Genetics of a Primaquin-resistant Yeast

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SUMMARY
Primaquin specifically inhibits mitochondrial function in yeast. Mutants resistant to primaquin have been isolated. Genetic analysis revealed that the expression of resistance in one of them was under the control of both a nuclear gene and a cytoplasmic factor (possibly a mitochondrial gene).

INTRODUCTION
Saccharomyces cerevisiae provides a good system to study the structure of mitochondrial DNA and the expression, in close association, of both nuclear and mitochondrial genetic information (Ephrussi, 1953). Yeast cells can grow on fermentable substrates when mitochondrial respiratory functions are not expressed. This property permits the isolation of many mitochondrial mutants. One type, 'petite', has been extensively used to study the structure and properties of the mitochondrial DNA (Carnevali & Leoni, 1972; Mehrotra & Mahler, 1968; Mounolou, Jakob & Slonimski, 1966; Nagley & Linnane, 1970, 1972). The study of mutants resistant to various antibiotics has allowed the analysis of mitochondrial heredity (for review see Howell et al. 1973). The many chemicals which interfere specifically with mitochondrial functions can be arranged in two classes: one consists of mutagens (Goldring et al. 1970; Goldring, Grossman & Marmur, 1971; Mahler, Mehrotra & Perlman, 1971; Perlman & Mahler, 1971) of mitochondrial DNA, like acriflavine and ethidium bromide; and drugs such as oligomycin and erythromycin, which inhibit the normal processes of mitochondrial functions (Avner et al. 1973; Avner & Griffiths, 1973; Stuart, 1970).

We have used the dye primaquin [primaquin diphosphate, i.e. 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline, diphosphate salt; Sigma], known for its intercalation into nucleic acids in vitro. This drug shares some interesting properties with other intercalating drugs. Like ethidium bromide, it prevents growth when functional mitochondria are essential, but unlike ethidium bromide it is not mutagenic (Rotman, 1971); it could therefore be a very useful tool to study the expression of mitochondrial DNA.

Mutant strains resistant to primaquin have been isolated. In one of them (194-5c/FP-R1) the expression of resistance appears to be under the control of both a nuclear gene and a cytoplasmic factor.

METHODS
Strains. Haploid strains of Saccharomyces cerevisiae sensitive to primaquin are listed in Table 1; they were from the Centre de Génétique Moléculaire, Gif-sur-Yvette, France. Strain 195-5c is unable to grow on plates containing glycerol and primaquin (see Growth conditions). This strain was u.v.-irradiated (survival 10⁻⁷) and plated, and the mutant strain was isolated as a colony after prolonged incubation (15 days).

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Table 1. Genotypes of the strains

<table>
<thead>
<tr>
<th>Strains sensitive to primaquin</th>
<th>Nuclear genotype</th>
<th>Mitochondrial genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>194-5c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain type A:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DP1-18/514</td>
<td>a ade-6 his-4 ura-1</td>
<td>ρ+ ω+ (CAM-S) (ERY-S)</td>
</tr>
<tr>
<td>IL102-8D</td>
<td>α his-1 trp-1</td>
<td>ρ+ ω+ (CAM-S) (ERY-R)514</td>
</tr>
<tr>
<td>Strain type B:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c982-19d</td>
<td>α his-1 trp-1</td>
<td>ρ+ ω+ (CAM-S) (ERY-S)</td>
</tr>
<tr>
<td>IL8-8C</td>
<td>α his-1 trp-1</td>
<td>ρ+ ω+ (CAM-R)321 (ERY-R)514</td>
</tr>
</tbody>
</table>

Strain resistant to primaquin: 194-5c/FP-R1

a ade-6 his-4 ura-1 ρ+ ω+ (CAM-S) (ERY-S)

Abbreviations: a, α, cellular mating type; ade, adenine auxotrophy; his, histidine auxotrophy; ura, uracil auxotrophy; trp, tryptophane auxotrophy; ρ+, respiratory competent cell, 'grande'; ω+, ω−, mitochondrial mating type; (CAM-S), (CAM-R), sensitivity and resistance to chloramphenicol; (ERY-S), (ERY-R), sensitivity and resistance to erythromycin.

Growth conditions. The basic medium contained: yeast extract, 1% (w/v); peptone, 1% (w/v); Na-K phosphate buffer, 0.1 M, pH 7.0; agar, 2% (w/v). The carbon source was either glucose (2%, w/v) (YPG medium) or glycerol (3%, w/v). When only glycerol was available in the medium, the addition of primaquin (1-5 g/l) inhibited the growth of sensitive but not resistant cells (Rotman, 1971). Plates were incubated at 27°C. Primaquin-containing agar has to be used within 2 to 3 days.

Crosses. Crosses were performed between compatible haploid strains differing in their nutritional requirements. Zygotes and their diploid progeny were isolated on a minimal medium (containing: Difco yeast nitrogen base, 6.7 g/l; glucose, 2% (w/v); agar, 2% (w/v). The carbon source was either glucose (2%, w/v) (YPG medium) or glycerol (3%, w/v). When only glycerol was available in the medium, the addition of primaquin (1-5 g/l) inhibited the growth of sensitive but not resistant cells (Rotman, 1971). Plates were incubated at 27°C. Primaquin-containing agar has to be used within 2 to 3 days.

Results

Genetic analysis of the resistance to primaquin

To study the segregation of resistance to primaquin, the resistant strain 194-5c/FP-R1 was crossed with various sensitive strains and the phenotypes of the diploid cells formed and that of the haploid progeny after meiosis were examined. Primaquin resistance might be specified by nuclear or mitochondrial genes. The former would be supported by a 2:2 segregation of resistance and sensitivity at meiosis, the latter by the absence of segregation at meiosis and by mitotic segregation during the early divisions of the diploids heterogeneous for the cytoplasmic factor.

In the diploid populations derived from such crosses (some 20 cell generations after zygote formation) two types of cells were usually found: cells resistant to primaquin, and sensitive cells. Subsequent cloning and examination showed that sensitivity was stable. The proportion of sensitive cells in the diploid population varied according to the genotype of the sensitive parent and to the growth conditions of the diploids (Table 2).
**Genetics of a primaquin-resistant yeast**

Table 2. *Segregation of the resistance and sensitivity to primaquin in diploid progeny of the cross between haploid primaquin-resistant and sensitive strains*

<table>
<thead>
<tr>
<th>Haploid sensitive strains crossed with 194-5C-FP-R1</th>
<th>Culture conditions of the diploids</th>
<th>YPG</th>
<th>Sporulation medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP1-1B/514</td>
<td>Percentage diploid sensitive colonies observed</td>
<td>Number colonies observed</td>
<td>Percentage sensitive diploid*</td>
</tr>
<tr>
<td>IL102-8D</td>
<td>0.5</td>
<td>2750</td>
<td>0</td>
</tr>
<tr>
<td>C982-1d</td>
<td>4</td>
<td>2800</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2350</td>
<td>75</td>
</tr>
</tbody>
</table>

* Percentage in total population of resistant diploid after transfer to sporulation medium. At least 1000 colonies examined in each case.

Table 3. *Inheritance of primaquin resistance at meiosis*

<table>
<thead>
<tr>
<th>Random analysis*</th>
<th>Tetrat analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meiosis of resistant diploids</td>
<td>No. of spores</td>
</tr>
<tr>
<td>44</td>
<td>21:23</td>
</tr>
<tr>
<td>Meiosis of sensitive diploids</td>
<td>128</td>
</tr>
</tbody>
</table>

* See Methods.

These observations suggest that resistance and sensitivity phenotypes segregate among diploid cells.

Meiosis was induced in resistant diploid cells (with a poor efficiency – 10%) and gave a 2:2 Mendelian segregation among the haploid products (Table 3). On the other hand, the four meiotic products from sensitive diploids were sensitive to primaquin (Table 3) (in four tetrads examined the Mendelian segregation of known nuclear genes testified to the normal process of meiosis).

Thus crosses between primaquin-sensitive cells and the resistant mutant exhibited three features: a mitotic segregation of the phenotypes among diploid cells, an absence of segregation when sensitive diploids undergo meiosis, and a Mendelian segregation in meiotic products of resistant diploids. Various explanations of these results can be put forward:

Clearly the control of primaquin resistance is not determined by one or several nuclear genes but depends upon the joint effects of a nuclear gene and either some physiological non-genetic process or some cytoplasmic genetic determinant. It is possible that primaquin resistance depends on two genes: one nuclear (pri-r/pri-s), one cytoplasmic (FP-R/FP-S). The occurrence of the two mutated alleles, pri-r and FP-R, is required in a cell to express primaquin resistance. According to this hypothesis three genotypes are conceivable for a sensitive cell: I, pri-s, FP-S; II, pri-s, FP-R; and III, pri-r, FP-S.

* A priori, sensitive cells from our stocks can have any of these three genotypes. But the results of crosses between these sensitive cells and petites (see below) suggest a common genotype (pri-s, FP-S).
Fig. 1. Scheme for the inheritance of primaquin resistance. 

(a) Cross between resistant and sensitive cells; (b) crosses between sensitive haploid progeny. Spore 'A', sensitive spore issued from resistant diploids; spore 'B', sensitive spore issued from sensitive diploids. Abbreviations: (pri-r), (pri-s), resistance and sensitivity to primaquin specified by a nuclear gene; (FP-R), (FP-S), resistance and sensitivity to primaquin specified by a postulated cytoplasmic factor.
### Table 4. Crosses between sensitive haploid progeny of a cross between resistant and sensitive cells

<table>
<thead>
<tr>
<th>Spores ‘A’: genotype II (pri-s, FP-R)*</th>
<th>Spores ‘B’: genotype III (pri-r, FP-S)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>A₂</td>
</tr>
<tr>
<td>Spores ‘B’: genotype III (pri-r, FP-S)</td>
<td>B₁</td>
</tr>
<tr>
<td>B₂₃</td>
<td>+</td>
</tr>
<tr>
<td>B₂₈</td>
<td>+</td>
</tr>
<tr>
<td>Spores ‘B’: genotype I (pri-s, FP-S)</td>
<td>B₂₇</td>
</tr>
<tr>
<td>B₂₃</td>
<td>-</td>
</tr>
<tr>
<td>B₂₈</td>
<td>-</td>
</tr>
</tbody>
</table>

+ , Crosses giving resistant diploids; − , crosses giving only sensitive diploids (no. of cells counted ≥ 10⁶); spores ‘A’, sensitive haploids issued from resistant diploids, cf. Fig. 1; spores ‘B’, sensitive haploids issued from sensitive diploids, cf. Fig. 1. * Subscripts refer to independent spores.

If the resistant mutant, pri-r, FP-R, were crossed with a sensitive strain, pri-s, FP-S, the diploid cells would be heterozygous for the pri nuclear gene and heterogeneous for the cytoplasmic FP factor

\[
\left(\frac{\text{pri-r}}{\text{pri-s}}\right) \text{FP-R/FP-S}
\]

(Fig. 1a). Mitotic segregation between the FP-R and FP-S alleles would give sensitive diploid cells

\[
\left(\frac{\text{pri-r}}{\text{pri-s}}\right) \text{FP-S}
\]

and resistant diploid cells

\[
\left(\frac{\text{pri-r}}{\text{pri-s}}\right) \text{FP-R}
\]

Meiosis of these resistant cells would lead to Mendelian segregation of the pri-r/pri-s gene; the genotypes of the haploid products are pri-r, FP-R for the resistant cells and pri-s, FP-R for the sensitive ones (genotype II). Sensitive diploid cells would give rise through meiosis to sensitive haploid spores but of two different genotypes, I and III, in equal number.

According to this hypothesis, crosses between a haploid sensitive strain of genotype III and a haploid sensitive strain of genotype II would give diploid zygotes

\[
\left(\frac{\text{pri-r}}{\text{pri-s}}\right) \text{FP-R/FP-S}
\]

resistant to primaquin, with a mitotic segregation of FP-R and FP-S. None of the other possible crosses between sensitive strains would give resistant diploid cells.

Crosses of haploid sensitive cells derived from the meiosis of resistant diploids (‘A’ spores in Table 4; genotype II: pri-s, FP-R) with the haploid sensitive cells issued from the meiosis of sensitive diploids (‘B’ spores in Table 4; two possible genotypes: pri-r, FP-S and pri-s, FP-S) should fall into two classes: the cross pri-s, FP-R × pri-r, FP-S should form some diploid cells able to grow in the presence of primaquin, where the genotype of the diploids is

\[
\left(\frac{\text{pri-r}}{\text{pri-s}}\right) \text{FP-R/FP-S}
\]

while the cross pri-s, FP-R × pri-s, FP-S should not give any resistant diploid cells (Fig. 1b).
Table 5. Segregation of resistance and sensitivity to primaquin in diploid progeny of crosses between sensitive haploids*

The results are expressed as percentages of resistant cells among the total diploid cells; the numbers of colonies counted are given in parentheses.

<table>
<thead>
<tr>
<th>Spores 'A': genotype II (pri-s, FP-R)</th>
<th>A₁</th>
<th>A₂</th>
<th>A₃</th>
<th>A₅</th>
<th>A₂₆</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁</td>
<td>100 (475)</td>
<td>10 (592)</td>
<td>100 (537)</td>
<td>40 (499)</td>
<td>6 (558)</td>
</tr>
<tr>
<td>B₂₆</td>
<td>20 (278)</td>
<td>7 (359)</td>
<td>70 (271)</td>
<td>30 (380)</td>
<td>50 (247)</td>
</tr>
</tbody>
</table>

* See nomenclature in Table 4.

Table 6. Inheritance of primaquin resistance through meiosis of the resistant diploid progeny of the cross between sensitive haploids able to complement

<table>
<thead>
<tr>
<th>Crosses*</th>
<th>No. of spores</th>
<th>Resistant/</th>
<th>a/a†</th>
<th>No. of tetrads</th>
<th>Resistant/</th>
<th>a/a†</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₁ × A₁</td>
<td>41</td>
<td>19:22</td>
<td></td>
<td>4</td>
<td>2:2</td>
<td></td>
</tr>
<tr>
<td>B₁ × A₂</td>
<td>39</td>
<td>18:21</td>
<td></td>
<td>4</td>
<td>2:2</td>
<td></td>
</tr>
<tr>
<td>B₁ × A₅</td>
<td>45</td>
<td>22:23</td>
<td></td>
<td>4</td>
<td>2:2</td>
<td></td>
</tr>
</tbody>
</table>

* For nomenclature, see Table 4.
† Inheritance of the cellular mating type as a control of Mendelian segregation.

All possible crosses were made (Table 4). Eight sensitive strains out of 17 sensitive ‘B’ spores examined were able to give primaquin-resistant diploids when crossed with sensitive ‘A’ spores, whereas no crosses between ‘A’ spores or crosses between ‘B’ spores yielded any resistant diploids. These results are consistent with the hypothesis of three different genotypes responsible for primaquin sensitivity.

When complementation occurred in diploids (cross pri-s, FP-R × pri-r, FP-S) mitotic segregation of resistance and sensitivity was observed as in the original crosses (Table 5).

The genotype of these resistant diploids segregants should be

\[
\left( \frac{\text{pri-r}}{\text{pri-s}} \right) \text{FP-R}.
\]

To substantiate this prediction Mendelian segregation of the pri-r/pri-s gene should then appear at meiosis. Results shown in Table 6 are in agreement with this.

All these results are consistent with resistance to primaquin being controlled by the interaction of a nuclear gene and a cytoplasmic genetic determinant; they do not support physiological non-genetic control of the expression of the pri nuclear gene.

The identity of the cytoplasmic factor

As resistance and sensitivity to primaquin are revealed only when functional mitochondria are necessary for growth, the resistance factor could be located on mitochondrial DNA.
Table 7. Transmission of primaquin resistance by petites issued from the resistant grande mutant

<table>
<thead>
<tr>
<th>p- Strains crossed with p+ sensitive strain (DP1-1B/S14)</th>
<th>Percentage resistant cells among the diploid grande colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Spontaneous petites</td>
<td>0</td>
</tr>
<tr>
<td>5 Petites (ethidium bromide)</td>
<td>100</td>
</tr>
<tr>
<td>4 Petites (acriflavin)</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 8. Comparative segregation of mitochondrial genes (ERY-R)/(ERY-S) and (CAM-R)/(CAM-S), and FP-R/FP-S cytoplasmic factor in the cross between haploid primaquin-sensitive and resistant cells

<table>
<thead>
<tr>
<th>Cross of 194-5c/FP-R by dp1-1b/s14 [w+ (CAM-S) (ERY-S) FP-R]</th>
<th>[w+ (CAM-S) (ERY-R)]s14 FP-S</th>
<th>100 60</th>
<th>100 30</th>
<th>100 30</th>
<th>100 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL102-8d [w- (CAM-S) (ERY-R)]221 FP-S</td>
<td></td>
<td>4000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL8-8c [w+ (CAM-R)]321 (ERY-R)s14 FP-S</td>
<td></td>
<td>3500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control cross of 194-5c by IL8-8c [w- (CAM-S) (ERY-R) FP-S]</td>
<td></td>
<td>1500</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Induction of the petite mutation which alters the mitochondrial DNA sequence can be used to decide whether a cytoplasmic factor is a mitochondrial gene. In principle, two main situations can be encountered with respect to a mitochondrial gene when the cells have been made petite: either the sequence of this gene is not altered and the petite (p-) is able to transmit it in a cross with a grande (p+), or the sequence of this gene is grossly altered or lost and the petite is no longer able to transmit any information at this locus (Bolotin et al. 1971).

A few independent petites obtained from the mutant resistant to primaquin were studied; nine of them (induced by ethidium bromide or acriflavin) had retained the ability to transmit resistance when crossed with a sensitive grande, while three (spontaneous petites) had lost this ability (Table 7). No attempt was made to determine if the cytoplasmic factor was lost in grande cells.

These results are consistent with the FP factor being located on mitochondrial DNA although they do not constitute proof.

As shown by Goldring et al. (1970) and by Nagley & Linnane (1972), prolonged treatment by ethidium bromide induces the loss of mitochondrial DNA. That nine petites induced by ethidium bromide or acriflavin had kept the ability to transmit primaquin resistance is therefore rather puzzling. If this were confirmed for all petites inducible in the primaquin-resistant mutant, then the FP factor could not be located on mitochondrial DNA.

The characterization of the cytoplasmic FP factor as a mitochondrial gene would be substantiated if genetic linkage were found between this factor and the well-known mitochondrial genes that control resistance to chloramphenicol and erythromycin, (CAM-R)/
Table 9. Comparative mitotic segregation of mitochondrial genes (ERY-S)/(ERY-R) and FP-S/FP-R cytoplasmic factor in the diploid progeny of crosses between haploid parents (ERY-R) and (ERY-S), both primaquin sensitive but able to complement

<table>
<thead>
<tr>
<th>Crosses*</th>
<th>Percentage primaquin-resistant cells in diploid colonies</th>
<th>Percentage primaquin-resistant cells in diploid colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ERY-R) in total diploid colonies</td>
<td>(ERY-R) in total diploid colonies</td>
</tr>
<tr>
<td></td>
<td>(ERY-S) in total diploid colonies</td>
<td>(ERY-S) in total diploid colonies</td>
</tr>
<tr>
<td>B₁×A₁</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td>B₁×A₂</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>B₁×A₃</td>
<td>36</td>
<td>100</td>
</tr>
<tr>
<td>B₂×A₁</td>
<td>40</td>
<td>20</td>
</tr>
</tbody>
</table>

* Haploid parents as in Table 4: strains ‘B’ (ERY-S), and strain ‘A’ (ERY-R).

Table 10. Comparative inheritance of mitochondrial genes (ERY-R)/(ERY-S) and FP-S/FP-R factor through meiosis of the diploid progeny (erythromycin, primaquin resistant) of the cross between haploid parents (ERY-R) x (ERY-S)

<table>
<thead>
<tr>
<th>Crosses*</th>
<th>No. of spores</th>
<th>(ERY-R):(ERY-S)</th>
<th>Primaquin resistant/ sensitive</th>
<th>No. of tetrads</th>
<th>(ERY-R):(ERY-S)</th>
<th>Primaquin resistant/ sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁×B₁</td>
<td>39</td>
<td>39:00</td>
<td>18:21</td>
<td>4</td>
<td>4:0</td>
<td>2:2</td>
</tr>
<tr>
<td>A₂×B₁</td>
<td>45</td>
<td>45:0</td>
<td>22:23</td>
<td>4</td>
<td>4:0</td>
<td>2:2</td>
</tr>
</tbody>
</table>

* For nomenclature, see Table 9.

(CAM-S) and (ERY-R)/(ERY-S). In crosses between the mutant resistant to primaquin but sensitive to erythromycin and chloramphenicol and strains resistant to one or both of these antibiotics, mitotic segregation of the mitochondrial genes occurs in the diploid progeny, independent of the FP factor (Tables 8 and 9).

Furthermore, as can be seen in the last two lines of Table 8, primaquin resistance does not affect the recombination between the CAM and ERY mitochondrial genes. In the homosexual crosses (ω+ x ω+), the ratio of recombinants,

\[
\text{CAM-R ERY-S} \\
\text{CAM-S ERY-R'}
\]

is similar among primaquin-resistant diploids and among sensitive diploids, being 0.51 and 0.48 respectively. On the other hand, when meiosis is induced in pure diploids no segregation is observed for the mitochondrial gene (ERY-R)/(ERY-S), although primaquin resistance and sensitivity segregate (Table 10). The inheritance of the mitochondrial genes (CAM) and (ERY) is independent of that of the FP cytoplasmic factor.

However, the fact that different petites transmit primaquin resistance in different ways argues against a purely nuclear genetic control and against the involvement of a physiological process. Moreover, the absence of primaquin-resistant diploids derived from a cross between a sensitive grande parent and a spontaneous petite originated from the resistant mutant, shows that the sensitive grande does not carry the cytoplasmic FP factor and substantiates the genotype postulated for the original sensitive cell, namely pri-s, FP-S.
DISCUSSION

The experimental results support dual genetic control of primaquin resistance. The expression of this phenotype is jointly controlled by a nuclear gene (pri) and a cytoplasmic (possibly mitochondrial) factor, FP. (The occurrence of two allelic forms of the FP factor has been postulated, although the experimental results are also consistent with loss of the FP-R determinant.)

The occurrence of the cytoplasmic factor is of interest. Two genetic criteria have been used to establish mitochondrial inheritance: (i) segregation at mitosis in heterogeneous yeasts and absence of segregation at mitosis or meiosis of pure diploid yeasts, and (ii) linkage with known mitochondrial genes and modifications of mitochondrial DNA (petites).

In the case of the FP factor, the process of mitotic segregation in diploid yeasts varies according to the growth conditions and to the genotype of the sensitive haploid parents. Great variability of mitotic segregation of the FP factor is observed in crosses between haploid sensitive strains of various genotypes derived from meiosis of diploid yeasts (Table 5). The influence of nuclear genotype upon the selective transmission and recombination of mitochondrial genes has been reported in yeast by Howell et al. (1973), in Paramecium aurelia (Sainsard, 1973) and in other organisms (Tilney-Basset, 1973). On the other hand the importance of the physiology of the cell on the mitotic segregation of the petite phenotype in diploid cells (suppressiveness) was emphasized by Ephrussi, Jakob & Granschamp (1966).

The only evidence for the mitochondrial location of the FP factor is given by the different results obtained with petites, though the number of petites examined was too small to draw a firm conclusion from the observed loss of primaquin resistance in 3 of them. However, if one can show that any petite induced by ethidium bromide, even when all the mitochondrial DNA is lost (Nagley & Linnane, 1972; Perlman & Mahler, 1971), is still able to transmit primaquin resistance, the location of the FP factor will have to be re-examined. Such situations have already been described for the killer character (Al-Aidros, Somers & Bussey, 1973) and the utilization of ureidosuccinic acid (Lacroute, 1971).

If the FP factor is a mitochondrial gene, its complete genetic independence from the (CAM) and (ERY) genes can be taken as an argument against the location of the primaquin factor on the same DNA molecule as the (CAM) and (ERY) genes (Wolf, Dujon & Slonimski, 1973).

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REFERENCES


