
**Correction.** In the article entitled “Organelle Mutations and Their Expression in Chlamydomonas reinhardi”, by Stefan J. Surzycki and Nicholas W. Gillham, which appeared in the June 1971 issue of *Proc. Nat. Acad. Sci. USA*, 68, 1301–1306, the following changes should be made. On p. 1303, Table 2, footnote c, should read: “Streptomycin-dependent”. On p. 1304, left-hand column, beginning 14 lines from bottom, should read: “should exhibit a wild-type phenotype . . .”, not “should inhibit a wild-type phenotype . . .”. On p. 1305, Table 4, column head should read: “Location of mutation (Chlp./Mit./Nuc.)”, not “Location of mutation (Chlp./Mit./Nuc.)”.

Correction. In the article “Photo-Affinity Labels for Adenosine 3’:5’-Cyclic Monophosphate”, by D. J. Brunswick and Barry S. Cooperman, which appeared in the August 1971 issue of *Proc. Nat. Acad. Sci. USA*, 68, 1801–1804, in the structure shown on p. 1801, the fourth (IV) derivative of cAMP had a subscript 2 omitted and should read: IV R₂ = R₄ = -CO(CH₃)₂CH₃.
**Organelle Mutations and Their Expression in Chlamydomonas reinhardi**

*(protein-synthesizing systems/antibiotics)*

STEFAN J. SURZYCKI AND NICHOLAS W. GILLHAM

Department of Botany, University of Iowa, Iowa City, Iowa 52240, and Department of Zoology, Duke University, Durham, N.C. 27706

*Doomunicated by M. M. Rhoades, March 15, 1971*

**ABSTRACT** A new method for classifying Mendelian and non-Mendelian antibiotic-resistant mutants in Chlamydomonas reinhardi is proposed. This classification takes into account the cellular site of expression of the resistant phenotype, the location of genetic information for resistance in the cell, and the pattern of inheritance of the different mutants. The classification is consistent with the idea that C. reinhardi contains three protein-synthesizing systems, two of which are sensitive to antibiotic-resistant systems and located in the chloroplast and mitochondria, respectively. The information for resistance can be coded either in the chloroplast, the mitochondria, or the nucleus. A new experimental approach to the problem of cellular localization of mutations to antibiotic resistance is prescribed and used for the classification of known resistant mutations.

It is now possible to study mutations which affect the mitochondrion and which may, in fact, be located in this organelle in Saccharomyces cerevisiae (1). There is reason to believe that non-Mendelian, respiratory-deficient and antibiotic-resistant mutations in S. cerevisiae arise in mitochondrial DNA (2). From the time of Carl Correns, mutations affecting chloroplast phenotype and exhibiting non-Mendelian inheritance have been known in higher plants. They have been assumed to be mutations of chloroplast rather than nuclear genes. However, as pointed out by Rhoades (3, 4), these mutations could as well be mutations of the mitochondria or some other extranuclear genome that affects plastid phenotype. These possibilities assume more significance in view of what is known today. There is good reason to believe that both the chloroplasts and mitochondria of green plants contain genetic information and their own distinctive protein-synthesizing systems (5).

The unicellular, haploid, heterothallic green alga Chlamydomonas reinhardi is well suited for the study of organelle inheritance in green plants. It contains only a single chloroplast and has well-defined Mendelian and non-Mendelian genetic systems (6). C. reinhardi is an obligate aerobe which can be grown in the light with carbon dioxide (CO₂) as the sole carbon source (*phototrophic growth*), in the dark with sodium acetate as the carbon source (*heterotrophic growth*), or in the light in the presence of both acetate and CO₂ (*mixotrophic growth*). Numerous Mendelian mutations are known in C. reinhardi which cannot grow phototrophically because they are unable to carry out one or more of the reactions associated with photosynthesis (7). On the other hand, heterotrophic growth and mixotrophic growth can be obtained without these functions. Since C. reinhardi is an obligate aerobe, heterotrophic growth cannot be obtained without those mitochondrial functions associated with respiration, but it is not altogether clear whether this is also true of mixotrophic and phototrophic conditions. It may be that photophosphorylation provides enough energy to make growth possible without mitochondrial respiratory functions. A suggestion that this may be the case is the observation (unpublished data) that obligate photoautotrophic mutants are readily isolated in C. reinhardi.

Both Mendelian and non-Mendelian mutations are known in C. reinhardi which make the alga resistant to or dependent upon antibiotics that inhibit protein synthesis by 70S ribosomes in bacteria (6). The non-Mendelian mutations show uniparental inheritance, and are transmitted in the majority of zygotes only by the mating type plus (mt⁺) parent (6). These mutations have been attributed to the chloroplast by Sager and Ramains (8) and to the mitochondria by Schimmer and Arnold (9, 10).

In higher plants maternal inheritance of the type seen in C. reinhardi is customarily attributed to the unequal contribution of cytoplasm (and consequently, organelles) to the fertilized egg by the male and female parents. However, C. reinhardi is an isogamous species and both parents contribute an equal amount of cytoplasm and a single chloroplast to the zygote, after which both chloroplasts and nuclei fuse. These observations rule out the classical explanation used in higher plants, but an alternative possibility is that organelle genetic information from one of the parents is destroyed in the zygote. If the destruction of chloroplast or mitochondrial satellite DNA could be correlated with the behavior of the uniparental genes in crosses, the localization of these genes would be explicitly proved. The results of experiments by Chiang (11) indicate the lack of destruction of any DNA contributed by either parent in the zygote of C. reinhardi. Thus, the problem of localization of non-Mendelian genes remains open despite the fact that some of the informational content of chloroplast DNA in this organism is known (12–14).

In this paper a classification of Mendelian and non-Mendelian mutations to antibiotic resistance in C. reinhardi is proposed which consists of three parts. First, the previously defined growth conditions are used as guides to the sites of phenotypic expression of the mutations within the cell. Second, a new experimental approach is used to localize these genes within the cell which takes advantage of the fact that the antibiotic rifampicin is a specific inhibitor of chloroplast DNA transcription (12). Third, the mode of
segregation of the genes is taken into account. The lack of certain types of mutations on the basis of this classification is discussed and methods for selecting these mutants are proposed.

MATERIALS AND METHODS

Organism and culture conditions

Strain 137c of *C. reinhardtii* wild-type and mutant strains derived from it were used in experiments. Tris-minimal (TM) and Tris-acetate–phosphate (TAP) media used in experiments were buffered with Tris-HCl at pH 7.0 and prepared as described previously (15). Cells were grown phototrophically on TM medium at a light intensity between 4000 and 5000 lux, mixotrophically on TAP medium at the same light intensity, and heterotrophically in dark on TAP medium. Both liquid and solid media were used.

The streptomycin-resistant mutants *sr*-2-80 and *sr*-1-6 (16), the spectinomycin-resistant mutants *spA*-1 and *spA*-2, and the kanamycin-resistant mutant, *kan*-1, were isolated as spontaneous mutations under mixotrophic conditions on medium containing the appropriate drug. The *spA*-1 and *spA*-2 mutants were isolated from TAP medium containing 50 and 100 μg/ml of spectinomycin, respectively. The mutant *spA*-1 is resistant to 50 μg/ml of spectinomycin and *spA*-2 to 100 μg/ml of the antibiotic when grown under heterotrophic or mixotrophic conditions, but both strains are as sensitive to spectinomycin as wild-type when grown phototrophically.

All other mutations were induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and selected under mixotrophic conditions on plates supplemented with antibiotic (17). The streptomycin-dependent mutant, *sd*-3-18, is not only resistant to 500 μg/ml of streptomycin, but also requires at least 20 μg/ml of antibiotic for growth. The erythromycin-resistant mutants, *ery*-12, *ery*-14, *ery*-21 and *ery*-22, are all resistant to at least 100 μg/ml of erythromycin under mixotrophic conditions. *Spr*-1-27 is resistant to at least 100 μg/ml of spectinomycin under mixotrophic and phototrophic conditions.

Treatment with antibiotics

For the preparation of rifampicin-containing media, the antibiotic was dissolved in sterile 0.01 M phosphate buffer, pH 4.5, at a concentration of 100 mg/ml and stirred overnight in the dark. Enough antibiotic-containing solution was added to the appropriate medium to give a final concentration of 250 μg/ml. Rifampicin was added after the medium had been autoclaved and allowed to cool to about 50°C. Other antibiotics were added in the same way, but without prior overnight stirring or special attention to light or dark conditions. To test the cells for growth in the presence of rifampicin and other antibiotics, we first grew the appropriate strains for 3 days under mixotrophic conditions on agar plates and afterwards suspended them in TM medium to a concentration of about 0.5 × 10^6 cells/ml. A 0.1-ml aliquot of this suspension was streaked onto appropriate testing medium. When rifampicin was present in the testing medium, the plates were incubated under heterotrophic conditions because of the light sensitivity of rifampicin and were scored for the presence or absence of growth after 2 weeks. In some experiments the cells were grown heterotrophically in liquid medium containing rifampicin for 5 days (about six generations) before being used. The scoring for growth on media other than rifampicin-containing media was carried out after 1 week for phototrophically and mixotrophically grown cells and after 2 weeks for heterotrophically grown cells.

RESULTS

Experiments with wild-type cells

The sensitivity of wild-type cells to different concentrations of antibiotics inhibiting protein synthesis on bacterial-type (70S) ribosomes was tested under phototrophic, mixotrophic, and heterotrophic growth conditions. Among the antibiotics tested, only spectinomycin had a differential effect (Table 1). Cells grown phototrophically in the presence of spectinomycin were more sensitive to the antibiotic than cells grown heterotrophically or mixotrophically. These results can be interpreted in one of the following ways. (a) The cytoplasmic (80S) ribosomes of *C. reinhardtii* are sensitive to all of the antibiotics used except spectinomycin. (b) All the antibiotics except spectinomycin affect some cell function necessary for cell survival other than protein synthesis. (c) The cells of *C. reinhardtii* contain only one protein-synthesizing system sensitive to antibiotics that is located in the mitochondria. The mitochondrial respiratory functions are indispensable under all growth conditions. (d) All the antibiotics except spectinomycin affect protein synthesis on organelle ribosomes, and *C. reinhardtii* wild-type cells possess two such protein-synthesizing systems. One of these is located in the chloroplast and the other in the mitochondria. Both systems are equally affected by all the antibiotics except spectinomycin, which has a preferential effect on the chloroplast system.

Interpretations (a–c) can be eliminated for chloramphenicol and spectinomycin for the following reasons. The 80S ribosomes of *C. reinhardtii* are not sensitive to either antibiotic, whereas 70S chloroplast ribosomes are (18, 19). The primary effects of chloramphenicol and spectinomycin in *C. reinhardtii* appear to be on protein synthesis (12, 18). In the case of streptomycin it is known that 80S ribosomes are insensitive to the effects of the antibiotic in an *in vitro* protein-synthesizing system (6). Also, it has recently been shown that mutants resistant to streptomycin have altered chloroplast but normal 80S ribosomes (20).

Experiments with synchronous cultures of *C. reinhardtii* showed that streptomycin, erythromycin, and chloramphenicol prevent the usual increase in photosynthetic capacity.
and greatly reduced respiratory capacity when added for a period of less than one cell cycle (13, 14). All the above facts are consistent with the interpretation (d), namely that chloramphenicol, spectinomycin, and streptomycin affect protein synthesis involving organelle (chloroplast and mitochondrial) ribosomes.

**Experiments with antibiotic-resistant mutants**

If wild-type cells of *C. reinhardtii* contain protein-synthesizing systems in the chloroplast and the mitochondria sensitive to the same set of antibiotics, three classes of antibiotic-resistant mutants are theoretically possible. Class I mutations would make the chloroplast protein-synthesizing system resistant to antibiotics. Class II mutations would make the mitochondrial protein-synthesizing system resistant to antibiotics. Class III mutations would make both systems resistant to antibiotics. Although all three classes may, in fact, exist, they can only be detected if phototrophic and mixotrophic growth are obtained without respiratory function and heterotrophic and mixotrophic growth are obtained without photosynthetic function. If this is the case, class I mutants having a resistant chloroplast would be recognized by the fact that they would be unable to grow in the presence of antibiotic under heterotrophic conditions. Class II mutants having resistant mitochondria would be unable to grow phototrophically in the presence of antibiotic. Class III mutants would grow under all three sets of growth conditions in the presence of antibiotic.

A number of antibiotic-resistant mutants were tested for resistance to antibiotic under all three conditions of growth (Table 2). All the mutants tested, except the spectinomycin-resistant mutants *spA-I* and *spA-2*, were resistant under all growth conditions, which indicates that they belong to class III and that both organelle protein-synthesizing systems were protected against the antibiotics to which the mutants are resistant. The *spA-I* and *spA-2* mutants were resistant to spectinomycin only under heterotrophic and mixotrophic growth conditions, but were as sensitive as wild-type to spectinomycin under phototrophic growth conditions. These mutations belong to class II and should have a resistant mitochondrial protein-synthesizing system but a sensitive chloroplast protein-synthesizing system. Class I mutants, which would have a resistant chloroplast protein-synthesizing system and a sensitive mitochondrial system, have not been found.

Why should class III mutations predominate? The simplest explanation is that a class III mutant changes the permeability of resistant cells to a given drug. Such mutations would make organelle protein synthesis appear resistant to antibiotic by virtue of lowering the internal concentration of antibiotic in the cell. This explanation does not apply to streptomycin-resistant mutants since (a) Sager has shown (21) that these mutants do not differ from wild-type cells in their uptake of the antibiotic; (b) the demonstration of phenotypical changes in chloroplast ribosomes in streptomycin-resistant and -dependent strains indicates that these mutations may alter chloroplast ribosome structure (20); and (c) it is difficult to see how streptomycin dependence can be explained in terms of altered cell permeability (20). Also, this explanation cannot apply to the spectinomycin-resistant mutant, *spr-I-27*, which not only has phenotypically altered chloroplast ribosomes but, as Burton has shown (19), these ribosomes do not bind spectinomycin whereas wild-type ribosomes do. The above data suggest that, in some cases of antibiotic resistance, ribosomes of the organelle become resistant as the result of mutation. However, it should be pointed out that the hypothesis of two antibiotic-sensitive organelle protein-synthesizing systems does not require that mutations resulting in resistance to a given antibiotic affect the ribosomes of the organelle. It requires only that, as a result of a mutation, the protein-synthesizing system of the organelle is in some way protected against the action of the antibiotic.

If reduced permeability to antibiotics cannot explain the high frequency of class III mutants, is there an alternative possibility? The most reasonable explanation seems to be that these mutants are the ones expected to grow most rapidly and form the biggest colonies under the mixotrophic conditions employed for the isolation of antibiotic-resistant mutants. The growth rate of wild-type cells under mixotrophic conditions is about twice that of phototrophically or heterotrophically grown cells. Mutants with unimpaired photosynthetic and respiratory functions would be expected to grow more rapidly on antibiotic-containing media under mixotrophic conditions than mutants in which one system or the other is impaired. Direct support for this hypothesis comes from the discovery of the two class II mutants, *spA-I* and *spA-2*. These mutants were isolated as the result of deliberate selection for slow-growing mutants.

**Experiments with rifampicin**

The site of phenotypic expression of an antibiotic-resistant mutation tells nothing about the location of the genes coding the information for the resistant phenotype. The data

---

**Table 2. Resistance level of antibiotic-resistant mutants under different conditions of growth**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Phototrophic</th>
<th>Heterotrophic</th>
<th>Mixotrophic</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mendelian</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sr-1-5 (+)*</td>
<td>100 (20)*</td>
<td>100 (20)*</td>
<td>100 (20)*</td>
</tr>
<tr>
<td>sr-1-5 (−)*</td>
<td>100 (10)</td>
<td>100 (10)</td>
<td>100 (10)</td>
</tr>
<tr>
<td>ery-12b</td>
<td>10 (10)</td>
<td>50 (30)</td>
<td>50 (30)</td>
</tr>
<tr>
<td>ery-21b</td>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>Non-Mendelian</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sr-2-60 (+)*</td>
<td>500 (20)</td>
<td>500 (20)</td>
<td>500 (20)</td>
</tr>
<tr>
<td>sr-2-60 (−)*</td>
<td>100 (50)</td>
<td>100 (50)</td>
<td>100 (50)</td>
</tr>
<tr>
<td>kan-1*</td>
<td>100 (10)</td>
<td>100 (10)</td>
<td>100 (10)</td>
</tr>
<tr>
<td>ery-2y (−)*</td>
<td>100 (10)</td>
<td>100 (30)</td>
<td>100 (30)</td>
</tr>
<tr>
<td>spr-1-27 (+)*</td>
<td>10 (500)†</td>
<td>10 (500)†</td>
<td>10 (500)†</td>
</tr>
<tr>
<td>spr-1-27 (−)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sl-3-19*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


† In parentheses, resistance of wild-type strain under the same testing conditions (see Table 1).
presented suggest that a Chlamydomonas cell contains protein-synthesizing systems in the chloroplast and mitochondria both of which are sensitive to the same set of antibiotics. The genes coding for resistance to these antibiotics could be located in the nucleus, the chloroplast, the mitochondria, or any combination of two or perhaps all three organelles.

The cellular location of a mutation cannot be determined on the basis of the pattern of genetic segregation since little is known about the means and mechanisms of distribution of "nunnuclear" organelle DNA into daughter cells. It is obvious that any chromosomal gene must exhibit a Mendelian pattern of inheritance, but any gene that exhibits a Mendelian pattern of inheritance is not necessarily located in a nuclear chromosome (7). The Mendelian segregation of characters cannot be taken a priori as evidence for their nuclear location.

The chloroplast localization of information for resistance can be established by the use of rifampicin, which is a specific inhibitor of chloroplast DNA transcription (12). This antibiotic is lethal to C. reinhardtii only under phototrophic conditions, which shows that chloroplast DNA transcription is dispensable when cells are grown heterotrophically or mixotrophically.

If expression of a resistant mutation requires transcription of chloroplast DNA, the resistant phenotype of the mutant should be lost in the presence of rifampicin and the mutant should exhibit a wild-type phenotype. However, when transcription of chloroplast DNA is not required for the expression of the resistant phenotype, the mutant would continue to exhibit the resistant phenotype in the presence of rifampicin.

The mutants of class III, however, present a special problem. If there is more than one source of information for resistance in the cells, these mutants could be double or triple mutants or they could be single mutants whose product is preferentially used to make both sensitive protein-synthesizing systems resistant to the effects of a given antibiotic. In the former case the resistance mutations would occur in both the mitochondria and chloroplasts and there would be no need to postulate that gene products from the chloroplast were used to make the mitochondrial protein-synthesizing system resistant to antibiotics or vice versa. In the latter case the mutation must occur in either the mitochondria, the chloroplasts, or the nucleus, and it would be necessary to postulate that resistant gene products from one system were used to protect both systems against antibiotics.

The antibiotic-resistant mutants were tested for resistance in the presence of rifampicin (Table 3). Mutants exhibiting Mendelian inheritance and belonging to class III (sr-1, ery-11, ery-12, ery-14, ery-21) are resistant when grown heterotrophically in the presence of rifampicin, which means that they are either nuclear or mitochondrial in location.

The class III uniparental mutations can be divided into two groups on the basis of their response to antibiotic in the presence of rifampicin (Table 3). The first group contains a single mutant, spr-1-27, which is resistant to spectinomycin when grown heterotrophically in the presence of rifampicin. This mutant has chloroplast ribosomes resistant to spectinomycin (19).

The results with mutant spr-1-27 can be explained in at least three different ways. First, the mutation may be of mitochondrial origin and so transcription of the information for resistance is not inhibited by rifampicin. If this explanation is correct, it means that mitochondrial genetic information or gene products can be transmitted to the chloroplast. Second, spr-1-27 may be a double mutant in which both the chloroplast and the mitochondria or nucleus contain mutations to resistance. In this case heterotrophic growth of the mutant in the presence of rifampicin is not lethal because mitochondrial protein synthesis remains resistant to the effects of spectinomycin and chloroplast protein synthesis is, of course, dispensable. Third, the resistant phenotype of spr-1-27 may be a consequence of a mutation resulting in the lack of a peptide chain specified by the wild-type allele. In this case transcription of the gene is not necessary for the expression of a resistant phenotype, and the presence of rifampicin does not affect expression of resistance. A clear distinction between these three possibilities is important: (a) If explanation three is eliminated, one would be forced to conclude that uniparental genes in C. reinhardtii belong to at least two independent genetic systems, one of which is rifampicin-sensitive and the other of which is not. (b) If explanation one proves to be correct, it means that mitochondrial genetic information can be used to make chloroplast ribosomes resistant to the effects of spectinomycin. Explanation three is readily open to experimental test, but a distinction between explanations one and two is a far more difficult task at the moment.

The remaining uniparental mutations to antibiotic resistance and dependence tested to date (ery-2y, sr-2-60, sr-3-18, kan-1) belong to the second group. These mutants are sensitive to antibiotics when grown heterotrophically in the presence of rifampicin but are resistant under these conditions in the absence of rifampicin (Table 3). According to our hypothesis, the information for resistance in these mutants resides in chloroplast DNA. In the absence of rifampicin, resistant information is used preferentially to protect both the chloroplast and mitochondrial protein-synthesizing systems, but in the presence of rifampicin, sensitive information must be used for the mitochondrial system since rifampicin blocks the production of resistant information.

### Table 3. Effect of rifampicin on antibiotic-resistant mutants

<table>
<thead>
<tr>
<th>Class</th>
<th>Strain and segregation</th>
<th>Resistance to antibiotic in presence of rifampicin (250 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>Mendelian</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sr-1-5 (+)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>sr-1-5 (-)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>ery-14</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>ery-21</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>ery-12</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Non-Mendelian</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ery-2y</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>sr-2-60 (+)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>sr-2-60 (-)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>kan-1</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>spr-1-27 (+)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>spr-1-27 (-)</td>
<td>Yes</td>
</tr>
<tr>
<td>II</td>
<td>Mendelian</td>
<td></td>
</tr>
<tr>
<td></td>
<td>spa-1</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>spa-2</td>
<td>No</td>
</tr>
</tbody>
</table>
It follows from this hypothesis that one should be able to convert phenotypically resistant cells to sensitive cells by prolonged growth in the presence of rifampicin alone. During this period the mutant cells would be forced to use the sensitive source of information in constructing the mitochondrial protein-synthesizing system. Upon transfer to antibiotic-containing medium lacking rifampicin, the treated cells should remain phenotypically sensitive to antibiotic despite the fact that they carry a mutation to resistance in the chloroplast, for there will be a delay in the expression of this mutation. This prediction has been confirmed in transfer experiments with sr-2-60, sd-3-18, and ery-2y, all of which lose their resistance to antibiotics after five generations of growth in the presence of rifampicin alone.

The class II, Mendelian, spectinomycin-resistant mutants, spA-1 and spA-2, appear to be located in the chloroplast because they lose their resistance to spectinomycin in the presence of rifampicin. However, they have chloroplast ribosomes that are sensitive to spectinomycin, since these mutants are unable to grow phototrophically in the presence of spectinomycin and their chloroplast ribosomes bind the antibiotic (19). These results suggest that the spA-1 and spA-2 mutations may affect a component of the mitochondria, other than ribosomes, that protects mitochondrial protein synthesis from the effect of the antibiotic. This component appears to be coded by chloroplast DNA.

DISCUSSION

The general hypothesis presented in this paper assumes that cells of Chlamydomonas reinhardtii contain three protein-synthesizing systems. One of these is located in the cytoplasm and is resistant to antibiotics that affect protein synthesis by bacterial ribosomes. The other two, located respectively in the chloroplast and the mitochondria, are sensitive to these antibiotics. Direct evidence for this hypothesis has been cited for the chloroplast and cytoplasmic systems and indirect evidence consistent with the hypothesis has been presented for mitochondria in this paper. If we take into account both the hypothesis and the experiments presented here, the antibiotic-dependent and -resistant mutants of Chlamydomonas reinhardtii can be classified by use of the following criteria: (a) site of phenotypic expression (chloroplast, mitochondrial, or both); (b) site of information for resistance (chloroplast versus nuclear or mitochondrial); and (c) mode of genetic segregation (Mendelian or uniparental). The results of such a classification are presented in Table 4.

Examination of Table 4 and analysis of the results suggests that there may be an exchange of information between chloroplasts and mitochondria on the phenotypic level. If there were not this exchange, class IIA and IIIB mutants, which are probably of chloroplast origin but also affect the mitochondria, should never be obtained. Whether such an exchange can occur in the reverse direction is less certain, for the behavior of the sole existing class IID mutant, spr-1-27, is open to several interpretations which have already been discussed. Finally, Mendelian mutations, such as sr-1, that belong to class IIC appear to make both organelles resistant at the phenotypic level.

The fact that rifampicin causes the loss of antibiotic resistance in most of the uniparental mutants (class IIIB) implies that they are located in the chloroplast. However, this correlation is not absolute since the mutant spr-1-27, which belongs to class IID, is resistant to spectinomycin in the presence of rifampicin, although it is uniparentally inherited. If spr-1-27 is, indeed, a single gene mutation, one is forced to conclude that uniparental mutations belong to at least two independent genetic systems, one of which is located in the chloroplast and the other of which is not. On the other hand, uniparental inheritance may not be characteristic of all chloroplast genes since the class IIA strains, spA-1 and spA-2, show a loss of antibiotic resistance in the presence of rifampicin, but segregate in a Mendelian fashion in reciprocal crosses. The important point is that in our present state of knowledge we must regard pattern of inheritance and loss of resistance in the presence of rifampicin as equally valid operational criteria for establishing genotypic
homogeneity of a given class of mutants. The lack of concordance between the two systems of classification indicates that one or the other of them is not absolutely specific.

We thank Drs. J. Boynton, U. Goodenough, R. P. Levine, J. R. Preer, Jr., and M. M. Rhoades for reading and commenting on various drafts of this paper, and Dr. George Whitfield of the Upjohn Co., Kalamazoo, Mich. for the generous gifts of spectinomycin sulfate, erythromycin, lincomycin-HCl, and pactamycin. Paromomycin sulfate was a gift from Parke Davis and Co., Detroit, Mich.

The work was supported by a grant to R. P. Levine from the National Science Foundation (GB 18666) and by a grant to Drs. J. Boynton and N. W. Gillham from the National Science Foundation (GB 22769). S. J. Surzycki was a Maria Moors Cabot Foundation Fellow at Harvard University.