EXTRACHROMOSOMAL ELEMENTS IN A SUPER-SUPPRESSION SYSTEM OF YEAST

II. RELATIONS WITH OTHER EXTRACHROMOSOMAL ELEMENTS

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1. Introduction

Super-suppression in yeast is probably caused by mutations in genes coding for minor species of transfer RNA. The mutant transfer RNA molecules are then able to translate one (or more) of the " stop " codons, UAA, UAG or UGA, occurring internally in the messenger RNA transcribed from suppressible alleles (Hawthorne, 1969*a*, *b*; Manney, 1968; Gilmore, Stewart and Sherman, 1968; Hawthorne and Mortimer, 1968). There is evidence that this suppression may be modified by alleles of extrachromosomal heredity determinant(s), termed " ψ " (Cox, 1965, 1971; Young and Cox, 1971). This would suggest that ψ is involved in the mechanism of protein synthesis.

These determinants are also of interest in furthering our knowledge of the extrachromosomal genome of yeast which contains genes which can confer " resistance " to a variety of antibiotics (Thomas and Wilkie, 1968*a*, *b*; Linnane, Saunders, Gingold and Lukins, 1968) as well as some of the genes responsible for respiratory competence (Ephrussi, 1953; Ephrussi, Hottingeur and Roman, 1955). The extrachromosomal genes for respiratory competence and for antibiotic resistance are probably located in mitochondrial DNA (Mounolou, Jakob and Slonimski, 1966; Thomas and Wilkie, 1968*b*) but it is not clear that they are linked (Gingold, Saunders, Lukins and Linnane, 1969).

This paper deals with the genetical relationships between the extrachromosomal determinant involved in suppression and those involved in erythromycin resistance and in respiratory competence. The demonstration of linkage between ψ and either of the latter determinants, both of which have mitochondrial function and perhaps location, would be a valuable clue in any attempt to build a plausible model for the function of ψ.

2. Materials and methods

(i) *Media*

As well as the complete (YC) sporulation (SM) and basal (YNB) media described previously (Young and Cox, 1971), two others were used in this investigation. CG is a complete medium in which the glucose has been replaced by glycerol (2 per cent. v/v). EG is the same as CG except for the addition of erythromycin lactobionate (Abbott Laboratories) to a final concentration of 50 μg. ml.$^{-1}$. The antibiotic was filter-sterilised and added to the medium shortly before plates were poured.

(ii) *Strains and Nomenclature*

The term " suppressive " is used only in the context of the suppressive form of respiratory deficiency first described by Ephrussi *et al.* (1955).

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Genetic nomenclature follows the suggestions proposed in the *Yeast Genetics Supplement to Microbial Genetics Bulletin No. 31* (November 1969).

The strains used are described in table 1. The erythromycin-resistant strains were obtained by plating aliquots of stationary phase cultures on EG plates. Since erythromycin specifically inhibits the synthesis of cytochromes a_1, a_3, b and c_1, producing a phenocopy of the petite mutation (Clark-Walker and Linnane, 1966), only resistant mutants will be able to grow on

TABLE 1

Yeast strains used

Strain	Chromosomal genotype	Extrachromosomal genotype	Phenotype
163/9c white	: α, *ade2-1*, S_{Q5}	[*psi*+, *ery-s*, *rho*+]	white, ADE+
163/9c red	: α, *ade2-1*, S_{Q5}	[*psi*−, *ery-s*, *rho*+]	red, ade$^-$
170/2c white	: *a*, *ade2-1*, S_{Q5}	[*psi*+, *ery-s*, *rho*+]	white ADE+
170/2c red	: *a*, *ade2-1*, S_{Q5}	[*psi*−, *ery-s*, *rho*+]	red ade$^-$
193/1b	: *a*, *ade2-1*, S_{Q5}	[*psi*−, *ery-s*, *rho*+]	red ade$^-$
1c	: α, *ade2-1*, *met*, *tyr*, S_{Q5}	[*psi*+, *ery-s*, *rho*+]	white, prototrophic
Er^1, derived from *170/2c* white		[*psi*+, *ery-r*, *rho*+]	erythromycin resistant strains
Er^2, Er^3, Er^4, from *170/2c* red		[*psi*−, *ery-r*, *rho*+]	
409/4c, from Er^2 by crossing	: α, *ade2-1*, S_{Q5}	[*psi*+, *ery-r*, *rho*+]	
RD_{21} derived from *1c*		[*psi*+, *ery-s*, *rho*−(60%*s*)]	suppressive respiratory deficient strains
193/1bρ^{80}, derived from *193/1b*		[*psi*−, *ery-s*, *rho*−(80%*s*)]	
193/1bρ^-, derived from *193/1b*		[*psi*−, *ery-s*, *rho*−(*n*)]	" neutral " respiratory deficient strain

The genotype symbols: *a* and α: mating type alleles; *ade2-1*, met, tyr: alleles for requirements for adenine, methionine and tyrosine, all suppressible by the super-suppressor S_{Q5}; *ade2-1* gives a red pigmentation to a colony when it is unsuppressed; [*psi*+] and [*psi*−] extrachromosomal alleles controlling the activity of S_{Q5}; [*ery-r*] and [*ery-s*]: extrachromosomal alleles conferring resistance or sensitivity to erythromycin on non-fermentable substrates; [*rho*+], [*rho*−(*n*)] and [*rho*−(%)]: extrachromosomal alleles conferring respiratory competency, neutral deficiency or suppressive deficiency respectively.

N.B.: [*psi*+] and [*psi*−] have previously been designated ψ^+ and ψ^- (Cox, 1965, 1971; Young and Cox, 1971) but are brought into line with the suggestions for the nomenclature of determinants proposed in the yeast genetics supplement to *Microbial Genetics Bulletin No. 31* (November 1969). Similarly [*rho*] replaces the former symbol ρ (Sherman and Ephrussi, 1962). Square brackets are intended to indicate the extrachromosomal genome.

EG medium, since this contains glycerol, a non-fermentable substrate, as carbon source.

The suppressive petite RD_{21} was obtained from *1c* in the following manner. Cells at appropriate dilution were spread on complete medium containing glycerol (2 per cent. v/v) and glucose (0·2 per cent. w/v). Spontaneously-occurring petites will produce much smaller colonies than the wild type and may be selected visually. Several of the small colonies were picked and crossed to *170/2c* white and six zygotes from each cross were isolated by micromanipulation. Those *1c* isolates which are suppressive will impose their respiratory deficiency on a proportion of the diploid clones, which depends on the " degree of suppressiveness " of the particular suppressive mutant. The proportion was estimated by testing inocula of each diploid clone on CG, isolate RD_{21} being found to have a degree of suppressiveness of about

50 per cent. [though see Results section (ii) (a)]. The suppressive petite *193/1b* red was selected by the method of Sherman and Ephrussi (1962). Spontaneous petite colonies of *193/1b* were identified by replica plating on CG. Several such colonies were mated with a respiratory competent strain having complementary auxotrophic requirements and diploids were selected by plating the zygotes on YNB. The proportion of petite diploids from each cross was estimated by the tetrazolium overlay technique of Ogur, St John and Nagai (1957). *193/1bρ*^{80} was found to be 80 per cent. suppressive.

Diploid clones were tested for the presence of erythromycin resistant cells by spotting cell suspensions on to CG and EG plates with a sterile glass rod or with a 5 μl. micro-pipette. This is a rapid qualitative method of detecting segregation of erythromycin resistance and sensitivity within such clones (Thomas and Wilkie, 1968*a*). Alternatively, 0·1 ml. aliquots of cells at suitable dilution were spread on EG and CG plates. Haploid cultures were tested by the spotting method. No attempt was made to determine whether mitotic segregation of resistance and sensitivity occurred within clones.

3. Results

(i) *Digenic extrachromosomal crosses*

(a) *Er*^{1} [*psi*+, *ery-r1*] × *163/9c*[*psi*−, *ery-s*]

Zygotes were isolated from a mass-mating mixture on YC and were transferred to a CG plate. This eliminates any petite diploids, which would not be testable for erythromycin phenotype (see materials and methods). Four zygotes gave rise to diploid clones, inocula from which were suspended in saline and spread on CG and EG plates. All colonies on both sets of plates were white and a sample, when tested on YNB, proved to be prototrophic. Table 2 gives the percentages of colonies which can be deduced to have arisen

Table 2

The segregation of suppressed and erythromycin phenotypes in diploid clones from the cross Er^{1} ([psi+ ery-r]) × 163/9c *red* ([psi−, ery-s])

Diploid clone	Nos. of colonies on: CG medium	EG medium	% erythromycin sensitive cells	% suppressed cells
1	83	8	90	100
2	50	74	0	100
3	91	44	52	100
4	confluent	separate colonies	~90	100

from sensitive cells. The appearance of *both* resistant and sensitive phenotypes within a clone and the different proportions of the two types in different clones is characteristic of erythromycin resistant × sensitive crosses. (Thomas and Wilkie, 1968*a*; Linnane *et al.*, 1968). This pattern is not observed in the super-suppression characteristics of the four clones, for all diploid colonies are suppressed. This is the expected behaviour for [*psi*+] × [*psi*−] crosses (Cox, 1965). Overall it can be seen clearly that phenotypes have reassorted in many of the offspring to give a novel suppressed, erthromycin-sensitive phenotype.

Tetrad analysis proved to be impossible in this cross, for although the diploids sporulated well on SM, the spores gave very poor germination on YC.

(b) *Er*^{2}, *Er*^{3} *and Er*^{4}[*psi*−, *ery-r*] × *lc*[*psi*+, *ery-s*]

In these crosses, the extrachromosomal markers were in the reciprocal arrangement compared to the first cross. Ten zygotes per cross were isolated from a mass-mating mixture on YC and transferred to CG. The diploid clones which grew up were tested by spotting on EG and CG plates. As in the first cross, there was considerable variation in the proportion of resistant cells between different clones in each cross, although the exact ratios were not obtained. Tetrad analyses were carried out using one diploid clone from each of the three crosses. The spore-clones were tested for erythromycin resistance by spotting on to CG and EG plates and for prototrophy by replication on to YNB plates (table 3). The 4 : 0 suppressed : non-suppressed

TABLE 3

The segregation of suppressed and erythromycin phenotypes in tetrads from crosses of Er^2, Er^3 or Er^4 ([psi−, ery-r]) × lc ([psi+, ery-s])

Ery parent	Erythromycin resistant: sensitive	Suppressed: non-suppressed	Mating type
Er^2	4 at 0 : 4	4 at 4 : 0	4 at 2 : 2
Er^3	4 at 4 : 0	4 at 4 : 0	4 at 2 : 2
Er^4	4 at 4 : 0	4 at 4 : 0	not tested

segregations are typical of [*psi*+] × [*psi*−] crosses (Cox, 1965); similarly the 4 : 0 and 0 : 4 sensitive : resistant segregations are those expected from erythromycin sensitive × resistant crosses (Thomas and Wilkie, 1968*a*; Linnane *et al.*, 1968). The failure to observe both 4 : 0 and 0 : 4 segregations for resistance and sensitivity in the same set of tetrads, despite the mixed phenotype of the diploid clones from which the tetrads arose, is not surprising in view of the few tetrads dissected. However, it can be seen that in two of the three crosses, a new phenotype has arisen by reassortment of the parental phenotypes, namely suppressed, erythromycin resistant.

These reciprocal digenic crosses reveal that the suppression and drug resistant phenotypes freely reassort. Alternatively we might say that the segregation patterns for the two phenotypes are independent.

(ii) *Trigenic extrachromosomal crosses*

(a) *RD*$_{21}$ [*psi*+, *ery-s*, *rho*−(*60 per cent. s*)] × *Er*^{2}[*psi*−, *ery-r*, *rho*+]

Respiratory deficient diploids are unable to sporulate (Ephrussi, 1953) but this restriction may be circumvented if zygotes are transferred to sporulation medium as soon as they have formed. Accordingly, a mass-mating mixture of *RD*$_{21}$ and *Er*^{2} was set up on YC, *Er*^{2} having been grown previously on CG to eliminate respiratory deficient cells. After about 6 hours, by which time zygotes had formed, an inoculum from the mating mixture was transferred to SM. Fourteen tetrads were tested initially for respiratory competence and for prototrophy. Those spore-clones that proved to be respiratory competent were then tested by spotting on EG plates. The results in table 4 show that two new phenotypes have appeared at high frequency:

22 spore-clones from a total of 56 are suppressed and respiratory competent.

13 clones are suppressed and erythromycin resistant.

TABLE 4

Segregation of three extrachromosomal phenotypes in tetrads from the cross RD_{21} ([psi+, ery-s, rho−(60%s)] × Er^2 ([psi−, ery-r, rho+])

	Suppressed: non-suppressed	Respiratory competent: deficient	Erythromycin resistant: sensitive
	14×4 : 0	5×4 : 0	2×4 : 0
			2×2 : 2
			1×1 : 3
		2×1 : 3	2×0 : 1
		7×0 : 4	not testable
Totals	56 : 0	22 : 34 (61% "suppressive")	13 : 9

Mating type alleles segregated 2 : 2 in all cases except one of the 0 : 4 respiratory competent : deficient tetrads.

It was of some interest to see whether the spore clones which were respiratory deficient still harboured any determinants for erythromycin resistance, brought into the cross by *Er*². Thomas and Wilkie (1968*a*), on inducing respiratory deficiency with euflavine in formerly resistant strains found that the ability to transmit resistance determinants was lost. Spontaneously arising respiratory deficient mutants in erythromycin resistant strains seem to be able to transmit such determinants however (Gingold *et al.*, 1969). In the cross reported in this paper, the respiratory deficient offspring are the progeny of a suppressive petite parent. To test whether or not these offspring could transmit resistance determinants, 16 respiratory deficient spore-clones from four tetrads were crossed on YC to either *163/9c* white or *170/2c* white, both of which had been grown on CG to eliminate respiratory deficient cells. Three zygotes from each of the 16 crosses were isolated and 42 of the 48 zygotes gave rise to diploid clones. These were tested by spotting on CG and EG plates. At least four of the respiratory deficient spore clones were capable of transmitting determinants for erythromycin resistance (table 5). This is probably an underestimate, for in some

TABLE 5

Analysis of diploid clones from crosses involving respiratory deficient segregants from the cross $RD_{21} \times Er^2$ *(see table 4) and respiratory competent, erythromycin sensitive strains*

Spore clone parent	No. of diploid clones tested	Respiratory competent	Erythromycin resistant
Tetrad 2 *a*	3	2	0
b	2	1	1
c	2	0	n.t.*
d	3	0	n.t.
Tetrad 3 *a*	2	1	1
b	2	0	n.t.
c	3	0	n.t.
d	3	0	n.t.
Tetrad 5 *a*	3	0	n.t.
b	3	1	0
c	3	0	n.t.
d	2	1	1
Tetrad 7 *a*	3	2	0
b	2	1	0
c	3	0	n.t.
d	3	1	1
Totals	42	10 (76% suppressive)	4 from 10 testable clones

* Not testable.

crosses no respiratory competent diploid clone was available for testing. It can also be seen that the " degree of suppressiveness " has been inherited by the petite segregants, since 32 of the 42 diploid clones (76 per cent.) were themselves respiratory deficient.

(b) *193/1bρ*^{80}*[psi−, ery-s, rho−(80 per cent. s)]* × *409/4c[psi+, ery-r, rho+]*

This cross has the suppression and suppressive respiratory deficient phenotypes in the reciprocal arrangement to that in cross (a). The same mating, sporulating, and phenotype-testing procedures were followed as for cross (a). The results are shown in table 6 where it can be seen that there was

TABLE 6

Segregation of three extrachromosomal phenotypes in tetrads from the cross 193/1bρ^{80} ([psi−, ery-s, rho−(80%s)] × 409/4c ([psi+, ery-r, rho+)]

(i) *Tetrad data*

Suppressed: non-suppressed	Respiratory competent: deficient	Erythromycin resistant: sensitive
11 × 4 : 0	1 × 4 : 0	1 × 4 : 0
	1 × 3 : 1	1 × 3 : 0
	9 × 0 : 4	n.t.
3 × 3 : 1	1 × 4 : 0	1 × 4 : 0
	2 × 0 : 4	n.t.
2 × 2 : 2	1 × 4 : 0	1 × 4 : 0
	1 × 0 : 4	n.t.
1 × 0 : 4	1 × 0 : 4	n.t.

Mating type segregated 2 : 2 in all tetrads.

(ii) *Spore data obtained from above tetrads*

	Suppressed	Non-suppressed	Totals
Respiratory competent	12	3	15
Respiratory deficient	45	8	53
Totals	57	11	68

P for a 2 × 2 contingency table: 0·26. (Fisher's exact test.)

segregation for the suppression phenotypes. This is *not* the typical pattern of segregation from [*psi*+] × [*psi*−] crosses (Cox, 1965) in which the non-suppressed phenotype never reappears, although it has been observed in crosses involving [*psi*−] strains derived from a strain U_{16} containing the nuclear allele *R* (Young and Cox, 1971). However, it allows us to make an unequivocal test as to whether or not the suppression phenotype is segregating independently of the respiratory deficient phenotype. Using Fisher's exact treatment for 2 × 2 contingency tables (Bailey, 1959), P for the hypothesis that the phenotypes are unlinked is 0·26.

The appearance of non-suppressed offspring is not a consequence of strain *193/1bρ*^{80} being a suppressive respiratory-deficient strain for when the same [*psi*+ *ery-r*] strain, *409/4c*, was crossed to the original *rho*+ culture of *193/1b* or to a neutral petite mutant of it, *193/1bρ*$^{-}$, non-suppressed clones segregate in a similar frequency (tables 7 and 8). Again it is possible to check the segregation of the various phenotypes to see whether or not they are assorting independently. The results are compatible with the hypothesis

TABLE 7

Segregation of three extrachromosomal phenotypes in tetrads from the cross 193/1bρ^- ([psi−, ery-s, rho−(n)]) × 409/4c ([psi+, ery-r, rho+])

(i) *Tetrad data*

Suppressed: non-suppressed	Respiratory competent: deficient	Erythromycin resistant: sensitive
6×4:0	2×4:0	1×4:0 1×3:1
	4×3:1	3×3:0 1×1:2
7×3:1	3×4:0	2×3:1 1×2:2
	2×3:1	1×3:0 1×2:1
	1×2:2	1×2:0
	1×1:3	1×0:1
6×2:2	2×4:0	1×4:0 1×3:1
	3×3:1	2×3:0 1×2:1
	1×2:2	1×2:0

Mating type segregated 2 : 2 in all except one tetrad.

(ii) *Spore data from the above tetrads*

	Sup-pressed	Non-suppressed	Totals		Sup-pressed	Non-suppressed	Totals
Respiratory competent	47	13	60	Erythromycin resistant	40	9	49
Respiratory deficient	10	6	16	Erythromycin sensitive	7	4	11
Totals	57	19	76	*Totals*	47	13	60
	P = 0·11.				P = 0·13.		

that the suppression phenotype assorts independently of the respiratory and the erythromycin phenotypes (P values varying from 0·11 to 0·30).

Table 9 brings together the data of the segregation of the respiratory phenotype from the erythromycin phenotype in tables 4, 6, 7 and 8. In sharp contrast to the segregation of the suppression phenotype, the percentage of erythromycin-resistant offspring is clearly affected by the respiratory phenotype of the erythromycin-sensitive parent. This is most marked in the bottom two crosses of the table which involve sensitive parents differing only in their respiratory phenotype.

4. DISCUSSION

Initially we assumed that the independence of *patterns of inheritance* for different determinants in digenic and trigenic crosses would be a sufficient proof of independent assortment. Thus, for example, in the cross *Er*^{1} × *163/9c* red (table 2), the suppression phenotype appeared in 100 per cent. of the diploid offspring while the erythromycin phenotypes segregated within clones. Both patterns of inheritance are typical for their respective determinants and this is strong circumstantial evidence that the determinants are not linked. However, we cannot state categorically that the [*psi*]

TABLE 8

Segregation of extrachromosomal phenotypes in tetrads from the cross 193/1bρ^+ ([psi−, ery-s, rho+]) × 409/4c ([psi+, ery-r, rho+])

(i) *Tetrad data*

Suppressed: non-suppressed	Respiratory competent: deficient	Erythromycin resistant: sensitive
7 × 4 : 0		1 × 3 : 1
	5 × 4 : 0	1 × 1 : 3
		3 × 0 : 4
	1 × 3 : 1	1 × 1 : 2
	1 × 2 : 2	1 × 0 : 2
4 × 3 : 1	1 × 4 : 0	1 × 0 : 4
		1 × 3 : 0
	3 × 3 : 1	1 × 2 : 1
		1 × 0 : 3
3 × 2 : 2	1 × 4 : 0	1 × 0 : 4
	1 × 3 : 1	1 × 1 : 2
	1 × 1 : 3	1 × 1 : 0
3 × 1 : 3	1 × 4 : 0	1 × 1 : 3
	1 × 3 : 1	1 × 0 : 3
	1 × 0 : 4	not testable
1 × 0 : 4	1 × 4 : 0	1 × 4 : 0

Mating type segregated 2 : 2 in all tetrads.

(ii) *Spore data from the above tetrads*

	Sup-pressed	Non-suppressed	Totals		Sup-pressed	Non-suppressed	Totals
Respiratory competent	40	17	57	Erythromycin resistant	10	3	13
Respiratory deficient	9	6	15	Erythromycin sensitive	30	14	44
Totals	49	23	72	*Totals*	40	17	57
	P = 0·18.				P = 0·30.		

TABLE 9

Data on the segregation of suppression, respiratory and erythromycin phenotypes from the crosses reported in tables 4, 6, 7 and 8

Cross	% Respiratory competent	Respiratory competent offspring which are erythromycin resistant: sensitive		% sensitive (recombinant class in crosses 1-3)	% suppressed
1. $RD_{21} \times Er^2$ [*psi+, ery-s, rho−(60%s)*] × [*psi−, ery-r, rho+*]	39	13	9	41	100
2. *193/1b*$\rho^{80} \times$ *409/4c* [*psi−, ery-s, rho−(80%s)*] × [*psi+, ery-r, rho+*]	22	15	0	0	84
3. *193/1b*$\rho^- \times$ *409/4c* [*psi−, ery-s, rho−(n)*] × [*psi+, ery-r, rho+*]	79	49	11	18	75
4. *193/1b*$\rho^+ \times$ *409/4c* [*psi−, ery-s, rho+*] × [*psi+, ery-r, rho+*]	79	13	44	77	68

determinants are not affecting the inheritance of the [*ery*] determinants unless we compare the results with those from a cross in which both parents are [*psi*+] but allelic for [*ery*].

This difficulty was resolved for us by the segregation of non-suppressed phenotypes in the crosses, involving strains of *193/1b*, in which zygotes were sporulated soon after formation (tables 6, 7 and 8). By means of statistical comparisons *within* each cross we can state that the suppression phenotype segregates independently of both the erythromycin and respiratory phenotypes. Furthermore, we can make qualitative comparisons *between* crosses (table 9). It is clear that the percentage of offspring which are suppressed is high in all crosses despite wide variations in the percentage of offspring which are (*a*) respiratory competent, or (*b*) erythromycin resistant. This is particularly striking when the segregation of erythromycin resistance is compared in tables 7 and 8. The ratios of erythromycin resistant and sensitive segregants are almost reciprocal, without affecting the proportion of " suppressed " phenotypes in each class. Thus fluctuations in the apparent transmission of [*ery*] and [*rho*] do not impose similar fluctuations in the transmission of [*psi*]. This would suggest that [*psi*] is not linked to either of the other determinants. These results, coupled with our previous observation that the nuclear allele " *R* " interferes with the transmission of [*psi*] but not with that of [*ery*] or [*rho*], leads us to propose that [*psi*] is on a separate " extrachromosomal chromosome " (Young and Cox, 1971).

The segregation of suppressed and non-suppressed phenotypes, while not typical for [*psi*+] × [*psi*−] crosses, nevertheless confirms the extrachromosomal location of the [*psi*] determinant since non-Mendelian segregations of both 0 : 4 and 4 : 0 suppressed : non-suppressed are observed within two crosses (tables 6 and 8).

The crosses reported in this paper reveal some of the complexities in the genetic transmission of [*rho*] and [*ery*] determinants. Firstly, table 9 (crosses 1-3) shows that [*ery*] and [*rho*] are linked, but that the tightness of linkage is variable. This may reflect different cytological distances between [*ery*] and the different [*rho*−] markers used in the three crosses. Secondly, some respiratory deficient haploid segregants from a cross involving a suppressive petite parent can still transmit erythromycin resistance determinants in crosses to erythromycin-sensitive strains (table 5). These segregants must be recombinant since the resistance and respiratory deficiency markers came into the cross in repulsion (table 4). Our findings complement those of Gingold *et al.* (1969) who found that petites arising spontaneously in formerly erythromycin-resistant strains could still transmit the resistance marker in crosses to sensitive, respiratory-competent strains. Thirdly, the frequency of erythromycin-resistant haploid offspring from any [*ery-r*] × [*ery-s*] cross would appear to be dependent upon the respiratory phenotype of the [*ery-s*] parent. This is seen most clearly in table 9 in which crosses 3 and 4 are genetically identical except that the [*ery-s*] parent in cross 3 is [*rho*−] and the offspring are predominantly [*ery-r*]. Two explanations occur to us. If it is assumed that in a heteroplasmon formed between *193/1b*ρ^{+} and *409/4c*, the extrachromosomal determinants from the former parent are preferentially transmitted to the haploid offspring, then the majority of these offspring would be [*ery-s*, *rho*+] as is the case in cross 4 (77 per cent.). On the other hand, in cross 2 involving strain *193/1b*ρ^{80}, which is respiratory deficient, the majority of offspring should be [*rho*−].

The observed value is 78 per cent. Finally, in strain *193/1bρ⁻*, we have to assume since it is a neutral petite that the preferential transmission characteristic has been lost along with respiratory competence so that in cross 3 the extrachromosomal determinants are mainly derived from *409/4c* and the haploid offspring would be mainly [*ery-r*, *rho*+] as indeed they are (82 per cent.). The second explanation is based on the observation of Gingold *et al.* (1969) that the ability to transmit erythromycin-resistance determinants was *gradually* lost on sub-culture of an [*ery-r*, *rho*−] strain. If the [*ery-s*, *rho*−] strains in table 9 were losing the ability to transmit [*ery-s*] in crosses to [*ery-r*] strains, then the recovery of [*ery-r*] offspring would be enhanced. However, this would not explain the observed preferential transmission of [*ery-s*] determinants to the haploid offspring in cross 4.

Finally, it is interesting to note that the degree of suppressiveness of a suppressive petite is inherited by its petite meiotic progeny. This is a formal demonstration that the cytoplasmic determinants for the petite and the suppressive phenotypes are linked.

5. Summary

1. Crosses have been performed using a variety of extrachromosomally determined phenotypes, namely the activity of the super-suppressor S_{Q5}, various types of respiratory deficiency and the ability to grow on a non-fermentable substrate supplemented with the antibiotic erythromycin.

2. The segregation of phenotypes compared *within* and *between* crosses indicates that the determinant for suppressor activity, [*psi*], segregates independently of the determinants for respiratory [*rho*], and erythromycin, [*ery*] phenotypes. It is suggested that [*psi*] may be on a different " extrachromosomal chromosome " from [*ery*] or [*rho*].

3. The segregations of 0 : 4 and 4 : 0 suppressed: non-suppressed phenotypes in some crosses lend support to the hypothesis that [*psi*] is indeed extrachromosomal.

4. The determinants [*ery*] and [*rho*] are linked. Furthermore it is suggested that transmission of an [*ery*] allele may be dependent on the preferential transmission of the [*rho*] allele to which it is coupled.

5. In a cross between a suppressive petite and an erythromycin resistant strain, some suppressive petite haploid segregants are capable of transmitting the [*ery*−*r*] determinant by mating to an [*ery*−*s*, *rho*+] strain.

6. The " degree of suppressiveness " of a suppressive petite strain is transmitted through meiosis.

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