The *ery*-M2 Group of *Chlamydomonas reinhardtii*: Cold-sensitive, Erythromycin-resistant Mutants Deficient in Chloroplast Ribosomes

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Erythromycin-resistant mutants mapping to the Mendelian *ery*-M2 locus of *Chlamydomonas reinhardtii* were cold-sensitive for growth at 15 °C. The phenotypes erythromycin-resistance and cold-sensitivity could not be separated in crosses.

Mutants at the *ery*-M2 locus were previously found to have chloroplast ribosomes with altered [¹⁴C]erythromycin binding capacity (Mets & Bogorad, 1971). When grown at 25 °C, haploid strains containing *ery*-M2 alleles alone or in combination with other *ery*^r loci had fewer chloroplast ribosomes than the wild type. A further reduction occurred when *ery*-M2 strains were shifted to 15 °C. Diploids containing both *ery*-M2 and its wild-type allele contained normal amounts of chloroplast ribosomes at 25 and 15 °C, and were cold-resistant and antibiotic-sensitive.

The inability of chloroplast ribosome-deficient *ery*-M2 strains to grow at 15 °C in the presence of a reduced carbon source indicates that chloroplast protein synthesis is essential for cellular growth even when photosynthesis is not essential.

INTRODUCTION

Mutants with alterations in the level of chloroplast ribosomes can be used to evaluate the importance of these ribosomes to cellular activities. A number of *Chlamydomonas* mutants with reduced numbers of chloroplast ribosomes are known (Boynton, Gillham & Chabot, 1974; Goodenough *et al.*, 1971; Harris, Boynton & Gillham, 1974). These chloroplast ribosome-deficient mutants were selected for a deficiency in photosynthesis expressed as slow growth in the absence of a reduced carbon source (acetate). A type of mutant which has been useful in studying bacterial ribosomes (Davies & Nomura, 1972; Flaks *et al.*, 1966) is the temperature-sensitive ribosome assembly-defective mutant.

Mets & Bogorad (1972) had previously found that erythromycin-resistant *ery*-M2 *Chlamydomonas reinhardtii* mutants had reduced amounts of chloroplast ribosomes when grown at the usual culture temperature of 25 °C. Chloroplast ribosomes from *ery*-M2 mutants have altered [¹⁴C]erythromycin binding (Mets & Bogorad, 1971). There is evidence that a basic, low molecular weight protein is altered in the large chloroplast subunit of an *ery*-M2 mutant (Mets & Bogorad, 1972).

Any alteration in a ribosomal protein has the potential of affecting ribosome assembly and/or stability. A number of bacterial ribosome mutants have multiple phenotypes, e.g. temperature-sensitivity and antibiotic-resistance (Nashimoto *et al.*, 1971; Pardo & Rosset, 1974; Feunteun *et al.*, 1974). We therefore screened a collection of erythromycin-resistant mutants of *C. reinhardtii* for temperature-sensitivity at 15 and 35 °C. Five mutants belonging to the *ery*-M2 group were cold-sensitive for growth at 15 °C. We now report an analysis of the cold-sensitive phenotype by determination of growth characteristics and ribosome content and by genetic studies.

METHODS

Strains and genetic analysis. The erythromycin-resistant mutants of *C. reinhardtii* used represented one uniparental (U) and three Mendelian (M) loci. Mutants *ery-M1a*, *b*, *c* and *d*, *ery-U1a* and *ery-M2a*, *b*, *c* and *d* were erythromycin-resistant strains described by Mets & Bogorad (1971). Mutant *ery-M2e*, mating type (+), originally *ery 12* (+), was obtained from N. W. Gillham and found to be linked to the *ery-M2* mutants by Davidson (1976). Mutant *ery-M3a* was originally *ery 2y* from N. W. Gillham, and represents a third Mendelian locus unlinked to *ery-M1* or *ery-M2* (Davidson, 1976).

Standard techniques for crossing and tetrad analysis of *C. reinhardtii* were used (Levine & Ebersold, 1960). Vegetative diploids were isolated according to the method of Ebersold (1967) and Gillham (1963).

Growth tests. The minimal high salts (HS) medium used was similar to that of Sueoka (1960). Growth of strains at 15, 25 and 35 °C was compared on solid HS medium or HS medium supplemented with acetate (HSA) or with yeast extract, peptone and acetate (YPA HS). Media containing Tris buffer were not used because the pH of this buffer is strongly temperature-dependent.

Resistance to erythromycin (Sigma), cleocin (Upjohn, Kalamazoo, Michigan, U.S.A.) and lincomycin (Upjohn) was evaluated on solid HS media containing various concentrations of these filter-sterilized antibiotics.

Drop growth tests were carried out by placing strains in liquid HS medium for 2 to 6 h before adding drops to test plates. Relative growth was evaluated after 1 week at 35 °C, 2 weeks at 25 °C and 3 weeks at 15 °C.

Minimum inhibitory concentration determinations for erythromycin were done as described by Davidson & Bogorad (1977). Tubes were scored for growth after 2 weeks incubation at 25 °C.

Preparation and analysis of cellular fractions. Despite attempts by numerous laboratories, no satisfactory method for the isolation of chloroplasts from *Chlamydomonas* has yet been described. Therefore, no attempts were made to analyse chloroplast ribosomes prepared from isolated chloroplasts.

Strains were grown in liquid HS medium at 15 or 25 °C under 5 % (v/v) CO₂ and 4 to 8 klx illumination. Cultures were harvested in a Sorvall G3 rotor, washed and resuspended to 2×10^8 to 5×10^8 cells ml⁻¹ in 25–25–25–5 buffer (25 mM-MgCl₂, 25 mM-KCl, 25 mM-Tris/HCl, 5 mM-dithiothreitol, pH 7.5). Cells were broken by two passages through a Yeda press at 1500 lbf in⁻² (9.4 MPa). Small-scale preparations for analysis of rRNA by polyacrylamide gel electrophoresis were sometimes made by the sonicator-bath technique of Simon (1974). The homogenate was centrifuged at 17000 rev. min⁻¹ for 20 min in a Sorvall SS34 rotor. The supernatant was centrifuged again at 17000 rev. min⁻¹ for 20 min. The pellets from both centrifugations were combined and resuspended in 25–25–25–5 buffer. The final supernatant (S17) and the combined pellets (P17) were stored in liquid nitrogen after adding RNAase-free sucrose to 5 % (w/v).

Ribosomal subunits in S17 and P17 fractions were analysed on exponential sucrose gradients at 4 °C. Ribosomal RNAs were analysed by polyacrylamide gel electrophoresis as described by Hanson & Bogorad (1977b).

Total RNA analysis. Phenol extracts of total cellular RNA were made according to the method of Cattolico & Jones (1972). Gel electrophoresis of a sample containing 1 to 2 A₂₆₀ units was done as described by Hanson & Bogorad (1977b). Before electrophoresis, 0.1 vol. 250–250–250–50 buffer (250 mM-MgCl₂, 250 mM-KCl, 250 mM-Tris/HCl, 50 mM-dithiothreitol, pH 7.5) was added to the sample. Relative amounts of different species of rRNA were calculated from absorbance scans of gels at 260 nm. Relative peak areas were obtained by weighing pieces of Xerox copies of gel scans. The relative areas were converted to mol % using the *Chlamydomonas* rRNA molecular weight values given by Cattolico, Senner & Jones (1973).

RESULTS

Cold-sensitivity and erythromycin-resistance of haploid strains containing ery-M2 alleles

Wild-type, as well as mutant strains *ery-M1a*, *b*, *c*, *d*, *ery-M3a* and *ery-U1a* grew well at 15 and 35 °C. However, the *ery-M2* mutants did not grow at 15 °C on minimal medium nor on media supplemented with acetate, yeast extract and peptone (Table 1). The *ery-M2* mutants apparently have a narrow optimum growth range; the mutants did not grow as well at 30 to 35 °C as at 25 °C.

The haploid double mutants *ery-M2d ery-U1a* and *ery-M2d ery-M3a* were also erythromycin-resistant and cold-sensitive on minimal and on supplemented media (Tables 1 and 2). Presence of both the *ery-M2d* and *ery-U1a* alleles resulted in a strain which grew slowly even at 25 °C with no antibiotic (Table 2). The double mutant class *ery-M1 ery-M2* was not tested because the combination of these two alleles is lethal (Mets & Bogorad, 1971).

Table 1. Growth of *C. reinhardtii* strains at 15 °C on solid media

Strain	Relative growth*		
	HS medium	YPA HS medium	HS medium with 100 µg erythromycin ml ⁻¹
Wild type	++++	++++	0
<i>ery</i> -M2a	0	0	0
<i>ery</i> -M2b	0	0	0
<i>ery</i> -M2c	0	0	0
<i>ery</i> -M2d	0	0	0
<i>ery</i> -M2e	0	0	0
<i>ery</i> -U1a	++++	++++	++++
<i>ery</i> -M2d <i>ery</i> -U1a	0	0	0
<i>ery</i> -M3a	++++	++++	++++
<i>ery</i> -M2d <i>ery</i> -M3a	0	0	0
+/ <i>ery</i> -M2b	++++	++++	0
+/ <i>ery</i> -M2d	++++	++++	0
<i>ery</i> -M1b + / + <i>ery</i> -M2d	++++	++++	0+
<i>ery</i> -M1b	++++	++++	++++
+/ <i>ery</i> -M1b	++++	++++	+

* 0, No growth; 0+, very slight growth; +, ++, +++, +++++, relative growth scores in increasing order, with +++++ being the amount of growth of wild type after 3 weeks at 15 °C in the absence of antibiotics.

Table 2. Growth of *C. reinhardtii* strains at 25 °C on minimal medium containing various concentrations of erythromycin

Strain	Erythromycin concn (µg ml ⁻¹)				
	30	50	100	200	500
Wild type	++++*	0	0	0	0
<i>ery</i> -M2d	++++	++++	++++	++++	++
+/ <i>ery</i> -M2d	++++	0	0	0	0
<i>ery</i> -U1a	++++	++++	++++	++++	++++
<i>ery</i> -M2d <i>ery</i> -U1a	++	++	++	++	++
<i>ery</i> -M1b	++++	++++	++++	++++	++
+/ <i>ery</i> -M1b	++++	++++	++++	+	0
<i>ery</i> -M1b + / + <i>ery</i> -M2d	++++	++++	++	0+	0
<i>ery</i> -M3a	++++	++++	++++	++++	++++
<i>ery</i> -M2d <i>ery</i> -M3a	++++	++++	++++	++++	++

* Symbols for relative growth are as in Table 1, except that +++++ is the amount of growth of wild type after 2 weeks at 25 °C.

Association of the *ery^r* and *cld^s* phenotypes

Erythromycin-resistance (*ery^r*) and cold-sensitivity (*cld^s*) are very closely linked and appear to result from a single mutation. A cross between wild type and *ery*-M2d yielded over 95 % germination of zygotes and over 97 % progeny survival. Analysis of progeny from 63 zygotes did not reveal any *cld^r ery^r* or *cld^s ery^s* recombinants. Crosses of mutants with *arg*-2, *arg*-7, *nic*-15, *nic*-1, and *arg*-4 yielded no *cld^r ery^r* or *cld^s ery^s* recombinants in 150 tetrads examined.

To examine more progeny for recombination between *cld^s* and *ery*-M2, a form of random spore analysis (Davidson, 1976) was used. Mutant *arg*-2 was crossed with *ery*-M2d and zygotes were poured on to Petri dishes containing minimal medium. Mature zygotes were allowed to germinate into four or eight progeny on minimal medium. Progeny were then removed and plated at 25 and 15 °C on minimal medium containing 100 µg erythromycin ml⁻¹. Cells of genotype *arg*-2 cannot grow on minimal medium and cells of genotype

Table 3. Growth of *C. reinhardtii* strains at 25 °C on minimal medium containing various concentrations of lincomycin and cleocin

Strain	Lincomycin concn ($\mu\text{g ml}^{-1}$)		Cleocin concn ($\mu\text{g ml}^{-1}$)		
	300	500	10	20	40
Wild type	0*	0	0	0	0
<i>ery</i> -M2d	++++	++++	++++	++	+
+/ <i>ery</i> -M2d	0	0	0	0	0
<i>ery</i> -U1a	+	0	0	0	0
<i>ery</i> -M2d <i>ery</i> -U1a	++	++	++	++	++
<i>ery</i> -M1b	++++	++++	0	0	0
+/ <i>ery</i> -M1b	++++	+++	0	0	0
<i>ery</i> -M1b + / <i>ery</i> -M2d	++	++	0	0	0
<i>ery</i> -M3a	0	0	0	0	0
<i>ery</i> -M2d <i>ery</i> -M3a	++++	++++	+++	+++	+++

* Symbols for relative growth are as in Table 2.

cld^s ery^r cannot grow at 15 °C. Only *cld^r ery^r* recombinants could grow on 100 μg erythromycin ml^{-1} at 15 °C.

Growth was apparent on control plates which contained arginine and no erythromycin at 15 °C, or contained erythromycin at 25 °C. However, no growth, and thus no *cld^r ery^r* recombinants, were detected among approximately 10000 progeny tested. This result is strong evidence that the same mutation is conferring cold-sensitivity and erythromycin-resistance. Another reason that the *ery*-M2 mutants are not likely to be double mutants is that ethyl methanesulphonate was used during their isolation (Mets & Bogorad, 1971). Ethyl methanesulphonate, unlike nitrosoguanidine, does not induce closely linked mutations at high frequency (Guerola, Ingraham & Cerda-Olmeda, 1971; Guerola & Cerda-Olmeda, 1975).

Reversion/suppression frequency

Revertants or suppressed clones did not arise from *ery*-M2 mutants at a high frequency. Dilutions of *ery*-M2 strains grown at 25 °C in HS medium were plated on HS or YPA HS media at 15 °C and examined after 3 and 5 weeks. In several separate experiments, over 1×10^8 total cells were examined and only one cold-resistant colony was detected. Cells derived from this colony were as sensitive to erythromycin as the wild type and contained the normal amount of chloroplast ribosomes.

Complementation analysis at the *ery*-M2 locus

The characters antibiotic-resistance and cold-sensitivity conferred by the mutant *ery*-M2 allele are recessive. Heterozygous diploids (+/*ery*-M2) grew well at 15 °C (Table 1) and were inhibited by low concentrations of erythromycin (Table 2). The *ery*-M2 mutants are cross-resistant to lincomycin and cleocin, but +/*ery*-M2 diploids appear completely sensitive to these antibiotics on solid media (Table 3).

Minimum inhibitory concentration determinations in liquid culture on wild type, *ery*-M2d and +/*ery*-M2d revealed that the heterozygous diploid is slightly more resistant to erythromycin than the wild type (Table 4). However, the +/*ery*-M2 diploid is much less resistant than the +/*ery*-M1 diploids (Table 4 and Hanson & Bogorad, 1977a).

Ribosomal RNA present in cellular fractions of haploid strains

Ribosomal RNAs from cold-sensitive *Chlamydomonas* mutants were examined because bacterial mutants with this phenotype are known to be ribosome assembly-defective and to contain increased amounts of precursor forms of rRNAs (Nashimoto *et al.*, 1971; Feunteun *et al.*, 1974).

Table 4. Minimum inhibitory concentrations (m.i.c.) of erythromycin for *C. reinhardtii* strains

Strain	M.i.c. in liquid medium (μ g erythromycin ml ⁻¹)
Wild type	10
<i>ery</i> -M2d	250
+ / <i>ery</i> -M2d	20
<i>ery</i> -M1b	> 200
+ / <i>ery</i> -M1b	> 50

Fig. 1. Gel electrophoretic analysis of rRNA in S17 and P17 fractions from wild type and *ery*-M2d grown at 25 °C. L₇₀, rRNA from large subunit of 70S chloroplast ribosome; S₇₀, rRNA from small subunit of 70S chloroplast ribosome. The other two peaks represent the rRNA from the large and small subunits of the cytoplasmic (80S) ribosomes.

Precursor forms of chloroplast rRNAs were not detected in haploid strains containing *ery*-M2 alleles nor in wild type at 25 and 15 °C. However, quantitative alterations in chloroplast rRNAs were detected in *ery*-M2 strains.

Ribosomal RNA content could be used as a measure of ribosome content because no free rRNA could be detected in homogenates. Also, because the absolute amount of rRNA from cytoplasmic (80S) ribosomes per cell did not vary in different strains, rRNA from 80S ribosomes could be used as an internal standard for comparison of amounts of chloroplast rRNA.

The supernatant (S17) fractions from cells of wild type and *ery*-M1 mutants contained chloroplast rRNA as 35 to 40 mol% of the total rRNA. The S17 fractions from *ery*-M2d cells grown on either minimal or acetate-supplemented medium contained only 13 to 18 % chloroplast rRNA (Fig. 1). Unlike the *Chlamydomonas* mutant strain studied by Goodenough *et al.* (1971), the presence of acetate in the medium had no effect on the chloroplast ribosome content of *ery*-M2 mutants.

Because a mutant lacking chloroplast rRNA (ribosomes) in the supernatant fraction could possibly have a defect in the release of chloroplast ribosomes from membranes, the membrane (P17) fractions of mutants were also examined for rRNA content. However, the P17 fractions of *ery*-M2 mutants were not enriched in chloroplast rRNA in comparison with those of the wild type after growth at 25 °C (Fig. 1) or after incubation at 15 °C.

The rRNA contents of S17 fractions of wild type, erythromycin-resistant mutants and

Fig. 2. Gel electrophoretic analysis of rRNA in S17 fractions from wild type, *ery-M3a* and *ery-U1a* grown at 25 °C, and from *ery-M2d*, *ery-M2d ery-U1a* and *ery-M2d ery-M3a* grown at 25 °C and after 24 h incubation at 15 °C. Abbreviations as in Fig. 1.

double mutants after growth at 25 °C and after 24 h at 15 °C are shown in Fig. 2. The rRNA content of wild-type cells is identical after growth at 25 or 15 °C. All of the strains carrying *ery-M2d* have reduced amounts of chloroplast rRNA at 25 °C compared with wild type at 25 °C, and further reduction occurs at 15 °C. The double mutant *ery-M2d ery-M3a* has less chloroplast rRNA at 25 °C than any of the *ery-M2* mutants.

Fig. 3. Gel electrophoretic analysis of total cell RNA phenol extracts from wild type, *ery*-M2d and the heterozygous diploids $+/ery$ -M1b and $+/ery$ -M2d grown at 25 °C. Abbreviations as in Fig. 1.

Chloroplast ribosomal RNA content of heterozygous diploids

Total cell RNA from wild type, *ery*-M2 mutants, $+/ery$ -M1 diploids and $+/ery$ -M2 diploids was analysed by gel electrophoresis. Preparations from all these strains, except for the haploid *ery*-M2 mutants, had 35 to 40 mol % chloroplast rRNA. The *ery*-M2 mutants again had only 13 to 18 % chloroplast rRNA (Fig. 3). Thus, cold-sensitivity (Table 1), antibiotic-resistance (Table 2) and reduction in chloroplast RNA is recessive in $+/ery$ -M2 diploids. This association of cold-resistance with normal amounts of chloroplast rRNA supports the hypothesis that the *cld^s* phenotype results from the absence of sufficient numbers of chloroplast ribosomes for cell survival and multiplication at 15 °C.

Ratio of small to large subunits of chloroplast ribosomes in ery-M2 mutants

Gel electrophoretic analyses of rRNA shown in Figs 1 to 3 indicate that the small and large subunit of the chloroplast ribosome are present in approximately equimolar amounts in the *ery*-M2 mutants. Although it is the large subunit which appears to have an altered ribosomal protein in the *ery*-M2 mutants (Mets & Bogorad, 1972), both the large and small subunits are considerably reduced in these mutants.

To determine whether small differences exist in the amounts of small and large subunits of chloroplast ribosomes in the *ery*-M2 mutants, we employed sucrose gradients with salt conditions adjusted so that most chloroplast ribosomes remained associated as 70S monomers. Under these conditions, a small difference in the ratio of small to large subunits would be detected more easily.

The sucrose gradient profiles of ribosomes of S17 fractions from wild type and two *ery*-M2 mutants grown at 25 °C (Fig. 4) revealed that the ratio of small to large subunits is slightly greater than 1 in the mutants. All five *ery*-M2 mutants had similar gradient profiles. This small difference in subunit ratio in the mutants was not detected by Mets & Bogorad (1972) because the low-magnesium sucrose gradients they employed resulted in nearly complete dissociation of the chloroplast ribosomes into subunits.

After 12 h at the restrictive temperature of 15 °C, a further reduction in the large subunit of the chloroplast ribosome had occurred in the *ery*-M2 mutants (Fig. 5). The ribosome content of the membrane (P17) fraction was similar at both temperatures (Fig. 5). Evidently,

Fig. 4. Sucrose gradient analysis of S17 fractions from wild type, *ery-M2d* and *ery-M2c* grown at 25 °C. Gradients contained 25–500–50 buffer (25 mM-MgCl₂, 500 mM-KCl, 50 mM-Tris/HCl, pH 7.5). Gradient profiles of *ery-M2a*, *ery-M2b* and *ery-M2e* were similar to those of the *ery-M2* mutants shown. S₇₀, Small subunit of the 70S chloroplast ribosome; L₇₀, large subunit of the 70S ribosome; M₇₀, chloroplast ribosome monomer; S₈₀, small subunit of the 80S ribosome; L₈₀, large subunit of the 80S ribosome; M₈₀, monomer of the 80S ribosome.

Fig. 5. Sucrose gradient analysis of S17 and P17 fractions from *ery-M2d* grown at 25 °C and from *ery-M2d* after 12 h at 15 °C. The S17 and P17 fractions were dissolved in 25–25–25–5 buffer and run on gradients containing 25–500–50 buffer. Abbreviations as in Fig. 4.

there is a high degree of, but not a perfect, co-ordination of the amount of the chloroplast subunits in the *ery-M2* mutants. Whether or not this co-ordinate reduction is a general feature of chloroplast ribosome-deficient mutants having alterations in the large subunit remains an open question until such mutants at other genetic loci have been isolated and characterized. Although no mutants have been described which are specifically deficient in the large subunit of the chloroplast ribosome, Harris *et al.* (1974) have described mutants specifically deficient in the small subunit.

Table 5. Doubling times of phototrophically grown *C. reinhardtii* mutants having reduced amounts of chloroplast ribosomes

Genotype	Doubling time (h)	% of normal amount of chloroplast monomers*	Reference
Wild type	12.8	100	Boynton <i>et al.</i> (1974)
<i>ac-20</i>	18.9	32	Boynton <i>et al.</i> (1974)
<i>cr-1</i>	21.1	42	Boynton <i>et al.</i> (1974)
<i>ac-20, cr-1</i>	19.9	24	Boynton <i>et al.</i> (1974)
Wild type	8.5	100	This paper
<i>ery-M2d</i>	22	30–45	This paper

* The total amount of chloroplast monomer ribosomes which would be present if all subunits were associated was calculated from areas under chloroplast monomer and subunit peaks in absorbance scans of sucrose gradients.

Effect of reduced chloroplast ribosome content on growth rate

Although some degree of chloroplast protein synthesis appears necessary for vegetative growth even when a reduced carbon source is available, *Chlamydomonas* cells can continue to grow when chloroplast protein synthesis is much reduced. In the absence of an exogenously supplied carbon source, slow growth can be maintained even in mutants which have abnormally few chloroplast ribosomes. The *ery-M2* mutants, as well as *ac-20*, *cr-1*, *cr-2*, *cr-3* and *cr-4*, can all grow phototrophically, although with a lower doubling time than wild type has under the same conditions (Table 5).

DISCUSSION

The genetic data presented above indicate that the traits erythromycin-resistance and cold-sensitivity in *ery-M2* mutants of *Chlamydomonas* are the consequence of a single nuclear gene mutation which affects chloroplast ribosomes. Furthermore, the cold-sensitivity for growth appears to result from a deficiency of chloroplast ribosomes at the restrictive temperature.

The reduced ribosome content of *ery-M2* mutants at 25 and 15 °C could be explained if a mutant form of a ribosomal protein has decreased affinity for assembling ribosomes. Mets & Bogorad (1972) presented evidence for an altered chloroplast ribosomal protein in *ery-M2* mutants.

Decreased affinity of a ribosomal protein for an assembling ribosome could also explain the phenotype of the *+/ery-M2* diploids, which are much more sensitive to erythromycin than the *+/ery-M1* diploids. The *+/ery-M1* diploids are quite resistant to erythromycin although they contain only 30 to 40 % erythromycin-resistant chloroplast ribosomes (Hanson & Bogorad, 1977). Therefore, the high degree of erythromycin-sensitivity of *+/ery-M2* diploids indicates that few erythromycin-resistant ribosomes are present in these diploids. This situation would result if, in a diploid containing *ery-M2* and its wild-type allele, the mutant gene product is less likely to be incorporated into an assembling ribosome.

The cold-sensitivity for growth of the *ery-M2* mutants implies that chloroplast protein synthesis is not dispensable when cells are given a reduced carbon source, contrary to the suggestion of Boynton *et al.* (1974) and Harris *et al.* (1974). All haploid strains carrying mutations at the *ery-M2* locus, alone or in combination with *ery-M3a* and *ery-U1a*, were cold-sensitive even when provided with a reduced carbon source. Previous evidence for the importance of chloroplast protein synthesis in *C. reinhardtii* was that *ery-M1 ery-M2* recombinants are not viable – this combination of mutations in two genes affecting the chloroplast ribosome is lethal (Mets & Bogorad, 1971).

Chloroplast protein synthesis is apparently essential for growth of *Chlamydomonas* even under heterotrophic conditions, when photosynthesis is not essential for growth. This fact emphasizes the interdependence of genetic information storage and processing in the chloroplast and nuclear-cytoplasmic systems. It will be valuable to identify the products of chloroplast protein synthesis necessary for heterotrophic cellular growth.

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REFERENCES

- BOYNTON, J. E., GILLHAM, N. W. & CHABOT, J. F. (1974). Chloroplast ribosome deficient mutants in the green alga *Chlamydomonas reinhardtii* and the question of chloroplast ribosome function. *Journal of Cell Science* **10**, 267–305.
- CATTOLICO, R. A. & JONES, R. F. (1972). Isolation of stable ribosomal RNA from whole cells of *Chlamydomonas reinhardtii*. *Biochimica et biophysica acta* **269**, 259–264.
- CATTOLICO, R. A., SENNER, J. W. & JONES, R. F. (1973). Changes in cytoplasmic and chloroplast ribosomal RNA during the cell cycle of *Chlamydomonas reinhardtii*. *Archives of Biochemistry and Biophysics* **156**, 58–65.
- DAVIDSON, J. N. (1976). *Genes affecting erythromycin resistance in ery-M1 mutants of Chlamydomonas reinhardtii*. Ph.D. thesis, Harvard University, U.S.A.
- DAVIDSON, J. N. & BOGORAD, L. (1977). Suppression of erythromycin resistance in ery-M1 mutants of *Chlamydomonas reinhardtii*. *Molecular and General Genetics* (in the Press).
- DAVIES, J. & NOMURA, M. (1972). The genetics of bacterial ribosomes. *Annual Review of Genetics* **6**, 203–234.
- EBERSOLD, W. T. (1967). *Chlamydomonas reinhardtii*: heterozygous diploid strains. *Science* **157**, 447–449.
- FEUNTEUN, J., MONIER, R., VOLA, C. & ROSSET, R. (1974). Ribosomal assembly and defective mutants of *Escherichia coli*. *Nucleic Acids Research* **1**, 141–148.
- FLAKS, J. G., LEBOY, P. S., BIRGE, E. H. & KURLAND, C. G. (1966). Mutation and genetics concerned with the ribosome. *Cold Spring Harbor Symposia on Quantitative Biology* **31**, 623–631.
- GILLHAM, N. W. (1963). Transmission and segregation of a non-chromosomal factor controlling streptomycin resistance in diploid *Chlamydomonas*. *Nature, London* **200**, 294.
- GOODENOUGH, U. W., TOGASAKI, R. K., PASZEWSKI, A. & LEVINE, R. P. (1971). Inhibition of chloroplast ribosome formation by gene mutation in *Chlamydomonas reinhardtii*. In *Autonomy and Biogenesis of Mitochondria and Chloroplasts*, pp. 224–234. Edited by N. K. Boardman, A. W. Linnane and R. M. Smillie. Amsterdam: North-Holland.
- GUEROLA, N. & CERDA-OLMEDA, E. (1975). Distribution of mutations induced by ethyl methanesulfonate and ultraviolet radiation in the *Escherichia coli* chromosome. *Mutation Research* **29**, 145–147.
- GUEROLA, N., INGRAHAM, J. L. & CERDA-OLMEDA, E. (1971). Induction of closely linked multiple mutations by nitrosoguanidine. *Nature New Biology* **230**, 122–125.
- HANSON, M. R. & BOGORAD, L. (1977a). Complementation analysis at the ery-M1 locus in *Chlamydomonas reinhardtii*. *Molecular and General Genetics* **153**, 271–277.
- HANSON, M. R. & BOGORAD, L. (1977b). Effects of erythromycin on membrane-bound chloroplast ribosomes from wild-type *Chlamydomonas reinhardtii* and erythromycin-resistant mutants. *Biochimica et biophysica acta* **479**, 279–289.
- HARRIS, E. H., BOYNTON, J. E. & GILLHAM, N. W. (1974). Chloroplast ribosome biogenesis in *Chlamydomonas*. Selection and characterization of mutants blocked in ribosome formation. *Journal of Cell Biology* **63**, 160–179.
- LEVINE, R. P. & EBERSOLD, W. T. (1960). The genetics and cytology of *Chlamydomonas*. *Annual Review of Microbiology* **14**, 197–216.
- METS, L. J. & BOGORAD, L. (1971). Mendelian and uniparental alterations in erythromycin binding by plastid ribosomes. *Science* **174**, 707–709.
- METS, L. J. & BOGORAD, L. (1972). Altered chloroplast ribosomal proteins associated with erythromycin-resistant mutants in two genetic systems of *Chlamydomonas reinhardtii*. *Proceedings of the National Academy of Sciences of the United States of America* **69**, 3779–3783.
- NASHIMOTO, H., HELD, W., KALTSCHMIDT, E. & NOMURA, M. (1971). Structure and function of bacterial ribosomes. XII. Accumulation of 21S particles by some cold-sensitive mutants of *Escherichia coli*. *Journal of Molecular Biology* **62**, 121–138.
- PARDO, D. & ROSSET, R. (1974). Genetic studies of erythromycin resistant mutants of *Escherichia coli*. *Molecular and General Genetics* **135**, 257–268.
- SIMON, R. D. (1974). The use of an ultrasonic bath to disrupt cells suspended in volumes of less than 100 microliters. *Analytical Biochemistry* **60**, 51–58.
- SUEOKA, N. (1960). Mitotic replication of DNA in *Chlamydomonas reinhardtii*. *Proceedings of the National Academy of Sciences of the United States of America* **46**, 83–91.