternative metabolic pathways for bypassing the original genetic block, seem to occur frequently. The *meth 1 locus* of *A. nidulans* reverts frequently and spontaneously (Siddiqi and Putrament, 1963; Lilly, 1965; Alderson and Clark, 1966; Paszewski and Grabski, 1975) all of them being revertants of the suppressor type. Gajewski and Litwiska (1968) studying the *meth locus* and its suppressors found that all the *meth* suppressor *loci*, except one, were either recessive or partially dominant. The morphology of the suppressed strains was also altered (Lilly, 1965).

Bal *et al.* (1978) found spontaneous reversions in two different *loci* of *A. nidulans* (*ade A* and *meth G*) accounted for monogenic suppressor mutations giving evidence for the existence of a super-suppressor in *A. nidulans*. The high frequency of different suppressors related to the same *meth* mutation in *A. nidulans* suggests that methionine synthesis from cystein is not the only existing metabolic pathway.

In general, the presence of suppressors in fungi as *A. nidulans* and *S. cerevisiae* (Lewis and Casselton, 1975; Moon and Kang, 1982) and in bacteria (Schwartz, 1965, 1967) confers somatic instability on account of the association with anti-suppressor genes (Gallucci and Garen, 1966; Liebman and Sherman, 1976) or by involvement with a transposition element (Ball, 1967).

As a part of a comprehensive study on mutations affecting recombination, Zucchi (1986) developed a system that proved to be useful for selecting mutants bearing altered meiotic recombination frequencies in relation to the genetic markers of a given chromosomal segment. The present paper reports on the results obtained with a silent *meth* suppressor mutant isolated through the above cited system. Special emphasis is placed on how much it disturbs the recombination frequency in the *meth-w* interval of chromosome II of *A. nidulans*.

## MATERIAL AND METHODS

### Strains

According to the nomenclatural proposition of Clutterbuck (1970) the allele mutants of the strains used were:

**UT 448:** *w* A2 (II) white conidia; *ribo* A1, *paba* A124, *bi* A1 (I), with requirements for riboflavin, *p*-aminobenzoic acid and biotin, respectively; *Acr* A1 (II) resistant to acriflavin.

**UT 196:** *y* A2 (I) yellow conidia; *meth* A17 (II); *pyro* A4 (IV), with requirements for methionine and pyridoxine.

**UT 184:** *cha* A1 (VIII) "chartreuse" conidia; *pyro* A4 (IV); *s* B3 (VI); *nic* B8 (VII); *ribo* B2 (VIII) requirements for pyridoxine, sodium thiosulphate, nicotinamide

and riboflavin, *gal* A1 (III); *fac* A303 (V); *lac* A1 (VI), unable to grow in a medium containing galactose, acetate and lactose, respectively as the sole carbon source; *sul* A1 (I) and *Acr* A1 (II); resistant to sulphonylamide and acriflavin, respectively.

**Mutant 30:** Isolated from the *meth*<sup>+</sup> UT 448 strain after MNNG treatment (Zucchi, 1986). Same markers as in UT 448, except for the mutation which confers to it the *sup meth* character to be described.

### *Media and solutions*

Complete medium (CM) and minimum medium were based on Van de Vate and Jansen (1978). For solid medium 1.5% Bacto agar Difco was added.

### *Methods*

The general methodology followed Pontecorvo *et al.* (1953). The diploids were prepared by the method of Roper (1952). The location of the mutant alleles, duplication and suppressor on linkage groups was determined by haploidization of diploids (Forbes, 1959) after *p*-fluorophenylalanine (Morpurgo, 1961; Lhoas, 1961) or benlate (Hastie, 1970) treatment.

The incubation temperature was 37°C.

## RESULTS AND DISCUSSION

The mutant 30, isolated by Zucchi (1986), presents, among others, the following characteristics: slight mitotic instability giving rise to sectors with improved or deteriorated morphology, slightly reduced growth rate (Figure 1) and a high recombination frequency in the interval *meth* A17 - *w* A2.

The genetic markers of mutant 30 are: *ribo* A1, *paba* A124, *bio* A1; *Acr* A1, *w* A2. The improved and deteriorated sectors spontaneously arising in the colonies of this mutant were also isolated. The recombination frequency was very high in the *meth* A17 - *w* A2 region of chromosome II, as routinely measured in crosses with UT 196 (*y* A2; *meth* A17; *pyro* A4). The control strain (UT 448), the mutant 30 and its sectors were crossed to UT 196. The results in relation to *meth* and *w* are given in Table I which shows that the *meth-w* recombination frequency increased only in the "wild type" class of crossing over. Table II shows that the recombination frequencies in the *Acr-meth* interval of chromosome II are almost normal.

The abnormal meiotic behavior of the markers of chromosome II becomes more evident from the data in Table III which show that the control cross (UT 448 x UT 196) present two main recombinant classes (1) and (2), while mutant 30 and its sectors present four: (1), (2), (3) and (4).

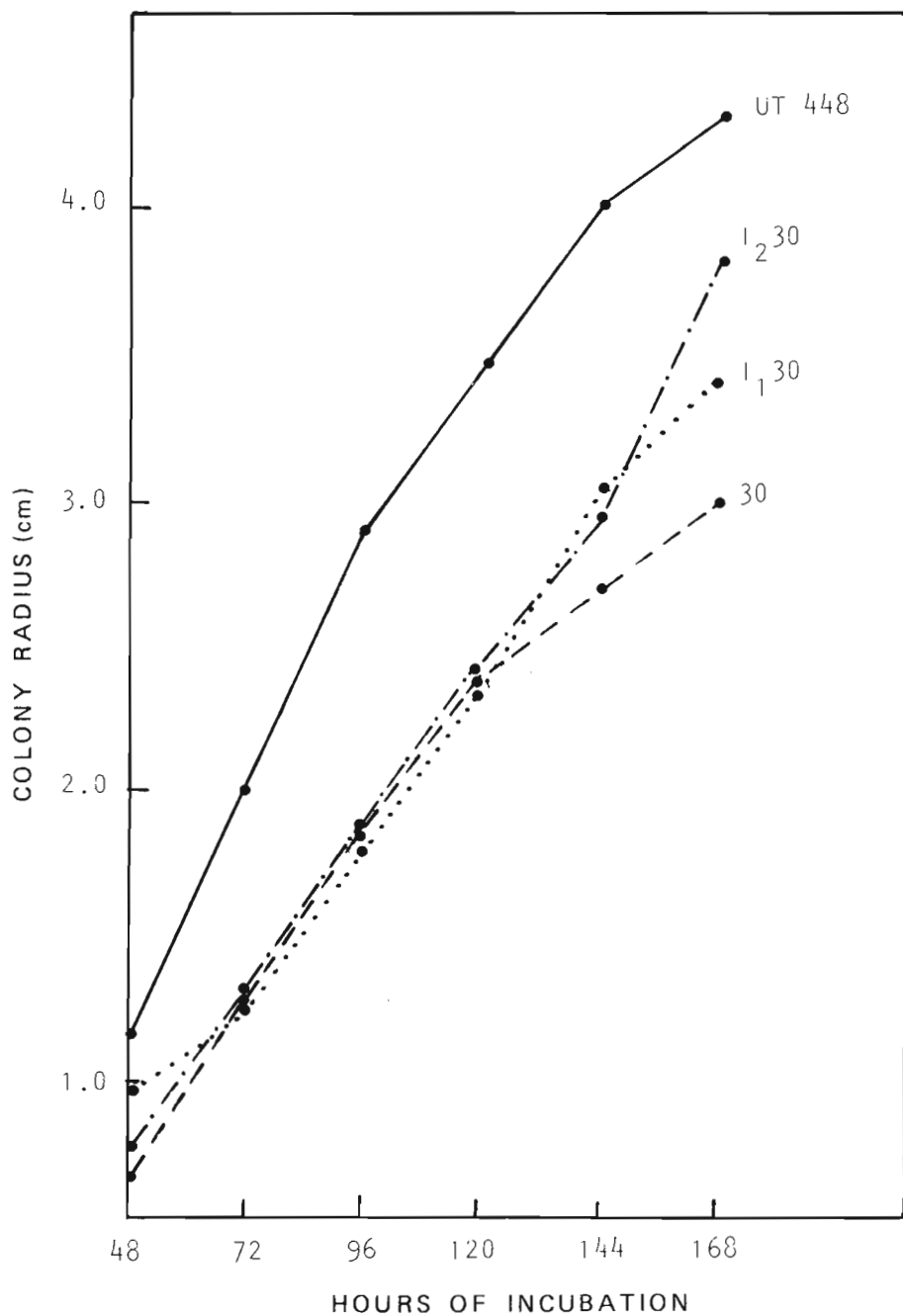


Figure 1 - Growth curves of the control strain UT 448, the 30 mutant and the spontaneous improved sectors: I<sub>1</sub>30 and I<sub>2</sub>30.

Table I - Meiotic recombination frequency in the *meth-w* interval of chromosome II. (Data from analysis of a random sampling of ascospores).

| Crosses                 | RF (%) | Paternal class             |                                   | Recombinant class                              |               |
|-------------------------|--------|----------------------------|-----------------------------------|--|---------------|
|                         |        | <i>meth w</i> <sup>+</sup> | <i>meth</i> <sup>+</sup> <i>w</i> | <i>meth</i> <sup>+</sup> <i>w</i> <sup>+</sup> | <i>meth w</i> |
| 448 x 196               | 1.8    | 241                        | 250                               | 7  | 1             |
| 30 x 196                | 24.0   | 118                        | 178                               | 90   | 3             |
| I <sub>1</sub> 30 x 196 | 13.0   | 143                        | 241                               | 56   | 3             |
| I <sub>2</sub> 30 x 196 | 20.0   | 57                         | 122                               | 45   | 1             |
| D <sub>1</sub> 30 x 196 | 15.0   | 73                         | 91                                | 28   | 1             |

\*I: improved sector; D: deteriorated sector.

Table II - Frequency of meiotic recombinants, interval *Acr-meth*, of chromosome II.

| Crosses                 | RF (%) | Paternal class               |                                     | Recombinant class                                |                 |
|-------------------------|--------|------------------------------|-------------------------------------|--|-----------------|
|                         |        | <i>Acr meth</i> <sup>+</sup> | <i>Acr</i> <sup>+</sup> <i>meth</i> | <i>Acr</i> <sup>+</sup> <i>meth</i> <sup>+</sup> | <i>Acr meth</i> |
| 448 x 196               | 27.3   | 95                           | 105                                 | 38   | 37              |
| 30 x 196                | 34.4   | 185                          | 128                                 | 106  | 58              |
| I <sub>1</sub> 30 x 196 | 31.4   | 196                          | 108                                 | 101  | 38              |
| I <sub>2</sub> 30 x 196 | 32.8   | 106                          | 45                                  | 61   | 13              |
| D <sub>1</sub> 30 x 196 | 25.0   | 76                           | 68                                  | 42   | 7               |

Table III - Classes observed after the crossing over among the markers of chromosome II.

|                           | 448 x 196 | 30 x 196 | I <sub>1</sub> 30 x 196 | I <sub>2</sub> 30 x 196 |
|---------------------------|-----------|----------|-------------------------|-------------------------|
| <i>Acr</i> + <i>w</i> (1) | 111       | 94       | 183                     | 94                      |
| + <i>meth</i> + (2)       | 92        | 94       | 102                     | 44                      |
| <i>Acr meth</i> +         | 18        | 24       | 42                      | 13                      |
| + + <i>w</i> (3)          | 22        | 84       | 46                      | 28                      |
| + <i>meth w</i>           | 0         | 0        | 1                       | 0                       |
| <i>Acr</i> + +            | 2         | 7        | 10                      | 11                      |
| + + + (4)                 | 3         | 83       | 46                      | 34                      |
| <i>Acr meth w</i>         | 3         | 3        | 1                       | 1                       |

\* I, improved sector.

### A test for the suppressor hypothesis

The *meth*<sup>+</sup> character of the recombinant is not totally methionine independent as in the wild strains: the colonies in SM without methionine are smaller and, in some crosses, the character *meth* segregates again. In Table I, indeed, the paternal class *meth*<sup>+</sup> *w* is more frequent than the other parental one. A hypothesis is presented in Figure 2, where the suppressor is called *sup* 30. The expected frequency of the *meth* A - *sup* *meth* A class is 25%, in agreement with the frequency given in Table I (2nd line, 24%).

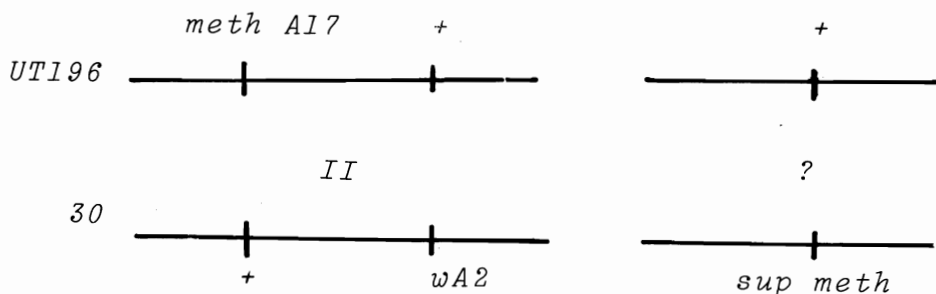


Figure 2 - The suppressor hypothesis.

This hypothesis can be easily tested by crossing the assumed "wild" cross-overs *w*<sup>+</sup> *meth*<sup>+</sup> with another strain (Master) *meth*<sup>+</sup>. If the suppressor hypothesis is correct, *meth* segregants are expected to occur in the progeny. Some of the phenotypically *meth*<sup>+</sup> *w*<sup>+</sup> recombinants were crossed with UT 184 and some others with the 602 strain: *paba* A124; *bi* A2; *arg* B2. The results are given in Table IV.

A large number of *meth* segregants appeared in crosses involving paternal *meth*<sup>+</sup>, thus providing evidence for the presence of a *sup meth* in the segregants (Table IV). Of these, only B13 and 3<sup>16</sup> are real *meth*<sup>+</sup> recombinants whereas all the others are indeed *meth sup* 30 strains. The results in Table V give full support to the suppressor hypothesis, suggesting that the character *hyper rec* 30 is the same as the *meth* suppressor, since they disappear simultaneously.

### Suppression mechanism

The option for the suppression hypothesis was reached merely through the use of formal arguments. Up to now there is no evidence concerning the suppressive mechanism or its dominant or recessive role.

Table IV - *meth* segregants among the progeny of *meth*<sup>+</sup> x *meth*<sup>+</sup> crosses.

| <i>meth</i> <sup>+</sup> w <sup>+</sup><br>apparent<br>crossing-over | Genotype  | Origin   | Progeny of the<br>crosses with 184 or 602 |                          |
|--|---|----------|---|--------------------------|
|  |   |          | <i>meth</i>                               | <i>meth</i> <sup>+</sup> |
| 1 <sup>3</sup>   | <i>yA2; Acr A1</i>                                    | 30 x 196 | 23  | 31                       |
| 3 <sup>6</sup>   | <i>yA2; Acr A1</i>                                    | 30 x 196 | 35  | 74                       |
| 15   | <i>ribo A1, yA2, bi A1; Acr A1</i>                    | 30 x 196 | 51  | 79                       |
| 1A   | <i>paba A124, bi A1</i>                               | 30 x 196 | 55  | 70                       |
| 30B  | <i>paba A124, yA2</i>                                 | 30 x 196 | 44  | 81                       |
| 6  | <i>ribo A1, paba A124, bi A1; Acr A1</i>              | 30 x 196 | 20  | 99                       |
| 8  | <i>yA2</i>  | 30 x 196 | 61  | 39                       |
| 12   | <i>ribo A1, yA2</i>                                   | 30 x 196 | 21  | 24                       |
| 17   | <i>ribo A1, paba A124, bi A1; Acr A1</i>              | 30 x 196 | 28  | 56                       |
| 22   | <i>yA2</i>  | 30 x 196 | 40  | 68                       |
| 28   | <i>ribo A1, paba A124, yA2</i>                        | 30 x 196 | 46  | 50                       |
| 3 <sup>16</sup>  | <i>ribo A1, bi A1; Acr A1,</i><br><i>wA2; pyro A4</i> | 30 x 196 | 0   | 123                      |
| A <sub>18</sub>  | <i>ribo A1, yA2; pyro A4</i>                          | 30 x 196 | 33  | 65                       |
| B <sub>12</sub>  | <i>yA2, pyro A4</i>                                   | 30 x 196 | 31  | 68                       |
| B <sub>13</sub>  | <i>ribo A2, bi A1; Acr A1; pyro A4</i>                | 30 x 196 | 0   | 98                       |

Table V - Segregants and recombination frequency in the offspring of *meth* strains crossed with *meth*<sup>+</sup> strains.

| Descendants<br>( <i>meth</i> ) | Genotype<br><i>meth A17</i><br>and                    | Crossed<br>with<br>( <i>meth</i> <sup>+</sup> ) | RF<br>(%) | <i>meth</i><br>w <sup>+</sup> | <i>meth</i> <sup>+</sup><br>w | <i>meth</i> <sup>+</sup><br>w <sup>+</sup> | <i>meth</i><br>w |
|--------------------------------|---|---|-----------|-------------------------------|-------------------------------|--|------------------|
| B <sub>2</sub>                 | <i>yA2; pyro A4; Acr A1</i>                           | 448   | 1         | 43                            | 56                            | 1  | -                |
| B <sub>9</sub>                 | <i>yA2; pyro A4</i>                                   | 448   | 1         | 40                            | 59                            | 1  | -                |
| D <sub>4</sub>                 | <i>yA2; pyro A4</i>                                   | 448   | 3         | 51                            | 47                            | 2  | -                |
| D <sub>15</sub>                | <i>yA2; pyro A4</i>                                   | 448   | 1         | 55                            | 44                            | -  | 1                |
| 1 <sup>23</sup>                | <i>yA2</i>  | 448   | 2.5       | 32                            | 85                            | 3  | 0                |
| 5 <sup>19</sup>                | <i>yA2; Acr A1, wA2</i>                               | 184   | 5.9       | 63                            | 49                            | 5  | 2                |
| 07                             | <i>ribo A1, bi A1;</i><br><i>Acr A1, wA2, pyro A4</i> | 602   | 0         | 46                            | 54                            | -  | -                |

A dominant suppression appears to be due to: a) a duplication of the *meth*<sup>+</sup> gene somewhere in the genome. Marin (1983) found such a case when studying another mutant which presented a *meth*<sup>+</sup> duplication in chromosome I, between *paba* and *y*; b) the use of a new metabolic pathway or c) a nonsense or missense suppressor. Conversely, a recessive suppressor could be due to: a) several kinds of regulatory mutations; b) gene dose effect.

To distinguish among these possibilities, diploids and heterokaryons were constructed by using a strain with the suppressor (*sup meth* A17) and a strain without the suppressor (*meth* A17). The results are summarized in Table VI, which shows that the suppressor (*sup 30*) is recessive although this characteristic is more evident in the heterokaryon than in the diploid.

Table VI - Test for suppressor recessivity.

| <i>sup meth</i><br>strain | Genotype  | Heterokaryon with 196     |  | Diploid with 196 |              |
|---------------------------|---|---------------------------|--|------------------|--------------|
|                           |   | Medium without methionine |  | with meth        | without meth |
| I <sub>18</sub> **        | <i>paba</i> A124;<br><i>meth</i> A17<br><i>sup 30</i> | No growth                 |  | 5.0 cm           | 1.8 cm*      |

\* Colony diameter.

\*\* I<sub>18</sub> represents a recombinant (30 x 196) bearing the *sup 30* trait.

### *Morphology of the suppressive strains*

In a medium lacking methionine the suppressive strains (*meth* A17) did not completely return to the wild morphology. They were smaller, with less conidiation, and the colony center was denser with a more transparent and irregular peripheral region. This fact was explored in the following analysis since it facilitated scoring the strains bearing the *meth* A17 suppressor, except for some cases discussed below.

### *The linkage group of sup 30*

In an attempt to determine the linkage group of *sup 30*, the haploids originating from the N17//184 diploid were analysed. N17 is a recombinant which originated from 30 x 196. Its genotype is *paba* A124, *y*A2; *meth* A17 *sup 30*; the phenotype of this strain is, of course, *meth*<sup>+</sup>.

The diploids were converted to haploids, by treatment with benlate and then were scored for every conspicuous marker. In the present case and since it requires previous knowledge of the genetic background of the haploids, the suppressive genotype was not scored. But even under these circumstances the observations showed a positive result. The combination of the chromosome markers (*meth* A<sub>17</sub> and *Acr* A<sub>1</sub>) as distributed among the members of the chromosomal pairs are given in Table VII. As indicated by the markers, the member 184 is placed at the left and the member N17 at the right (Table VII).

Table VII - Analysis of the haploid segregants of the N17//184 diploid after treatment with benlate.

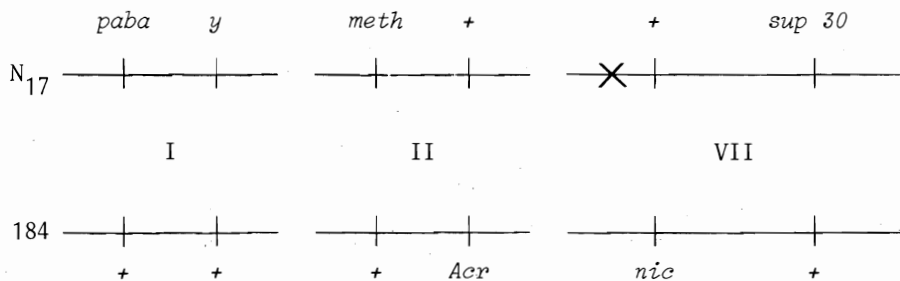
| Combinations<br>of the<br>chromosome |            | I  |    | III        |    | IV          |    | V          |    | VI         |    | VII        |   | VIII       |    |
|--------------------------------------|------------|----|----|------------|----|-------------|----|------------|----|------------|----|------------|---|------------|----|
| II markers                           |            | +  | y  | <i>gal</i> | +  | <i>pyro</i> | +  | <i>fac</i> | +  | <i>lac</i> | +  | <i>nic</i> | + | <i>cha</i> | +  |
| <i>meth</i>                          | +          | 20 | 10 | 12         | 18 | 15          | 15 | 14         | 16 | 20         | 10 | 30         | - | 11         | 19 |
| +                                    | <i>Acr</i> | 27 | 12 | 14         | 27 | 25          | 16 | 20         | 21 | 20         | 21 | 41         | - | 10         | 31 |
| +                                    | +          | 1  | -  | 1          | -  | -           | 1  | -          | 1  | -          | 1  | 1          | - | -          | 1  |
| <i>meth</i>                          | <i>Acr</i> | -  | -  | -          | -  | -           | -  | -          | -  | -          | -  | -          | - | -          | -  |

Since the *meth* progeny appears in the segregants shown in Table VII it is proved that: a) N17 is indeed a strain bearing a suppressor; b) *sup* 30 is not located on the II chromosome. Since the *meth* offspring cannot bear chromosome N17, which carries the suppressor, the data in Table VII, shows that *sup* 30 is located on chromosome VII.

The + *Acr* offspring should carry either the chromosome of 184 or the chromosome of N17. In addition, it also seems that the suppressor-bearing chromosome VII, is among the haploids selected against. It is assumed that this chromosome bears a genetical element sensitive to benlate (e.g. a duplication) and it remains to be proved whether or not the suppressor and the supposed genetical element are one and the same.

The + + offspring should contain the suppressed *meth*. On the basis of the selection against the chromosome bearing the suppressor, such a class should be very scanty. Since the *meth Acr* class requires mitotic crossing-over at the II chromosome, it is absent. These results are outlined in Figure 3.

On account of its ability to segregate fully *meth* haploids the N17//184 diploid is not fit for this analysis. In an attempt to improve this detrimental situation, the E<sub>2</sub>//B<sub>12</sub> and E<sub>10</sub>//B<sub>12</sub> diploids were employed. E<sub>2</sub> and E<sub>10</sub> are haploids from N17//184 and their genotypes are: *paba* A<sub>124</sub>, *yA*<sub>2</sub>; *meth* A<sub>17</sub>; *nic* B<sub>8</sub>; *ribo* B<sub>2</sub>; *cha* A<sub>1</sub>.



X selected against

Figure 3 - Partial representation of the  $N_{17}/184$  diploid.

Since  $E_2$  and  $E_{10}$  received their chromosome VII from the 184 strain (not from  $N_{17}$ ) they are methionine dependent since they do not bear the suppressor of the  $N_{17}$  strain.

The other element of the diploid is  $B_{12}$  whose genotype is  $yA_2$ ; *meth*  $A_{17}$ ; *pyro*  $A_4$ ; *sup 30* and thus the diploid is *meth*  $A_{17}$  homozygous and heterozygous for the suppressor. Therefore the suppressor segregation is detectable in the entire haploid progeny.

A partial representation of the diploids is given in Figure 4.

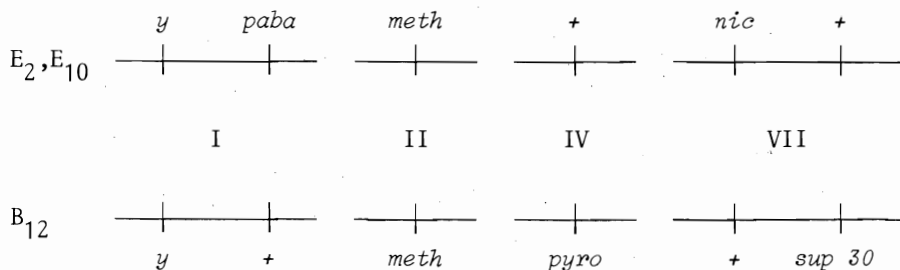


Figure 4 - Partial representation of the  $E_2//B_{12}$  and  $E_{10}/B_{12}$  diploids.

The results given in Table VIII give evidence for a strong selection against any chromosome VII bearing *nic*<sup>+</sup>. Since chromosome VII ( $B_{12}$ ) is being eliminated, the suppressor of *meth*  $A_{17}$  contained on it is also being eliminated.

Table VIII - Haploid segregants of the E<sub>2</sub>//B<sub>2</sub> and E<sub>10</sub>//B<sub>2</sub> diploids.

| Diploid                          | Haploids        |                             |                             |   |
|----------------------------------|-----------------|-----------------------------|-----------------------------|---|
|                                  | <i>meth nic</i> | <i>meth<sup>+</sup> nic</i> | <i>meth nic<sup>+</sup></i> | <i>meth<sup>+</sup> nic<sup>+</sup></i> |
| E <sub>2</sub> //B <sub>2</sub>  | 70              | -                           | 2                           | -                                       |
| E <sub>10</sub> //B <sub>2</sub> | 70              | -                           | 2                           | -                                       |

Thus most of the haploid segregants are methionine dependent, as evidenced in Table VIII. This is the feature under consideration which supports the proposed location of the suppressor.

If one considers that *sup meth A<sub>17</sub>* accounts for the observed high RF *meth-w*, one must conclude that when this suppressor is lost, the recombination frequency returns to normal levels. To check this conclusion, E<sub>2</sub> and E<sub>10</sub> were crossed with the normal strain 448 and the results obtained from this cross are given in Table IX.

Table IX - Segregants and recombination frequencies of the recombinants between markers of chromosome II, in meiotic crosses of E<sub>10</sub>, E<sub>2</sub> and 3<sup>11</sup>.

| Crosses               | RF            | Segregants                |                           |                                       |               |
|-----------------------|---------------|---------------------------|---------------------------|---------------------------------------|---------------|
|                       | <i>meth-w</i> | <i>meth-w<sup>+</sup></i> | <i>meth<sup>+</sup> w</i> | <i>meth<sup>+</sup> w<sup>+</sup></i> | <i>meth w</i> |
| E <sub>2</sub> x 448  | 2.7%          | 171                       | 187                       | 10                                    | -             |
| E <sub>10</sub> x 448 | 0.7%          | 167                       | 187                       | 3                                     | 1             |
| 3 <sup>11</sup> x 196 | 1.0%          | 43                        | 55                        | 0                                     | 1             |

The same kind of analysis was performed in relation to the selection of segregant 3<sup>11</sup>, obtained from the 30//184 diploid whose genotype is: *ribo A1*, *paba A124*; *bi A1*; *Acr A1*; *w A2*; *nic B8*; *ribo B2*; *cha A1*; i.e., all the chromosomes originate from the mutant 30, except VII and VIII; thus, 3<sup>11</sup> does not contain *sup 30* (VII). When meiotically crossed to 196, 3<sup>11</sup> gave an absolutely normal RF as happens to be the case for E<sub>2</sub> and E<sub>10</sub> (Table IX). Several other diploids were made and their analysis supported the results given above.

The 3<sup>10</sup> NM strain is a meiotic descendant of the cross between the normal strain 196 x 184 and was selected because it bears markers in chromosomes I, III, IV, V, VI and VIII, and because it permits demonstration of the mitotic *meth* and *w* cross-

ing-over of chromosome II. The full genotype of 3<sup>10</sup> NM is: *Acr A<sub>1</sub>*; *meth<sub>17</sub>*; *gal A<sub>1</sub>*; *pyro A<sub>4</sub>*; *fac A<sub>303</sub>*; *ribo B<sub>2</sub>*; *cha A<sub>1</sub>*; (NM means: New Master).

The analysis of the 3<sup>10</sup> NM//30 diploids showed a high recombination frequency between *meth* and *w* which represents an unusual fact, since genes so strongly linked are not supposed to give rise to recombinants in this region. Thus, they give *ca.* 10.4% recombination, instead of 0% (Table X).

Table X - Mitotic analysis of the 3<sup>10</sup>NM//30 (chromosome II).

| Diploid                 | RF            |                           | Progeny                   |               |                                       |
|-------------------------|---------------|---------------------------|---------------------------|---------------|---------------------------------------|
|                         | <i>meth-w</i> | <i>w<sup>+</sup> meth</i> | <i>w meth<sup>+</sup></i> | <i>w meth</i> | <i>w<sup>+</sup> meth<sup>+</sup></i> |
| 3 <sup>10</sup> NM//448 | 0%            | 29                        | 55                        | 0             | 0                                     |
| 3 <sup>10</sup> NM//30  | 10.4%         | 13                        | 20                        | 0             | 5*                                    |

\* Excessive number of recombinants.

Since crossing-over between very closely located genes constitutes a very rare event, the data in Table X show the presence of *sup 30* in the sense that it transforms part of the paternal *w<sup>+</sup> meth* class into *w<sup>+</sup> meth<sup>+</sup>* pseudo-recombinants. Analysis of the haploid segregants (Table XI) showed that *sup 30* is not present in chromosomes I, II, III, IV, V and VIII. Since Table VII also excludes chromosome VI, it is concluded that chromosome VII is the bearer of *sup 30*. Although indirectly, these results give further support to the suppressor hypothesis.

Except for VI and VIII, the *meth* progeny in Table XI segregate with all the linkage groups and the *w meth* class is absent since it requires crossing-over between very close markers.

The presence of a very low frequency of the *meth<sup>+</sup> w<sup>+</sup>* class, in the 30//3<sup>10</sup> NM diploid, corresponds exactly to the parental *w<sup>+</sup> meth<sup>+</sup>* class, bearing a suppressed *meth*.

This diploid (Figure 5) when haploidized, shows:

| Expected genotype               | Scored phenotype          |
|---------------------------------|---------------------------|
| <i>w<sup>+</sup> meth</i> +     | <i>w<sup>+</sup> meth</i> |
| <i>w meth<sup>+</sup> su 30</i> | <i>w meth<sup>+</sup></i> |
| <i>w<sup>+</sup> meth su 30</i> | <i>w<sup>+</sup> meth</i> |
| <i>w meth<sup>+</sup> +</i>     | <i>w meth<sup>+</sup></i> |

Table XI - Analysis of segregants of the 3<sup>10</sup>NM//30 and 3<sup>10</sup>NM//448 (control) diploids after *pFP* treatment.

| Diploids                             | Combinations<br>of<br>chromosome<br>II markers | I                      |           | III        |                         | IV        |                        | V          |                         | VIII       |                         |
|--------------------------------------|--|------------------------|-----------|------------|-------------------------|-----------|------------------------|------------|-------------------------|------------|-------------------------|
|                                      |  | <i>pa</i> <sup>+</sup> | <i>pa</i> | <i>gal</i> | <i>gal</i> <sup>+</sup> | <i>py</i> | <i>py</i> <sup>+</sup> | <i>fac</i> | <i>fac</i> <sup>+</sup> | <i>cha</i> | <i>cha</i> <sup>+</sup> |
| 30//3 <sup>10</sup> NM               | <i>w</i> <sup>+</sup> <i>meth</i>              | 9                      | 4         | 5          | 8                       | 6         | 7                      | 6          | 7                       | 9          | 4                       |
|                                      | <i>w</i> <i>meth</i> <sup>+</sup>              | 21                     | 9         | 17         | 13                      | 20        | 10                     | 19         | 11                      | ?          | ?                       |
|                                      | <i>w</i> <sup>+</sup> <i>meth</i> <sup>+</sup> | 3                      | 2         | 4          | 1                       | 2         | 3                      | 3          | 2                       | 5          | -                       |
|                                      | <i>w</i> <i>meth</i>                           | -                      | -         | -          | -                       | -         | -                      | -          | -                       | -          | -                       |
| 448//3 <sup>10</sup> NM<br>(control) | <i>w</i> <sup>+</sup> <i>meth</i>              | 12                     | 17        | 13         | 16                      | 16        | 13                     | 13         | 16                      | 23         | 6                       |
|                                      | <i>w</i> <i>meth</i> <sup>+</sup>              | 29                     | 26        | 23         | 32                      | 28        | 27                     | 28         | 27                      | ?          | ?                       |
|                                      | <i>w</i> <sup>+</sup> <i>meth</i> <sup>+</sup> | -                      | -         | -          | -                       | -         | -                      | -          | -                       | -          | -                       |
|                                      | <i>w</i> <i>meth</i>                           | -                      | -         | -          | -                       | -         | -                      | -          | -                       | -          | -                       |

At this point it is important to stress that chromosome I of E<sub>2</sub>, E<sub>10</sub> and 3<sup>11</sup> bears the markers:

|                                    |                          |             |                       |                        |
|------------------------------------|--------------------------|-------------|-----------------------|------------------------|
| E <sub>2</sub> and E <sub>10</sub> | <i>ribo</i> <sup>+</sup> | <i>paba</i> | <i>y</i>              | <i>bi</i> <sup>+</sup> |
| 3 <sup>11</sup>                    | <i>ribo</i>              | <i>paba</i> | <i>y</i> <sup>+</sup> | <i>bi</i>              |

and all them when crossed to a normal strain, 448 or 196 (Table IX) presented normal RF *meth-w* and obviously do not bear the suppressor. Thus, whatever the alteration present in chromosome I leading to its own elimination under *pFP*, the alteration is not *sup 30*. However, it is evident that a duplication of the genetic material should occur in chromosome I, but the evidence relating the duplication to any specific material from chromosome II, including any kind of relation to recombination frequency, deserves further attention. Furthermore, results showing selection against the VII chromosome were presented earlier.

The 3<sup>11</sup> segregant represents a case in which a translocation of genetic material from chromosome II to I is very likely to have occurred, during its isolation, but, even so, it presents altered RF *meth w*. Thus, something was added to chromosome I rather than the chromosome losing the determinant for high RF.

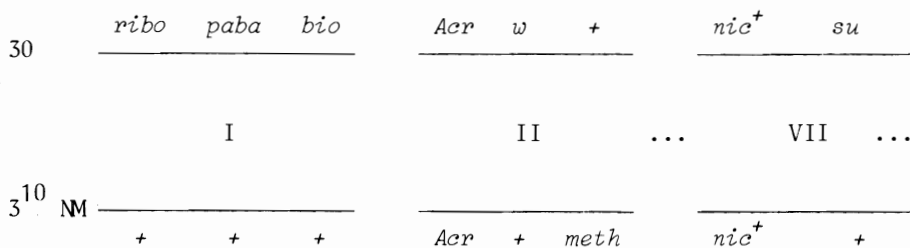


Figure 5 - Partial representation of the 30//3<sup>10</sup> NM diploid.

### *Mode of action of sup 30*

It is well known during the haploidization process that very close markers segregate together because mitotic crossing-over is very rare. Thus, linked markers such as *paba* and *bi* of chromosome I, *meth* and *w* of II, can combine in different ways but always without crossing-over.

The 3<sup>10</sup> NM//30 diploid presents the genotype presented in Figure 5.

The chromosomal combinations found in the haploids after *pFP* treatment are given in Table XII where it is shown that the *w* and *meth* markers, in the haploids from the mutant diploid (A), do not always segregate together. It was expected that the number of *w*<sup>+</sup> colonies would be similar to the number of *meth* (b and c) colonies and that, conversely, the frequency of *w* would be equal to the frequency of *meth* + . If so, the results would agree with the fact already stated that mitotic crossing-over between closely located markers are rare and, in the above instance, almost impossible.

Since previous analysis proved the presence of a suppressor, the *w*<sup>+</sup> *meth*<sup>+</sup> colonies arising among the haploids in Table XII can be understood to represent a fraction of the paternal *w*<sup>+</sup> *meth* class in which *meth* is suppressed by *sup 30*.

Thus the action of *sup 30* is restricted to the *meth* locus of chromosome II and has nothing to do with crossing-over in that region and is only dependent on the presence of *sup meth* in the VII chromosome. Unfortunately, the system used does not permit *sup 30* mapping since both strains used to make the diploids are *nic*<sup>+</sup> (VII).

### *Location of sup 30*

In a medium lacking methionine and using, as an indicator, the compact morphology of the *meth sup 30* colonies, the location of *sup 30* in chromosome VII was

possible. A meiotic segregant originating from the meiotic cross 30 x 196 and having the  $w^+$  *meth* (*sup meth*) genotype was crossed with 184 ( $w^+$  *meth*<sup>+</sup>), and demonstrated that *sup 30* is located 26.8 units from *nic B<sub>8</sub>*. Evidently this location is an approximation but crosses involving more adequate strains will certainly determine the exact position of *sup 30*.

Table XII - Action of *sup 30*.

| Genotypes of the haploid segregants                       |    |  | Number | Being:      |                          |
|---|----|--|--------|-------------|--------------------------|
|   |    |  |        | <i>meth</i> | <i>meth</i> <sup>+</sup> |
| 3 <sup>10</sup> NM//30diploid                             |    |  |        |             |                          |
| I   | II |  |        |             |                          |
| <i>paba bio w</i> (a)                                     |    |  | 8      | 0           | 8                        |
| <i>paba<sup>+</sup> bio<sup>+</sup> w<sup>+</sup></i> (b) |    |  | 12     | 9           | 3                        |
| <i>paba bio w<sup>+</sup></i> (c)                         |    |  | 6      | 4           | 2 (A)                    |
| <i>paba<sup>+</sup> bio<sup>+</sup> w</i> (d)             |    |  | 21     | 0           | 21                       |
| <i>paba bio<sup>+</sup> w</i> (e)                         |    |  | 1      | 0           | 1                        |
| 3 <sup>10</sup> NM//448diploid                            |    |  |        |             |                          |
| I   | II |  |        |             |                          |
| <i>paba bio w</i>   |    |  | 25     | 0           | 25                       |
| <i>paba<sup>+</sup> bio<sup>+</sup> w<sup>+</sup></i>     |    |  | 9      | 9           | 0                        |
| <i>paba bio w<sup>+</sup></i>                             |    |  | 17     | 17          | 0 (B)                    |
| <i>paba<sup>+</sup> bio<sup>+</sup> w</i>                 |    |  | 27     | 0           | 27                       |
| <i>paba bio<sup>+</sup> w</i>                             |    |  | 0      | 0           | 0                        |

## CONCLUSIONS

After using the system of induction and selection of mutants with altered recombination frequencies, in the *meth-w* interval of chromosome II, (Zucchi, 1986), it was possible to isolate several kinds of mutants, a very frequent one being the *sup meth A17* mutation. On the basis of their characteristics mutant 30, designated by *sup meth 30*, is the most representative among them. In the course of the analysis, it was evident that the high frequency of *meth*<sup>+</sup> *w*<sup>+</sup> recombinants is due to a *meth A17* suppressor, which is recessive and acts by suppressing the expression of *meth A17* mutation. The suppressor is a silent one, since mutant 30 is a *meth*<sup>+</sup> strain, and the

suppressive character of the mutation can only be expressed among segregants of crosses with methionine-deficient strains.

In a general sense, the presence of the suppressive mutation is detected by: a large number of  $meth^+ w^+$  recombinants, absence of the  $meth-w$  reciprocal class and reduction of the  $meth-w^+$  parental class in the same proportion as the  $meth^+ w^+$  class increases. The data presented here, showed that the segregation of  $meth^+ / meth$  is abnormal, but the markers  $w$  and  $Acr$  (close to  $meth$ ) are segregated quite normally in a Mendelian fashion (1:1).

This unlinked  $meth$  A17 suppressor is recessive and promotes a partial suppression of  $meth$  A17 since the suppressed strain does not recover the normal development of the wild type in a medium lacking methionine. This suppression should be caused by several kinds of regulatory mutations or by a gene dose effect.

According to Hartman and Roth (1973), a suppressor mutation is a kind of secondary mutation that modifies the phenotype of the original mutant gene, giving rise to phenotypically wild organisms due to the total or partial suppression of the mutation. Also, in contrast to conversion, suppression can be genetically separated from the mutation it suppresses.

Another source of misinterpretation of the recombination analysis is that the phenotypic effects of suppression of  $meth$  A17 can be confused with a  $meth^+$  duplication. The first case of genetic suppression was initially interpreted as genic duplication (Morgan *et al.*, 1925). It is also a fact that some duplications are really suppressors (Morgan *et al.*, 1925; Schultz and Bridges, 1932). This was also the case of mutant 118 V-1 which Marin and Zucchi (in preparation) called mutant Z1. This mutant, with a duplication bearing  $meth^+$  and  $w$  markers (Castro Prado and Zucchi, in preparation) presents a high frequency of recombination due to the  $meth^+$  duplication. Besides it presents several alterations close to the insertion point, between  $paba$  and  $y$  of chromosome I. Among these alterations there is the  $uvs$  character, which is very close to the  $meth^+$  duplication (Bonilha and Zucchi, in preparation).

In other methionine suppressors studied in several lower eukaryotes, it seems that in order to bypass a genetic block in the methionine metabolic pathway caused by the original mutation, the opening of an alternative metabolic pathway is a common occurrence.

The nature of this  $sup$  30 can be regulatory, since its recessive character, is more evident in the heterokaryon than in the diploid. We adopt the  $sup$  denotation (Demerec *et al.*, 1966; Sanderson, 1970; Taylor and Orton, 1970) because the mutation is typically intergenic and occurs out of the gene carrying the initial  $meth$  A17 (II) mutation. This new mutation makes chromosome VII more sensitive to benlate or  $pFP$  during haploidization of diploids bearing  $sup$  30.

The virtual elimination of the classes bearing the combined I and VII chromosomes of mutant 30, suggests some kind of interaction between at least two genetical factors present on them, one being responsible for the high RF  $meth w$ . It

was proved that this factor is the *sup meth* but the other defect remains unknown, although both make the haploid segregants from diploid 30//184 very sensitive to *pFP* or benlate used in the haploidization process. Another characteristic of the *sup meth* mutant was a slight mitotic instability. Which ever the factor evoking the instability, it is not the one responsible for the high recombination frequency.

It is known that the *locus meth* of *A. nidulans* reverts very frequent and spontaneously (Sidiqqi, 1962; Lilly, 1965; Alderson and Clark, 1966; Paszweski and Grabski, 1975) and almost all are not true revertants. The high frequency of different suppressors to the same mutation *meth A17* (*sup 30*, *sup 24* and *sup 29*; Zucchi, 1986) suggests that the methionine synthesis through cysteine is not the only possible metabolic pathway.

Also Wieber and Garner (1960) showed that in *Neurospora* there are several metabolic pathways to methionine synthesis. The regulation of methionine synthesis is very complex, as demonstrated by Paszewski *et al.* (1977) who showed that mutants of *A. nidulans* present two different biosynthetic pathways, regulated in an uncoordinated manner.

In the absence of biochemical characterization of our mutant 30, we suggest that the block caused by the *meth A17* mutation can be bypassed by *sup 30* thus opening a new metabolic pathway.

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## RESUMO

O mutante 30 foi selecionado principalmente pelo seu comportamento mitótico e meiótico, muito peculiar. A alta frequência de recombinação no intervalo *meth-w* do cromossomo II é devido a um supressor recessivo e silencioso de *meth A17*. A análise mitótica mostrou uma forte pressão seletiva contra os cromossomos I e VII. A morfologia da linhagem suprimida, em um meio sem metionina, permitiu a determinação da localização da mutação *sup 30* no cromossomo VII ligado ao *locus nic B8* (26,8%).

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