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Genetic evidence for a polycistronic unit of transcription in the complex locus '14' in *Podospora anserina*

II. Genetic analysis of informational suppressors

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SUMMARY

Forty-one suppressors obtained after NG and EMS mutagenesis of two 'polar' mutants of segment '29' in *Podospora anserina* were genetically analysed. Three classes of suppressor could be distinguished on spectrum pattern criteria. One representative suppressor of each class was demonstrated to be non-gene specific. The class I suppressor was dominant and only suppressed polar mutants in segment '29' and non-ICR-induced ones in genes where polarity cannot be determined. Class II and III suppressors were partially dominant and they suppressed polar, non-polar and even ICR-induced mutants. The difference between classes II and III seems to be only quantitative. According to whether class II and III suppressors are considered strongly or weakly allele-specific, two hypotheses are considered. First, *t*RNAs could be involved in all three classes of suppression: class I would be nonsense-specific and classes II and III would be nonsense-missense suppressors. Secondly, *t*RNA could be involved only in class I suppression, while ribosomal ambiguity could be responsible for class II and III suppression.

1. INTRODUCTION

One of the properties of polycistronic units of transcription is that comparison between genetic and complementation maps reveals two major classes of mutant. Some can be arranged into complementation groups which correspond to site clusters on the genetic map. Other mutants are non-complementing or display polarized complementation patterns. Non-complementing mutants show asymmetric localization on the genetic map, and suppressors of non-complementing mutants exhibit the properties of informational suppressors, i.e. allele and not gene specificities.

Segment '29' of locus '14' involved in ascospore pigmentation in *Podospora anserina* displays such properties, and is assumed to be a polycistronic unit of transcription. Comparison between genetic and complementation maps has been

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the object of previous communications (Picard, 1971; Toure & Picard, 1972). The main features of these maps are outlined in Fig. 1. The present paper is concerned with the genetic analysis of suppressors screened against two 'polar' mutants. Such an analysis involves dominance testing, chromosomal localization, observation of the action spectrum of suppressors on segment '29' mutants as well as on other mutants of functionally different genes, and consideration of the effects of suppression on the complementation pattern of the 'polar' mutants. The experimental data are discussed in relation to informational suppression.

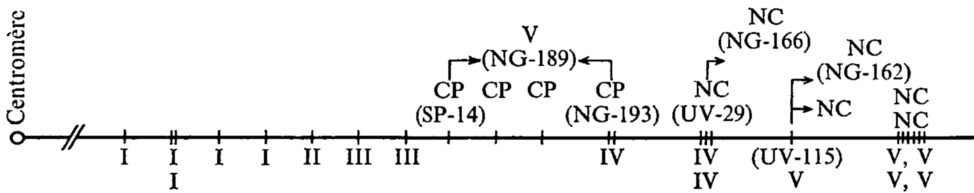

Fig. 1. Schematic genetic map of segment '29'. (Taken from Toure & Picard, 1972.) Each vertical dash corresponds to a mutant site. For simplification, the complementation group to which the localized mutants belong is indicated instead of the number of the mutants. Non-complementing mutants and mutants displaying a polarized complementation pattern are indicated respectively by the symbols NC and CP (above the map). Four mutants are only partially localized: they are indicated with arrows: for instance, mutant NG-166 lies on the right of mutant UV-29 and mutant NG-189 is localized between mutants SP-14 and NG-193. Since only the relative order is known, distances between mutants have been arbitrarily given.

2. MATERIAL AND METHODS

The main properties of and culture techniques for *Podospora anserina* were first described by Rizet & Engelmann (1949) and were recently reviewed by Esser (1969). The ascus of *P. anserina* contains four spores. Each one develops around two non-sister nuclei of the postmeiotic mitosis, so that both spores of each half ascus are genetically identical and each spore contains the genetic information of half a tetrad. This property allows dominance and complementation tests *in situ* because of the spontaneous formation of spores which are heterocaryotic for one or several pairs of alleles in the appropriate crosses (Picard, 1971, fig. 1).

The mutagenic origin and phenotypes of segment '29' mutants as well as mutagenesis and crossing techniques were described by Picard (1971). Every mutant of segment '29' was isogenized (over six generations).

In order to screen for suppressors of polar mutants, mutagenesis were carried out as follows: Nitrosoguanidine (NG), 40 min at a concentration of either 25 or 50 μg/ml; ultraviolet irradiation (UV), 3000, 6000 and 9000 ergs/mm^2; ethylmethane sulphonate (EMS), concentrations between 0·15 and 0·2 M for 4 h in phosphate buffer pH 7 containing 1% saccharose. At the present time, no suppressor strains have been isogenized except *su1-1*, *su2-1* and *su3-1* which were crossed to wild-type for six generations.

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Symbols for wild-type and mutant alleles of suppressor genes are: su^+ for the wild-type allele and *sux-n* for mutant alleles (x stands for the locus and n for the allele).

The origins of most suppressors are indicated in Table 1 except for the six which were screened under different conditions. Three of these appeared spontaneously during the numerous crosses involving mutant NG-193 (two belong to the *su1* locus and one belongs to the *su2* locus). Two others were obtained and analysed by students of the 'Diplôme d'études approfondies de Génétique': one is located

Table 1. *Reversions and suppressors screened on NG-193 and NG-166 mutants: mutagenic origin and distribution of the suppressors in the four loci*

(Independence of suppressors was assured by taking only one suppressor per treated dish. See Materials and Methods for information on six suppressors not included in this Table.)

				No. of independent		Suppressor loci				
Mutants	Mutagen	Dose	No. of treated dishes	Re-versions	Sup-pres-sors	*su1*	*su2*	*su3*	*su4*	Un-known
NG-166	None	—	100	0	0	.	.	.	.	.
	NG	25 µg/ml 40 min	250	5	3	1	1	.	.	1
	EMS	0·15 M, 4 h	100	0	1	.	.	.	1	.
	UV	3000–9000 ergs/mm²	280	0	0	.	.	.	.	.
NG-193	None	—	135	0	2	1	1	.	.	.
	NG	25 µg/ml	100	0	20	14	1	2	.	3
	EMS	0·15 M, 4 h	70	0	9	.	.	.	.	9
	UV	3000–9000 ergs/mm²	180	0	16	9	4	.	.	3
Total number of suppressors in each locus						25	7	2	1	.

at the *su1* locus and the other at the *su2* locus. Both were obtained after NG-mutagenesis (35 µg/ml, 40 min) on micronidia instead of mycelia. As for the first suppressor (*su1-1*), it was obtained by chance during the first NG mutagenesis (50 µg/ml) of the wild-type strain (it was present in the original strain carrying the mutant NG-199).

Genetic analysis of a suppressor-bearing strain requires essentially:

(*a*) Tests for the presence of a suppressor in this strain by crossing the strain (*m su*) with wild-type ($m^+ su^+$).

(*b*) Dominance tests which are performed by the cross $m\ su \times m\ su^+$ where m is the suppressed mutant.

(*c*) Determination of the number of suppressor loci by analysis of a cross such as $m\ su_x \times m\ su_y$, where su_x and su_y are two suppressors acting on the same mutant and obtained independently.

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Table 2. *Types of asci expected in some crosses involving suppressors in* P. anserina

	Crosses*			
	193 *su* × WT (a) Types of asci	193 *su* × 193 su^{+} (b) Types of asci	193 *sux* × 193 *suy* (c) Types of asci	*m* su^{+} × m^{+} *su* (d) Types of asci
I	2 spores 193 *su* / 193 *su* + 2 spores + + / + +	2 spores 193 *su* / 193 *su* + 2 spores 193 + / 193 +	2 spores 193 *sux* suy^{+} / 193 *sux* suy^{+} + 2 spores 193 sux^{+} *suy* / 193 sux^{+} *suy*	2 spores *m* + / *m* + + 2 spores + *su* / + *su*
II	2 spores 193 + / 193 + + 2 spores + *su* / + *su*	4 spores 193 *su* / 193 +	2 spores 193 *sux suy* / 193 *sux suy* + 2 spores 193 + + / 193 + +	2 spores *m su* / *m su* + 2 spores + + / + +
III	2 spores 193 *su* / 193 + + 2 spores + + / + *su*		2 spores 193 *sux suy* / 193 *sux* + + 2 spores 193 + *suy* / 193 + +	2 spores *m su* / *m* + + 2 spores + *su* / + +
IV			2 spores 193 *sux suy* / 193 + *suy* + 2 spores 193 *sux* + / 193 + +	2 spores *m su* / + *su* + 2 spores *m* + / + +
V			2 spores 193 *sux* + / 193 + *suy* + 2 spores 193 *sux suy* / 193 + +	2 spores *m* + / + *su* + 2 spores *m su* / + +
VI			4 spores 193 *sux suy* / 194 + +	4 spores *m su* / + +
VII			4 spores 193 *sux* + / 193 + *suy*	4 spores *m* + / + *su*

* *Cross* (a) permits the detection of a suppressor in a 'revertant' strain of NG-193 by the appearance of the mutant phenotype in type II asci (if the suppressor locus is genetically independent of locus '14') and in type III asci (if the suppressor locus shows some postreduction frequency and providing the mutant suppressor allele is not dominant over its wild-type allele). In *cross* (b) the postreduction frequency of the suppressor locus and the dominance test are both obtained by direct observation of type II asci. *Cross* (c) tests whether or not *sux* and *suy* belong to different loci. If they do, this cross provides information on the phenotypes of spores containing various doses of suppressors. *Cross* (d) is performed with many mutants (*m*) in order to elucidate the action spectrum of a suppressor. The type II ascus is most interesting: its frequency depends on the postreduction values of the mutant and of the suppressor loci and it implies that they are genetically independent.

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(*d*) Observation of the suppressor action spectrum among $m\ su^+ \times m^+\ su$ crosses where m are the mutants to be assayed.

These four stages of the genetic analysis of a suppressor are the most important and they are detailed in Table 2 where the different types of asci expected in *Podospora* are given for each type of cross. All these types of asci can be observed if the genetic markers lie on different chromosomes.

3. RESULTS

(1) *Reversions and suppressors*

All 'polar' mutants (except those induced by ICR) were submitted to UV-irradiation, NG and EMS treatments. Following mutagenesis, populations of ascospores from ripe perithecia were observed: all spores displaying some pigmentation (even very weak) were collected as 'revertants'. In order to retain independent events, only one pigmented spore was selected out of each treated dish.

The 'revertant' strains were then crossed with wild-type to test whether they were 'true' revertant or suppressed mutant strains. A cross between a mutant strain carrying a suppressor ($m\ su$) and the wild-type strain ($m^+\ su^+$) can lead to three types of ascus, as locus '14' lies so near to the centromere. In *Podospora*, the ascus structure and the binucleated spore formation lead to the three types of ascus marked in Table 2(a). Moreover, chromosome interference is nearly absolute for most chromosome arms. So, if the suppressor locus is linked to locus '14', non-parental ditype asci (type II of Table 2(a)) will never appear and such a suppressor will be detected in a revertant strain only if its wild-type allele is dominant.

However, the appearance of two uninucleate spores instead of a binucleate one in a low percentage of asci allows the detection of such suppressors unless they are closely linked to locus '14'. Therefore, the so-called reversions mentioned below could include true reversions, intragenic suppressors, and extragenic suppressors including a strongly linked dominant suppressor. However, all the revertant strains defined as such by crossing with wild type display a perfect wild-type phenotype, which never occurs in suppressed strains.

Reversions were found for almost all 'polar' mutants: mutants NG-155, NG-162, NG-166 and NG-177 reverted after NG-treatment; mutant NG-174 reverted after UV-irradiation; mutant UV-29 reverted both after UV and NG mutagenesis; spontaneous reversions were detected for mutants SP-14, UV-29 and NG-176; mutant SP-14 reverted only spontaneously and mutants NG-193 and NG-168 did not revert. None of these 'polar' mutants reverted on EMS treatment.

Suppressors were selected from two strains, NG-166 and NG-193 (Table 1). Spontaneous suppressors appeared in NG-193 and they all arose from premeiotic events. NG and UV (6000 ergs/mm^2) increased the frequency of appearance of suppressors in NG-193 by about 15-fold while no true reversion has as yet been detected for this mutant. On the other hand, the number of suppressors obtained

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in NG-166 was low and nearly equal to that of reversions obtained in the course of the same NG mutagenesis.

Fifty-seven suppressors have been screened and forty-one have now been analysed. Seven strains are still under study because they displayed either strong lethality, weak growth or abnormal segregation and require isogenization before analysis. The nine suppressors obtained after EMS mutagenesis of mutant NG-193 have not yet been studied.

(2) *Dominance tests*

In *Podospora*, as shown in Table 2(b), heterocaryotic spores appear spontaneously in a cross $m\ su \times m\ su^{+}$ as long as the postreduction frequency of the suppressor locus is not too low. The following characteristics were observed: two 'allelic' suppressors displayed no postreduction among several thousand asci observed; the suppressor screened on NG-166 after EMS treatment was dominant; all others were partially recessive: $\frac{193}{193}\frac{su}{su^{+}}$ spores displayed a phenotype intermediate between those of $\frac{193}{193}\frac{su^{+}}{su^{+}}$ and $\frac{193}{193}\frac{su}{su}$. This intermediate phenotype was closer to the mutant phenotype than to the suppressed one.

(3) *Number of suppressor loci and chromosome mapping*

The frequency of type II asci in Table 2, column *b*, is a direct measure of the postreduction frequency of a suppressor locus. At first, this criterion enabled them to be placed into three classes of suppressors showing 0, 30 and 75 % postreduction frequencies. The first three suppressors displaying these postreduction values were called *su3-1*, *su1-1* and *su2-1* respectively and they were used to test whether each postreduction class corresponds to one or several loci. For this purpose, several crosses were made with the available strains (see Table 2(c)). Genetic analysis of such crosses allowed the classification of the whole set of suppressors into four loci, *su1* to *su4* (80 % postreduction). The number of 'alleles' in each locus is indicated in Table 1 according to their origin.

Su1 and *su2* showed many 'alleles' and could be made up of several closely linked genes. This might be distinguishable by a classic complementation test; bearing in mind that the mutant forms are partially recessive, this test is *a priori* justified. In a cross such as 193 *su1-1* × 193 *su1-n*, the main types of expected asci are types I and VII as drawn in Table 2(c): 70 % of asci contain two $\frac{193}{193}\frac{su1\text{-}1}{su1\text{-}1}$ and two $\frac{193}{193}\frac{su1\text{-}n}{su1\text{-}n}$ spores, while 30 % of asci contain four $\frac{193}{193}\frac{su1\text{-}1}{su1\text{-}n}$ spores. The former type contain four suppressed spores. If the last type shows four mutants or nearly mutant spores, *su1-1* and *su1-n* may be assumed to belong to two different genes. But if such a cross leads to 100 % four suppressed spore asci, it is impossible to decide whether *su1-1* and *su1-n* belong to the same gene or to two genes with a

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similar function. In the latter instance the very weak dominance of the mutant suppressor alleles would only reveal a dosage phenomenon. For the locus *su1*, 20 pairwise combinations have been tested up to this time. In each case there was only one type of ascus containing four spores with a suppressed phenotype. Further recombination tests will perhaps make possible the classification of these mutants.

Loci *su1*, *su2* and *su3* were located on chromosomes IV, VII and V, respectively. *su4* also lies on chromosome IV, but on the other chromosome arm with respect to *su1*.

(4) *Allele specificity*

Each suppressor was tested with the 61 mutants of segment '29', by using crosses of the type $m\ su^{+} \times m^{+}\ su$, where m is a mutant of segment '29'. Because of the nearly 100% prereduction value of the mutants, the frequency of asci con-

Table 3. *Allele specificity of the suppressors with respect to mutants of segment '29'*

Suppressor classes	Distribution in suppressor loci				Fraction of suppressed mutants in each complementation group with respect to the mutagenic origin of the mutants					'Polar' mutants
	su1	*su2*	*su3*	*su4*	I	II	III	IV	V	
I	0	0	0	1	*2/14 NG*			0/3 NG	0/16 NG	*3/8 NG*
					0/5 ICR					0/5 ICR
						0/1 UV	0/2 UV	0/1 UV	0/3 UV	0/1 UV
									0/1 SP	0/1 SP
II	25	8	2	0	*2/14 NG*		.	0/3 NG	0/16 NG	*1/8 NG*
					0/5 ICR					0/5 ICR
						0/1 UV	0/2 UV	0/1 UV	0/3 UV	0/1 UV
									0/1 SP	0/1 SP
III	4	1	0	0	*5+2(?)/14 NG*		.	0/3 NG	*5+2(?)/16 NG*	*3/8 NG*
					2/5 ICR					0/5 ICR
						1/1 UV	*2(?)/2 UV*	*1(?)/2 UV*	*1+1(?)/3 UV*	0/1 UV
									1/1 SP	0/1 SP

Spectrum patterns allow one to distinguish three classes of suppressor. The distribution inside the four loci is given for each class of suppressors. Classes I and III suppress the same three polar mutants (NG-193, NG-166, NG-162) while class II only suppress NG-193. Both NG-mutants belonging to complementation group I and suppressed by classes I and II are the same and they are also included in the seven NG-group I mutants suppressed by class III suppressors. (?) means that these mutants do not seem to be suppressed every time the suppressor is present.

taining two $\frac{m}{m}\frac{su}{su}$ spores depends only upon the prereduction frequency of the suppressor (see Table 2(d)). This corresponds to 50, 35 and 12% of asci in the cases of *su3*, *su1* and *su2* suppressors respectively. *su4-1* is dominant, and $\frac{su}{su^{+}}\frac{m}{m}$ as well as $\frac{su}{su}\frac{m}{m}$ spores could be observed. Anyway, the number of asci needed in order to know if the suppressor is active upon the mutants is not very high. With

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suppressed spores, there are two ways of confirming the suppression. First, the percentage of asci showing the new 'suppressed' phenotype can be compared with the expected percentage. Secondly, a sample of asci can be used to analyse the relative segregation of the suppressor and the mutant. For nearly all crosses, the responses were unambiguous. As can be seen in Table 3 the whole set of suppressors showed allele specificity. Three classes which differ in spectrum and efficiency could be distinguished. However, the three spectra show a common part. Comparison of classes II and III suggests that suppressor efficiency and spectrum broadness are related: all the mutants which consititute the common part of class II and III spectra were better suppressed by class III suppressors than by those of class II. Some non-polar mutants of complementation groups II to V were suppressed by class III suppressors. On the contrary, only some group I and polar mutants were suppressed by class I and II suppressors.

(5) *Non-gene specificity*

In order to determine if the suppressors are informational, it is important to test their activity on mutants at other loci and especially on mutants that are functionally different from those of segment '29'. For this purpose, three suppressors were selected (*su1-1* for class II, *su1-25* for class III and *su4-1* for class I) and tested on mutants involved either in pigmentation, meiosis or cell incompatibility, and belonging to loci where numerous alleles are known.

Table 4. *Action spectra of suppressors* su4-1 (*class I*), su1-1 (*class II*) and su1-25 (*class III*) *on mutants of other genes involved in ascospore pigmentation.*

Suppressors	Proportion of suppressed mutants*	
	Gene '18'	Gene '122'
su4-1 (class I)	1/3 NG	2/5 NG
	0/1 UV	0/4 UV
	0/11 ICR	0/14 ICR
su1-1 (class II)	0/3 NG	2/5 NG
	0/1 UV	1/4 UV
	0/11 ICR	0/14 ICR
su1-25 (class III)	0/3 NG	4/5 NG
	1/1 UV	1/4 UV
	0/11 ICR	2/14 ICR

* The proportion of suppressed mutants is indicated for each gene with respect to the mutagenic origin of the mutants.

Four groups of mutants acting on ascospore pigmentation were tested: loci '30', '122' and genes '18' and 'vert foncé'. The last two belong to locus '14' and are located on different sides of segment '29'. They are thought not to be included in the segment '29' unit of transcription. Moreover, their mutant phenotypes differ from those of segment '29': gene '18' mutants show small green spores while every mutant spore of gene 'vf' is dark green. Locus '30' lies close to the centromere of

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chromosome VII, and mutant spores show dark pigmentation and variable size. On the contrary, locus '122' mutants (near the centromere of chromosome I) exhibit very weak pigmentation. Data observed for locus '122' and gene '18' mutants are listed in Table 4. Ten locus '30' mutants (8UV, 1 NG, 1 ICR) and 38 gene 'vf' mutants (10 UV, 10 NG, and 18 ICR) were tested but none seemed to be suppressed. All these mutants were probably pigmented too strongly for visual detection of weak suppression to be possible.

The non-gene specificity of these suppressors was even more apparent when functionally different genes were tested. Two genes involved in meiotic processes were investigated in collaboration with J. M. Simonet (*mei-1* and *mei-2*). Out of the three selected suppressors, only *su1-1* and *su4-1* are available for activity testing on meiosis mutants because *su1-25* exhibits strong female sterility (see paragraph (7)). Gene *mei-2* contains three recessive mutants (UV-557, NG-674, NG-782) and is localized near locus '122'. One of these mutants is 'leaky' and displays a pleiotropic phenotype: meiosis is blocked before the pachytene stage as in the other two non-leaky mutants, but in spite of this a few asci are formed. Genetic analysis of such asci reveals abnormal recombination effects. Furthermore, mutation rates are much higher in these strains than in wild type (Simonet, 1971; Simonet & Zickler, 1972). Two out of these three mutants were suppressed by *su1-1*: UV-557 was strongly suppressed and NG-674 (the leaky one) was slightly suppressed. But in this last case J. M. Simonet demonstrated not only that meiosis was completed more frequently than in the non-suppressed strain, but also that recombination processes were much less affected in the presence of the *su1-1* gene, even at the heterocaryotic stage. *su4-1* only suppresses the non-leaky mutant NG-557.

The other gene known to be involved in meiosis contains four recessive mutants (*mei-1*), all completely blocked before the pachytene stage. None seemed to be suppressed by *su1-1* or *su4-1*.

Action of one class II suppressor on mutants involved in cell incompatibility was analysed by J. Begueret (personal communication). Many mutants (even temperature-sensitive ones) of gene C (chromosome III) were suppressed by *su1-1* (class II). L. Belcour (personal communication) demonstrated that one of these mutants was better suppressed by *su1-25* (class III) than by *su1-1* (class II) and that it was not suppressed by *su4-1* (class I).

(6) *Complementation patterns of the suppressed polar mutants*

It is interesting to note the complementation patterns of the polar mutants in the presence of suppressors. Theoretically one just has to compare the phenotypes of, for instance, $\frac{193}{+}\frac{+}{m}\frac{su^+}{su^+}$ and $\frac{193}{+}\frac{+}{m}\frac{su}{su}$ spores ('*m*' being a non-suppressed mutant of any complementation group of segment '29'). Asci with two $\frac{193}{+}\frac{+}{m}\frac{su^+}{su^+}$ and two $\frac{193}{+}\frac{+}{m}\frac{su}{su}$ spores appear each time a postreduction occurs for 'segment 29' and

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a prereduction for the suppressor locus. However, in the case of strongly suppressed polar mutants, the suppressed mutant phenotype (193 *su*) was too dark to allow such complementation tests. It was then necessary to use double mutant strains such as 193.173 or 166.115 where NG-173 and UV-115 are two colourless group V mutants. As these mutants are also non-suppressed, spores 193.173 *su1* and 166.115 *su4* remain colourless. Such a phenotype allows accurate complementation tests. These tests were performed for every polar mutant, suppressed or not, and the results are shown in Table 5. NG-193 and NG-166 complementation was at least partially restored in the presence of a suppressor. Non-suppressed polar mutants remained non-complementing even in the presence of the suppressors.

Table 5. *Influence of suppressors on the complementation patterns of polar (suppressed or non-suppressed) mutants*

Strains used for the complementation tests	Group I mutants	Group II mutants	Group III mutants	Group IV mutants	Group V mutants
193	−	−	−	+	−
173	+ +	+ +	+ +	+ +	− or +
193.173	−	−	−	+	−
193.173.*su1-1*	+	+	+	+ +	−
166	−	−	−	−	−
115	+ +	+ +	+ +	+ +	− or +
166.115	−	−	−	−	−
166.115.*su4-1*	+	+	+	+ +	−
Non-suppressed 'polar' mutants	−	−	−	− or +	−
Non-suppressed 'polar' mutants +*su1* or +*su4*	−	−	−	− or +	−

+ +, means total complementation; +, partial complementation; and −, no complementation. Mutants NG-173 and UV-115 display white spores and are not polar. They belong to complementation group V and are not suppressed. They are used to test the complementation patterns of suppressed mutants because the pigmentation of 193 *su1* and 166 *su4* spores is too dark for proper testing.

(7) *Additivity phenomena between class II suppressors*

In the course of crosses such as 193 *su1-1* × 193 *su2-1*, various phenotypes appeared; they were different from those of (193 *su*) or (193 su^{+}) and sometimes nearer to wild type than that of (193 *su*). Genetic analysis of various asci obtained from 193 *su1-1* × 193 *su2-1* crosses revealed a pigmentation gradient corresponding to the following genotypes: $\frac{193}{193}$, $\frac{193\ su2}{193\ su2^{+}}$ or $\frac{193\ su1}{193\ su1^{+}}$, $\frac{193\ su2}{193\ su2}$ or $\frac{193\ su1}{193\ su1}$, $\frac{193\ su2\ su1}{193\ su2\ su1^{+}}$ or $\frac{193\ su1\ su2}{193\ su1\ su2^{+}}$ and finally $\frac{193\ su1\ su2}{193\ su1\ su2}$. The spores of the last genotype displayed the wild-type phenotype while $\frac{193\ su2}{193\ su2}$ and $\frac{193\ su1}{193\ su1}$ spores were only partially pigmented (green spores). This phenomenon was noticed in 193 *su1-1* × 193 *su2-1*, 193 *su1-1* × 193 *su3-1* and 193 *su2-1* × 193 *su3-1* crosses. Double and triple class II

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suppressor strains were then isolated. They had the same efficiency as class III suppressors. It was therefore of interest to determine the spectrum broadness of such poly suppressor strains with respect to class II and III spectra.

All the known properties of simple and complex suppressor strains are summarized in Table 6. Among the simple strains, class III suppressors displayed the recognizable phenotype of bad germination and female sterility. This last property was also found again for *su1-su2* and *su1-su2-su3* strains. Moreover, the spectra of these two complex strains are intermediate between those of class II and III.

Table 6. *Comparison of phenotypic properties and action spectra of strains bearing either one or several class II suppressors, and strains bearing class III suppressors*

Strains bearing the following suppressors	Female fertility	Germination	Level of suppression of mutant NG-193†	Action spectra*			
				m_1	m_2	m_3	m_4
su1-1 (class II)	Good	Good	+	±	−	−	−
su2-1 (class II)	Good	Good	+	±	−	−	−
su3-1 (class II)	Good	Good	±	±	−	−	−
su1-1.su3-1	Poor	Good	+ + +	+	−	−	−
su2-1.su3-1	Poor	Good	+ + +	+	−	−	−
su1-1.su2-1	Null	?	+ + +	+	±	−	−
su1-1.su2-1.su3-1	Null	?	+ + +	+	±	−	−
su1-25 (class III)	Null	Very poor	+ + +	+	±	+	−
su2-5 (class III)	Null	Very poor	+ + +	+	±	+	−

* −, no suppression; ±, very weak suppression; +, good suppression; and + + +, total suppression.

† m_1 mutants correspond to mutants which are suppressed by class II and III suppressors; m_2+m_3 mutants are only suppressed by those of class III. However, m_2 mutants are suppressed by strains bearing several class II suppressors (for instance, NG-193 is of the m_1 type, NG-166 and NG-162 belong to type m_2 and other polar mutants to that of m_4).

4. DISCUSSION

In summary, a study of the action spectra of 41 suppressors has led to the isolation of three classes of suppressor mapping in four different loci. One suppressor of each class was tested on mutants functionally different from those of segment '29'; this showed that these suppressors were not gene specific.

Su4-1, the only specimen of class I, was obtained after EMS mutagenesis. It was dominant, and with regard to segment '29', suppressed only polar mutants (NG-193, 166 and 162) and mutants of gene I (the last gene to be transcribed in this polycistronic unit of transcription).

Class II and III suppressors were obtained after UV and NG mutagenesis and were weakly dominant. Class II suppressors seemed to differ from those of class III in a quantitative way: strains carrying two or three class II suppressors displayed suppression patterns and phenotypic properties very similar to those of class III. Class III suppressors possessed the broadest action spectra: they suppressed not only polar and gene I mutants but also non-polar mutants. Class III suppressor-

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bearing strains showed some impairment in germination and fertility. Some class II and III suppressors belonged to the same loci (*su1* and *su2*).

Non-suppressed polar mutants remained non-complementing even in the presence of the suppressors, while suppressed polar mutants became complementing.

Table 7. *Sense anticodons which can lead to nonsense anticodons through a single base substitution*

	Mutational events leading to suppressors from sense anticodons					
Suppressor anticodons	(1) GC → AT	(2) AT → GC	(3) GC → CG	(4) GC → TA	(5) AT → TA	(6) AT → CG
CUA	CUG (glun)	.	GUA (tyr)	CUC (glu)	CUU (lys)	.
Amber specific	CCA (try)			CGA (ser)	CAA (leu)	
UUA	UUG (glun)	.	.	GUA (tyr)	UAA (leu)	.
Ochre – amber				UGA (ser)	UUU (lys)	
				UUC (glu)		
IUA						
Ochre specific	GUA (tyr)	.	.	.	.	.
Ochre + missense	.	.	.	IGA (ser)	.	.
UCA						
Opal	CCA (try)	.	.	.	.	.
Opal + missense	UCG (arg)	.	UGA (ser)	GCA (cys)	UCU (arg)	UAA (leu)
				UCC (gly)		
ICA						
Opal	GCA (cys)	.	.	.	.	.
Opal + missense	ICG (arg)	.	IGA (ser)	CCA (try)	.	.
				ICC (gly)		

The three classes of suppressors described above were not gene-specific, and are therefore presumed to be informational suppressors. Moreover, none were recessive. By analogy with the nonsense and missense suppressors in bacteria (Capecchi & Gussin, 1965; Carbon, Berg & Yanofsky, 1966; Garen, 1968) and the super-suppressors in yeast (Magni, Von Borstel & Steinberg, 1966; Gilmore & Mortimer, 1966; Hawthorne, 1969*a*) one hypothesis would be that these suppressor loci are genes coding for transfer RNAs.

The universality of the genetic code is now well established (i.e. Laycock & Hunt, 1969; Marshall, Caskey & Nirenberg 1967), and nonsense triplets appear to be the same in several organisms (Hawthorne, 1969*b*; Seale, 1971). The UGA codon seemed to make sense in some cases (Ycas, 1969) but it is also the most leaky nonsense triplet in bacteria (Model *et al.* 1969). The data observed in *Podospora* and that described in this paper will therefore be discussed on the basis that nonsense codons are UAA, UAG and UGA.

According to this hypothesis, the class I suppressor would be a nonsense suppressor. In agreement with this only non-leaky, non-ICR induced, polar mutants were suppressed by *su4-1*. Results obtained in complementation studies with

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polar (non-ICR induced) mutants of segment '29' suggest that suppressed and non-suppressed polar mutants probably have different nonsense triplets (Results part 6 and Table 5).

The study of strains carrying two class II suppressors showed that class II and III suppressors differ in a quantitative way (Results part 7 and Table 6). However, they were qualitatively different from class I. They suppressed not only the same three polar mutants but also non-polar mutants of segment '29' and leaky or temperature-sensitive mutants of other genes. Most of these mutants are probably missense. Class II and III suppressors might therefore be nonsense-missense suppressors. Such suppressors are predicted by the wobble mechanism (Crick, 1966). With the wobble type of codon – anticodon interactions, mutants NG-193, NG-166 and NG-162 could only be all ochre or all opal mutants. The presence of suppressors at the same locus displaying different efficiencies is not a real problem. *su1* and *su2* loci might correspond to one or several genes. In the first case, differences could be the consequence of the location of the mutations either in the anticodon or in another part of the *t*RNA structure. In the second case, differences in efficiency might, for instance, be due to different levels of transcription of the genes or to different wild-type *t*RNA structures. This hypothesis is well supported by all facts but two, namely the great proportion of missense suppressed mutants and the suppression of frameshift mutants. (ICR-170 induces frameshift mutants in *Podospora*, see Picard 1971.) According to the hypothesis which postulates that the three classes of suppressors are *t*RNAs, the suppressed missense mutants must *all* have the same codon (s): UAU and (or) UAC if class II and III suppressors specifically suppress ochre mutations, UGG or UGU and (or) UGC if they suppress opal mutations. The proportion of such mutant codons in locus '122', segment '29' and cell incompatibility gene C appears unusually high. Moreover, some extragenic frameshift suppressors were described in bacteria (Yourno, Barr & Tanemura, 1969), but in the cases studied they were not simultaneously nonsense and missense ones (Riddle & Roth, 1970).

The codon-specific characteristic of the class I suppressor is in good agreement with it being a nonsense suppressor. A different hypothesis is however necessary to account for the suppression pattern (non-codon specific) of classes II and III. Non-allele specificity is the principal feature of ribosomal suppression, at least in bacteria (see Gorini, (1970) for review). Nonsense, missense and frameshift mutants can be suppressed by misreading enhanced either by streptomycin or by mutation of a gene (*ram*) coding for a ribosomal protein (Rosset & Gorini, 1969; Zimmermann, Gavin & Gorini, 1971; Biswas & Gorini, 1972; Atkins, Elseviers & Gorini, 1972).

An attractive hypothesis is therefore that class II and III suppressors of *Podospora* are analogous to *ram* mutants of *E. coli*. Strains carrying the most efficient suppressors (class III or two class II) displayed abnormalities in their life-cycles (see Table 6): such properties might be predicted for ribosomal suppressors because of possible misreading of a great number of codons. However, class II and III suppressors did not suppress all the nonsense codons whereas *ram* does,

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although not to the same level. In one system, only UGA appeared to be suppressed (Rosset & Gorini, 1969). Also, some codons are refractory to streptomycin-induced misreading *in vitro* (Davies, Jones & Khorana, 1966). The genetic structure of *Podospora* may then be responsible for the suppression of only one nonsense codon by *ram*-like suppressors, although many experiments are needed to confirm this hypothesis. For instance, although *t*RNA suppressors were obtained by ICR mutagenesis (Magni *et al.* 1966), it is unlikely that such mutagens could induce ribosomal suppressors. Indeed, frameshift mutants of such proteins would most frequently be lethal, although the dicaryotic structure of *Podospora* ascospore might allow such lethal suppressors to be detected, assuming that they are dominant with regard to suppression. Only one *t*RNA suppressor has so far been obtained in *Podospora*. Failure to isolate other *t*RNA suppressors, especially for the remaining polar mutants of segment '29', can be explained in two ways. First, EMS and NG display some specificity in *Neurospora*, EMS acting preferentially on G-C pairs (Malling & De Serres, 1968), and NG leading mostly to G-C pairs (Malling & De Serres, 1970). If these mutagens act similarly in *Podospora*, NG would be a very poor mutagen when looking for *t*RNA suppressors via single base substitution in the anticodon (Table 7). Secondly, the genetic structure of *Podospora* is assumed to be responsible for the observed suppression of only one nonsense codon by class II and III suppressors. In the same way, the absence of *t*RNA suppressors for the remaining nonsense mutants could be explained by inherent restrictions, and, *t*RNA suppressors might be more easily obtained using strains carrying a *ram*-like mutation.

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