Ribosomal suppressors in *Podospora anserina*: evidence for two new loci by means of a new screening procedure

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SUMMARY

I describe here a new screening procedure to isolate ribosomal suppressors in *Podospora anserina*. I have used the sporulation defect displayed by an antisuppressor mutation *AS7-2*. The revertants able to sporulate are due to either true reversions or external mutations. The mutations which restore most efficiently the sporulation show all the properties of ribosomal suppressors and are localized in two new suppressor loci *su11* and *su12*.

INTRODUCTION

In *E. coli*, genetic and biochemical analyses have shown that alteration of ribosomal proteins can modify the translational ambiguity level (for reviews, see Gorini, 1974 and Piepersberg *et al.* 1980): some mutations (for example *str A* mutations) diminish the level of natural misreading (i.e. enhance the translational fidelity). They restrict the efficiency of informational (tRNA) suppressors. They are called *restrictive* mutations and can be considered as ribosomal *antisuppressors*. Antagonistic '*ram*' mutations were obtained which enhance the natural misreading and correspond to ribosomal suppressors.

In order to study the genetic control of translational fidelity in a eucaryotic organism, a genetic system has been developed in the fungus *Podospora anserina*. It was demonstrated that, as in bacteria, some mutations modify the ribosomal ambiguity level. Suppressors (*su*) and antisuppressors (*AS*) mutations analogous to *ram* and restrictive mutations of *E. coli* were previously described (see Picard-Bennoun, Coppin-Raynal & Dequard-Chablat, 1983 for a review). The mutations assumed to increase translational ambiguity (i.e. ribosomal suppressors) were localized in five loci: *su1*, *su2 su3*, *su5* and *su7*. They were isolated as informational suppressors (Picard, 1973; Arnaise and Mignotte, personal communication) while in *E. coli* most *ram* mutations were obtained as antagonists of restrictive mutations (Piepersberg *et al.* 1980). There are now biochemical arguments demonstrating that the *su* mutations increase translational ambiguity (Coppin-Raynal, 1982*a, b*). I report here the use of a new screening procedure for suppressors (*ram* like) mutations, which parallels that used in *E. coli*. The *AS7* strains were found to display a sporulation defect probably due to their high translational fidelity (Coppin-Raynal & Le Coze, 1982; Coppin-Raynal, 1982*a*;

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Table 1. *General characteristics of the strains used in this study*

Strains	Localization of mutation		Characteristics	References
	SDS %*	LG†		
AS7-1 AS7-2	0	V	AS, Pm^R, spo^- ‡	Coppin-Raynal (1982a) Coppin-Raynal & Le Coze (1982) Picard-Bennoun & Pereira-Vasquez (Personal communication)
AS3-1	40	IV	AS, Pm^R, reduced fertility	Picard-Bennoun (1976)
AS4-43	98	I	AS, Pm^R	
AS6-1	70	III	AS, Pm^R, ♀ sterile	Picard-Bennoun & Le Coze (1980) Picard-Bennoun (1981)
193	0	II	Unpigmented spores and mycelium, nonsense mutation	Picard (1971)
su1-1 193 su1-1	30	IV	Ribosomal suppressor, Pm^{HS}. Pigmented (green) spores	Picard (1973)
leu1-1	70	VII	Auxotrophy for leucine	Crouzet (personal communication)
su2-18 leu1-1 su2-18	70	VII	Ribosomal suppressor Prototrophic for leucine	Arnaise and Mignotte (Personal communication)
Su4-1	65	VII	tRNA type suppressor, spo^-	Picard (1973)
cs12-1	0	VII	Cold sensitive, spo^-, Pm^{HS}	Picard-Bennoun & Le Coze (1980)
PmA-1	98	I	Pm^{HS}, spo^-	Randscholt et al. (1982)
PmB-1	80	III	Pm^{HS}	
Pm1	98	I	Pm^R	Dequard et al. (1980)
Pm3(= mei3)	65	VII	Pm^R, spo^-	Coppin-Raynal & Le Coze (1982)

* SDS %, Second division segregation percentage (postreduction percentage).
† LG, Linkage group.
‡ Symbols are as follows: Pm^{HS} and Pm^R: respectively more sensitive and more resistant to paromomycin than wild-type; spo^-: defective in sporulation; *AS*: antisuppressor.

Dequard-Chablat & Coppin-Raynal, 1984). Among the revertants able to sporulate, three new ribosomal suppressors were characterized.

MATERIAL AND METHODS

(i) *Organism and strains*

Podospora anserina is a filamentous fungus. Its biology and culture techniques have been previously described (Rizet & Engelmann, 1949; Esser, 1974). The ascus of *Podospora* contains four spores. Each one develops around two non-sister nuclei of the post-meiotic mitosis. This property allows dominance and complementation tests in the appropriate crosses. A few asci (2–5 %) contain five spores: three binucleate and two small uninucleated ones. Crosses are generally performed between homocaryotic strains derived from the small spores. A pair of alleles (mt^+ and mt^-) controls the mating-type.

The *AS7-2* strain displays the same phenotypic properties as those described for *AS7-1* (Coppin-Raynal & Le Coze, 1982): paromomycin resistance, antisuppressor effect and sporulation defect. The characteristics of the other strains used in this study are listed in Table 1.

(ii) *Selection of AS7-2 revertants*

Strains homozygous for *AS7-2* and heterozygous for the mating-type locus (mt^+/mt^-) differentiate perithecia which do not contain any spores. In order to obtain revertants able to sporulate, small pieces of *AS7-2* mt$^+$/*AS7-2* mt$^-$ mycelium were inoculated on 3 cm cellophane disks plated on solid minimal medium (M_2) and grown for 2 days at 27 °C. The thalli were treated by ultraviolet irradiation (UV) at 250, 500 and 1000 ergs/mm^2 (respectively 30, 145 and 145 thalli). Fifty untreated thalli were kept for spontaneous reversion control. The disks were then transferred onto new M_2 dishes and put back at 27 °C in the light. After about 1 week, perithecia were formed and a systematic search for the appearance of asci was undertaken.

(iii) *Genetical analysis*

For each sporulating sector, a sample of 6–12 asci was picked up. The progeny in the revertant sector generally come from the cross of gametes carrying *AS7-2* revertant nuclei (AS7-2′) with gametes carrying the *AS7-2* parental ones. The analysis of the offspring allows verification of the genetic nature, to follow the segregation of the sporulation phenotype and to isolate the *AS7-2* revertant strain. Further crosses with the wild-type were made to distinguish between reversion (*AS7-2′ = AS7$^+$*) (or second site mutation closely linked to *AS7-2*) and external mutations (*AS7-2′ = AS7-2m*) (Table 2). In the latter case the *m* mutation was transferred from the *AS7-2* background and characterized.

(iv) *Estimation of the paromomycin resistance level* in vivo

Podospora anserina forms a relatively flat circular thallus growing only at the periphery. The growth curve measured by the increase in diameter of the thallus is linear. Growth curves of the various strains were determined at 27 °C on solid

minimal medium (M_2) and on M_2 containing 1 mM paromomycin. The diameters of at least four thalli were measured. In this study, the paromomycin resistance level was evaluated after 4 days of growth as the ratio of the average diameters of thalli on M_2 + antibiotic/M_2, expressed as a percentage.

Table 2. *Expected asci in the genetical analysis of the revertants*

	Crosses with			
	AS7-2*		*AS7*+ (wild-type)	
	2 (*spo*+) 2 (*spo*−)	4 (*spo*+)	2 (*spo*+) 2 (*spo*−)	4 (*spo*+)
Type of asci...				
Genotype				
AS7+	+	−	−	+
AS7-2	(No spore)		+	−
AS7-2' = *AS7*+ or = *AS7-2m* with *m* closely linked to *AS7-2*	+	−	−	+
AS7-2' = *AS7-2m*; *m* unlinked to *AS7-2*†	+	+	+	+

* *AS7-2* always segregates at the first meiotic division.

† The relative amount of each category of asci depends on the post-reduction frequency of the *m* mutation. The selection procedure of *AS7-2* revertants implies that the *m* mutations alleviating the sporulation defect must be at least semi-dominant.

(v) *Suppression tests*

The *193* mutation defective in spore pigmentation (white spores) is generally used to characterize informational suppressors (*su*). *193* '*su*' spores are more or less pigmented according to the efficiency of suppression. Other mutations altering spore pigmentation are known and their properties allow one to differentiate putative ribosomal suppressors and tRNA nonsense suppressor (Picard, 1973). We can distinguish between nonsense and missense mutations. Putative tRNA nonsense suppressors are only active on the former mutations, while ribosomal suppressors can suppress all kinds of mutations.

(vi) *Antisuppression tests*

They are based on the ability of the antisuppressor mutations to decrease the efficiency of a suppressor. Two strains were used: *193 su1-1* and *leu1-1.su2-18* (see Table 1). The *193 su1-1* strain forms pigmented spores. The addition of an antisuppressor mutation (AS) reduces the pigmentation level (Picard-Bennoun, 1976). The *leu1-1* mutation is auxotrophic for leucine. The auxotrophy is suppressed by ribosomal suppressors like *su2-18* and an antisuppressor mutation abolishes the prototrophy (Coppin-Raynal, 1981).

<div align="center">RESULTS</div>

(i) *Selection and characterization of AS7-2 revertants*

Strains homozygous for *AS7-2* and heterozygous for the mating-type locus differentiate perithecia which do not contain any spores. They were used to select revertants able to sporulate as described in Materials and Methods. Of the total of 320 irradiated thalli, we identified *17* sporulating sectors (respectively 1, 13 and 3 at 250, 500 and 1000 ergs/mm²). No spontaneous revertant was obtained. The revertants were analyzed as indicated (Materials and Methods). In every case, the (spo⁺) phenotype was recovered in the progeny with a mendelian segregation indicating single nuclear mutations. Crosses of the revertant strains by wild-type allowed differentiation between mutational events closely linked (back mutations or second site mutations), or unlinked to *AS7-2*. Eleven revertants belong to the former class. They have recovered wild-type sporulation and we were unable to separate the new mutation from *AS7-2* at the limit of the genetical analysis (between 25 and 50 asci analysed, according to the revertant). Two of them can be considered as true reversions because they have recovered a wild-type phenotype for all tested criteria (whole loss of antisuppressor effect and paromomycin resistance), while the nine others have kept a slight paromomycin resistance and antisuppressor effect on *193 su1-1* but not in *leu-1-1 su2-18* (see Material and Methods and Table 1). For these revertants, it is impossible at the moment to differentiate between reverse mutation which do not restore the wild-type sequence exactly and closely linked suppressor mutation. The six remaining revertants contained an external mutation (called *m*) unlinked to *AS7-2* which more or less restores the sporulation of *AS7-2* strain (Table 3). They were named *m4*, *m6*, *m22*, *m27*, *m35* and *m51*.

(ii) *Characteristics of the AS7-2 m strains*

Results of the crosses *AS7-2m* × *AS7-2* showed that the *m* mutations segregate mostly at the second meiotic division. As expected by the selection procedure they are at least semi-dominant. A dominance test based on sporulation showed that *m22* and *m27* were completely dominant. However, strains homozygote for *m4* sporulated better than heterozygotic strains and we can say that *m4* is partially dominant.

The three other mutations, *m6*, *m35* and *m51*, are linked to the mating-type, so comparison between homocaryotic and heterocaryotic strains is impossible. In terms of restoring the spo⁺ phenotype of *AS7-2* (Table 3), *m6* was the most efficient. *m22*, *m27* on the one hand, and *m35* and *m51* on the other hand, only partially relieved the sporulation defect but showed a cumulative effect (*m22* plus *m35* or *m51*, for example).

The phenotypic analysis of the revertants showed that some *m* mutations decreased the paromomycin resistance of *AS7-2* (Table 3). Moreover there was a good correlation between the effect of the mutation on sporulation and on the decrease of the resistance: While *m22* and *m27* displayed no effect, *m4* and *m6* notably reduced the paromomycin resistance of *AS7-2*, the *AS7-2 m6* strain even becoming more sensitive than the wild-type strain (Table 3).

Table 3. *General characteristics of the* m *mutations recovered from the revertants*

| | UV doses (erg/mm²) | Effect on AS7-2 | | Paromomycin resistance of m alone | Suppressor‡ effect on 193 | Localization |
		Sporulation*	Paromomycin† resistance in vivo			
m22	500	+	83	30	—	} Unlinked to the mating-type locus
m27	500	+	84	31	—	
m35	500	+	76	30	—	} Linked to the (§) mating type locus
m51	500	+	74	10	Very weak	
m4	1000	++	59	9	Weak	su11-1, linked to the PmB
m6	500	+++	22	No growth	Strong	su12-1, linked to the mating type locus (§)

* The effect of the *m* mutation on the sporulation of *AS7-2* is determined from the sporulation of a [(*AS7-2 m*/*AS7-2 m⁺*) (*mt⁺*/*mt⁻*)] strain. It is estimated by the number of asci projected during a day: +, about 100 asci; + +, 10000–100000 asci; + + +, more than 100000.

† The paromomycin resistance is estimated by the percentage of growth after 4 days on medium containing 1 mM paromomycin in comparison with the growth in absence of antibiotic (see Material and Methods). It is 32 % for wild-type and 84 % for *AS7-2* in the same experiment.

‡ The suppressor effect of a mutation is determined as described in Material and Methods: '—' means no visible effect. In the case of *m51*, the suppressor effect corresponds to the increase of the efficiency of a known ribosomal suppressor, *su1-1*.

§ For the moment there are no data available to determine the allelism between *m35*, *m51* and *m6* but they are very closely linked mutations and can be assumed to belong to the same locus (see in the text).

(iii) *Characteristics of the* m *mutations*

Effect on paromomycin resistance. The *m* mutations isolated from the *AS7-2* background have been characterized. They were tested for their paromomycin resistance (Table 3). *m22*, *m27* and *m35* are as sensitive as wild-type while *m4*, *m51* and *m6* (the most efficient suppressor) displayed a hypersensitivity towards paromomycin. We found a correlation between the efficiency of the *m* mutation in restoring the sporulation of *AS7-2* and its effect on the paromomycin resistance level. Moreover, *m6* produced a much altered phenotype with a very slow growth rate and female sterility. Its growth was a little improved at 30 °C and *m6* can be considered as a cold-sensitive mutation. However, *AS7-2 m6* strains grow well at 27 °C and is female fertile. The other *m* mutants manifested no particularly altered phenotype.

The m *mutations antagonize AS mutations.* In *Podospora*, the mutants unable to sporulate constitute a very heterogeneous group involving numerous genes (Simonet & Zickler, 1972; Zickler & Simonet, 1980). We investigated whether the *m* mutations could suppress different types of *spo⁻* mutations whose properties are listed in Table 1 (*Pm3*, *cs12*, *su4* and *PmA*). No suppression was found except for *AS7-1*. Recently, other *AS* mutations were found to be sporulation deficient (*AS4-43* and new *AS6* mutations). *m4* and *m6* are able to relieve this defect. This suggests that the specificity of the *m* mutations is limited to *AS* mutations. Furthermore *m4*, *m6* and *m51* decrease strongly or weakly the paromomycin resistance associated with several antisuppressor mutations (*AS4-43*, *AS3-1*, *AS6-1*). They also reduce the paromomycin resistance level of *Pm3* mutants which displays no antisuppressor effect.

The specificity of the *m* mutations at the sporulation level is limited to *AS* mutations while they interact with all mutations tested at the level of paromomycin resistance. In the same way, the *m6* growth defect seemed to be specifically alleviated by antisuppressor mutations (*AS6* mutations displayed a positive effect while *Pm3-1* had no effect).

m4, *m6* and *m51 display a suppressor effect.* The sporulation defect of *AS7-2* is assumed to be the consequence of its antisuppressor property, that is its high translational fidelity (Dequard-Chablat & Coppin-Raynal, 1984). In order to determine whether the *m* mutations interfered with translational ambiguity, we tested their effect on the *193* nonsense mutation, an allele generally used to characterize informational suppressors and antisuppressors in *Podospora*. The results are reported in Table 3. *m4* and *m6* suppressed the *193* mutation giving light green (*193 m4*) or green spores (*193 m6*). Moreover they displayed a suppression spectrum typical of ribosomal suppressors. They were called respectively *su11-1* and *su12-1*. In addition, their suppression efficiency on 193 is lowered by antisuppressor mutations (*AS7-2*, *AS3-3*, *AS4-43* and *AS6-1*) as previously observed for other ribosomal suppressors. The action of *m51* on *193* is not so obvious but it increases the suppressor effect of *su1-1*. It can be considered as a low efficiency suppressor.

The other mutations, *m22*, *m27* and *m35* had no suppressor effect. The antisuppression tests on *193 su1-1* or *leu su2-18* showed no antisuppression property.

(iv) *Localization of the* m *mutations*

The *m* mutations displayed post-reduction percentage between 60 and 100. They define three independent loci: a locus with *m35*, *m51* and *m6* closely linked to the mating-type locus; a locus with *m4*, and a third locus with *m22* and *m27* independent from the mating-type locus. *m6*, *m35* and *m51* are localized inside a very small region (less than 1 centimorgan) including the mating-type locus and different mutations altering the paromomycin phenotype (*PmA*, *Pm1*, *AS4*) (Table 1). It is difficult to know the allelic relationships between these mutations. In fact, the only available complementation test is based on paromomycin hypersensitivity which is a recessive character. This property is an unreliable criterion because we have found no or very poor complementation between unlinked hypersensitive mutations. Nevertheless, *m6* and *PmA-1* were found to recombine at a frequency about 10^{-4}. This indicated that they at least were localized at two different sites. The same result was found for *m51*. Owing to their related phenotype and genetic closeness, *m6* and *m51* are considered to belong to the same locus, *su12*. *m35* has no particular phenotype and can not be more precisely localized in this region.

Crosses with appropriate genetic markers including previously identified suppressor mutations placed *m4* close to *PmB* (Table 1 and 3) (no recombinant for 100 asci analysed). The previous remark on complementation tests is valid for these two mutations. However we can notice that neither *PmA-1* nor *PmB-1* relieved the sporulation defect of *AS7-2* indicating that if *PmA-1*, *m6* and *m51* on the one hand, and *m4* and *PmB-1* on the other hand belong respectively to single genes, these are mutations with different effects. *m22* and *m27*, displaying no particular phenotype, are not localized at this time.

In conclusion the *m* mutations define two *new* ribosomal suppressor loci: *su11* on chromosome III and *su12* on chromosome I. The other ribosomal suppressor loci known in *Podospora* are localized elsewhere (Marcou, Picard-Bennoun & Simonet, 1980).

DISCUSSION

The genetical analysis of 17 revertants of the *AS7-2* mutant revealed that the mutations which restore its sporulation belongs to two classes: the first class corresponds to mutations indistinguishable from *AS7-2*. Two of them are assumed to be true reversions while the others did not recover a completely wild type phenotype. In these cases we were unable in the limit of the genetical analysis to differentiate between intragenic revertants and closely linked suppressor mutations antagonistic to the *AS7-2* antisuppressor mutation. Indeed a ribosomal suppressor gene *su3* is known to be localized very close to *AS7* (Picard, 1973; Marcou *et al.* 1980; Coppin-Raynal & Le Coze, 1982). The second class corresponds to six external mutations (Table 3). The most efficient mutations can be considered as ribosomal suppressors localized in two new loci *su11* and *su12*. *In vivo* they display the same properties as the previously identified ribosomal suppressors (Picard, 1973; Coppin-Raynal, 1981): Suppression of the pigmentation defect caused by *193*, hypersensitivity to paromomycin, and interaction with *AS7-2* and other *AS* mutations as revealed by the reduction of the resistance level to paromomycin and

pigmentation of *193 su AS* spores. *In vitro* data confirm that *su11* and *su12* mutations increase the ribosomal ambiguity level, and electrophoretic analysis has shown that *su11-1* and *su12-1* affect proteins of the 40S ribosomal subunit (Dequard-Chablat & Coppin-Raynal, 1984).

These results suggest several remarks:

(1) The obtaining of 'true' reversions of the *AS7-2* mutant demonstrates that its various properties (antisuppression, paromomycin resistance, sporulation deficiency) are the result of a single mutation with pleiotropic effects. The fact that a high translational fidelity blocks sporulation in *Podospora* is discussed elsewhere (Dequard-Chablat & Coopin-Raynal, 1984) within the context of a more general hypothesis proposed by Picard-Bennoun (1982).

(2) Besides the three well defined external mutations localized in *su11* and *su12*, three mutations were found with a lower efficiency: *m35*, *m22* and *m27*. *m35* lies in the same region as *su12* but it does not display a suppressor effect or paromomycin hypersensitivity. On the contrary, unpublished *in vitro* experiments suggest that *m35* might correspond to a weak ribosomal antisuppressor mutation displaying an antagonistic action on *AS7-2*. In *Podospora*, antagonistic effects have already been described for suppressors (Picard-Bennoun, 1976; Coppin-Raynal & Le Coze, 1982). Further experiments are needed to know whether *m35* lies in the *AS4* gene which is closely linked to *su12*.

m22 and *m27* belong to the same locus and have no suppressor or antisuppressor effect *in vivo* (Table 3). Unreported data showed that, *in vitro*, they did not seem to modify the misreading level of the ribosomes. However they might have a slight effect on misreading level which could occur specifically during the sporulation process. This effect would not therefore be seen at the spore or vegetative level. An alternative explanation is that *m22* and *m27* alter some genes involved in sporulation.

(3) Until this work, all mutations increasing or assumed to increase the level of translational errors had been selected as suppressors of the *193* (Picard, 1973) or the *leu1-1* (Arnaise and Mignotte, personnal communication) nonsense mutations. These ribosomal suppressors were localized in five loci *su1*, *su2*, *su3*, *su5* and *su7*. Of these suppressors 90 %, particularly the most efficient ones, map in the *su1* and *su2* loci. In the present case, the suppressors have been selected against an antisuppressor, a procedure used in *E. coli* to isolate *ram* mutation (for a review, see Piepersberg *et al.* 1980). This screening procedure has allowed the identification of two new ribosomal suppressors in *Podospora*. Their expression required that they were epistatic to *AS7-2* (decrease of paromomycin resistance and translational fidelity, restoration of sporulation) and thus somewhat different from the previous suppressor mutations described. In effect, the *AS7-1* and *AS7-2* mutations are epistatic to *su1* and *su2* mutations both *in vivo* and *in vitro*. Even strong suppressor mutations like *su1-60* and *su2-5* cannot relieve the sporulation defect of *AS7-1* or notably decrease its paromomycin resistance. Moreover the ribosomes from the double mutants *su1-60 . AS7-1* or *su2-5 . AS7-1* behave like *AS7-1* ribosomes in *in vitro* experiments (Coppin-Raynal & Le Coze 1982; Coppin-Raynal 1982*a*, *b*). These data might explain why no *su1* and *su2* mutations were found in the *AS7-2* revertants.

In E. coli, mutations in two ribosomal genes can increase the misreading level.

They fall in the structural genes for ribosomal proteins *S4* and *S5*. In yeast, three omnipotent suppressor loci are now well characterized (Inge-Vechtomov & Andrianova, 1970; Hawthorne & Leupold, 1974; Gerlach 1975; Ono, Stewart & Sherman, 1981). So far, the alteration of one ribosomal protein (*S11*) has been identified, for *sup46* (Ishiguro *et al.* 1981). In *Podospora*, we have identified seven putative ribosomal suppressors loci. For three of them, we have evidence for the alteration of a ribosomal protein (Dequard-Chablat & Coppin-Raynal, 1984; Dequard-Chablat *et al.* in preparation). This result does not imply that the genetic control of translational fidelity is more complex in Podospora: Perhaps the variety of screening procedures used in this organism has allowed identification of a greater number of genes than in other organisms. The present results support this conclusion because a new screening procedure leads to two new ribosomal suppressor loci.

REFERENCES

COPPIN-RAYNAL, E. (1981). Ribosomal suppressors and antisuppressors in *Podospora anserina*: altered susceptibility to paromomycin and relationships between genetic and phenotypic suppression. *Biochemical Genetics* **19**, 729–740.

COPPIN-RAYNAL, E. (1982*a*). Ribosomal control of translational fidelity in *Podospora anserina*: a suppressor and an antisuppressor affecting the paromomycin-induced misreading *in vitro*. *Current Genetics* **5**, 57–63.

COPPIN-RAYNAL, E. (1982*b*). Thèse de Doctorat d'Etat, Université de Paris-Sud, Orsay (France). L'imprécision de la traduction: son contrôle par le ribosome et son rôle biologique chez le champignon *Podospora anserina*.

COPPIN-RAYNAL, E. & LE COZE, D. (1982). Mutations relieving hypersensitivity to paromomycin caused by ribosomal suppressors in *Podopsora anserina*. *Genetical Research* **40**, 149–164.

DEQUARD, M., COUDERC, J.-L., LEGRAIN, P., BELCOUR, L. & PICARD-BENNOUN, M. (1980). Search for ribosomal mutants in *Podospora anserina*: genetic analysis of mutants resistant to paromomycin. *Biochemical Genetics* **18**, 263–280.

DEQUARD-CHABLAT, M. & COPPIN-RAYNAL, E. (1984). Increase of translational fidelity blocks sporulation in the fungus *Podospora anserina*. *Molecular and General Genetics* **195**, 294–299.

ESSER, K. (1974). *Podospora anserina*. In *Handbook of Genetics*, vol. 1 (ed. R. C. King), pp. 531–551. New-York and London: Plenum.

GERLACH, W. L. (1975). Genetic properties of some amber-ochre super-suppressors in *Saccharomyces cerevisiae*. *Molecular and General Genetics* **138**, 53–63.

GORINI, L. (1974). Streptomycin and misreading of the genetic code. In *Ribosomes* (ed. N. Nomura, A. Tissières and P. Lengyel), pp. 791–803. New York: Cold Spring Harbor Laboratory.

HAWTHORNE, D. C. & LEUPOLD, U. (1974). Suppressor mutations in yeast. *Current Topics in Microbiology and Immunology* **64**, 1–47.

INGE-VECHTOMOV, S. G. & ADRIANOVA, V. M. (1970). Recessive super-suppressors in yeast. *Genetics* **6**, 103–115.

ISHIGURO, J., ONO, B. I., MASUREKAR, M., MCLAUGHLIN, C. S. & SHERMAN, F. (1981). Altered ribosomal protein S11 from the SUP46 suppressor of yeats. *Journal of Molecular Biology* **147**, 391–397.

MARCOU, D., PICARD-BENNOUN, M. & SIMONET, J. M. (1980). Genetic map of *Podospora anserina*. In *Genetic Maps*, vol. 1 (ed. S. J. O'Brien) pp. 151–159. Bethesda NCI-NIH.

ONO, B. I., STEWART, J. W. & SHERMAN, F. (1981). Serine insertion caused by the ribosomal suppressor *SUP46* in yeast. *Journal of Molecular Biology* **147**, 373–379.

PICARD, M. (1971). Genetic evidence for a polycistronic unit of transcription in the complex locus '14' in *Podospora anserina*. I. Genetic and complementation maps. *Molecular and General Genetics* **111**, 35–50.

PICARD, M. (1973). Genetic evidence for a polycistronic unit of transcription in the complex locus '14' in *Podospora anserina*. II. Genetic analysis of informational suppressors. *Genetical Research* **21**, 1–15.

PICARD-BENNOUN, M. (1976). Genetic evidence for ribosomal antisuppressors in *Podospora anserina*. *Molecular and General Genetics*. **147**, 299–306.

PICARD-BENNOUN, M. (1981). Mutations affecting translational fidelity in the eukaryote *Podospora anserina*: characterization of two ribosomal restrictive mutations. *Molecular and General Genetics* **183**, 175–180.

PICARD-BENNOUN, M. (1982). Does translational ambiguity increase during cell differentiation? *FEBS Letters* **149**, 167–170.

PICARD-BENNOUN, M., COPPIN-RAYNAL, E. & DEQUARD-CHABLAT, M. (1983). Translational ambiguity and cell differentiation in a lower eucaryote. In *Translational and post translational events* (ed. A. K. Abraham, T. S. Eikhom and I. F. Pryme), pp. 221–232. The Humana Press.

PICARD-BENNOUN, M. & LE COZE, D. (1980). Search for ribosomal mutants in *Podospora anserina*: genetic analysis of cold-sensitive mutants. *Genetical Research* **36**, 289–297.

PIEPERSBERG, W., GEYL, D., HUMMEL, H. & BÖCK, A. (1980). Physiology of ribosomal mutants – Physiology and Biochemistry of bacterial ribosomal mutants. In *Genetics and Evolution of Transcriptional and Translational apparatus*, pp. 359–377. Tokyo: Kodansha Scientific.

RANDSHOLT, N., IBARRONDO, F., DEQUARD, M. & PICARD-BENNOUN, M. (1982). Analysis of revertants of a ribosomal mutation in *Podospora anserina* evidence for new ribosomal mutations which confer hypersensitivity to paromomycin. *Biochemical Genetics* **20**, 569–584.

RIZET, G. & ENGELMANN, C. (1949). Contribution à l'étude génétique d'un ascomycète tétra-sporé: *Podospora anserina*. *Revue de Cytologie et de Biologie Végétale* **11**, 201–304.

SIMONET, J. M. AND ZICKLER, D. (1972). Mutations affecting meiosis in *Podospora anserina*. *Chromosoma* **37**, 327–351.

ZICKLER, D. AND SIMONET, J. M. (1980). Identification of gene-controlled steps of ascospores development in *Podospora anserina*. *Experimental Mycology* **4**, 191–206.