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# *GENETIC CONTROL OF PHOTOSYNTHESIS IN CHLAMYDOMONAS REINHARDI**

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An increasing knowledge of the physical and chemical events which attend the process of photosynthesis opens the way to an investigation of genetic controls of this fundamental phenomenon and permits us to consider the question of the degree of control over photosynthesis exerted by the nucleus and the chloroplast.

Genetic controls of photosynthesis can be envisaged as preceding along two pathways. First, the controls can be direct. For example, there may be genetic control over the synthesis of enzymes specifically involved in photosynthetic reactions. These controls may be independent of a second pathway in which genetic changes in chloroplast structure so alter the site of photosynthesis as to affect numerous photosynthetic reactions.

There are, at present, forty-two UV-induced mutants of the sexually reproducing unicellular green alga, *Chlamydomonas reinhardi*, which cannot grow in the light unless the minimal medium is supplemented with sodium acetate. Each of the mutants represents a simple genetic change in that it segregates in a one-to-one fashion when crossed to wild-type.

The requirement for a carbon source other than or in addition to carbon dioxide suggests at least three possibilities for genetic blocks in these mutants. First, the acetate mutants may be unable to carry out certain of the light-requiring reactions of photosynthesis and thus possess genetic blocks which lie along the first pathway mentioned above. Second, the acetate mutants may have altered pigments or they may be pigment-deficient, and therefore are mutants which act indirectly on photosynthesis by way of the second pathway. Third, the mutants may possess metabolic blocks at some non-photosynthetic step in their intermediary metabolism.

One mutant strain, *acetate-21* (*ac-21*), appears to be incapable of sufficient carbon dioxide fixation to grow photosynthetically, and thus it falls into the category of a mutant strain which may be blocked in some essential step of photosynthesis.

Such a mutant provides a means for initiating an investigation into direct genetic controls of photosynthesis in sexually reproducing green algae. Accordingly, it is the purpose of the present paper to describe some of the phenomena associated with the growth and photosynthetic capabilities of *ac-21* in comparison with the wild-type strain.

*Growth Rate of ac-21.*—The growth rates of *ac-21* and wild-type were compared in both the light and the dark. *C. reinhardi* is not an obligate photoautotroph as,

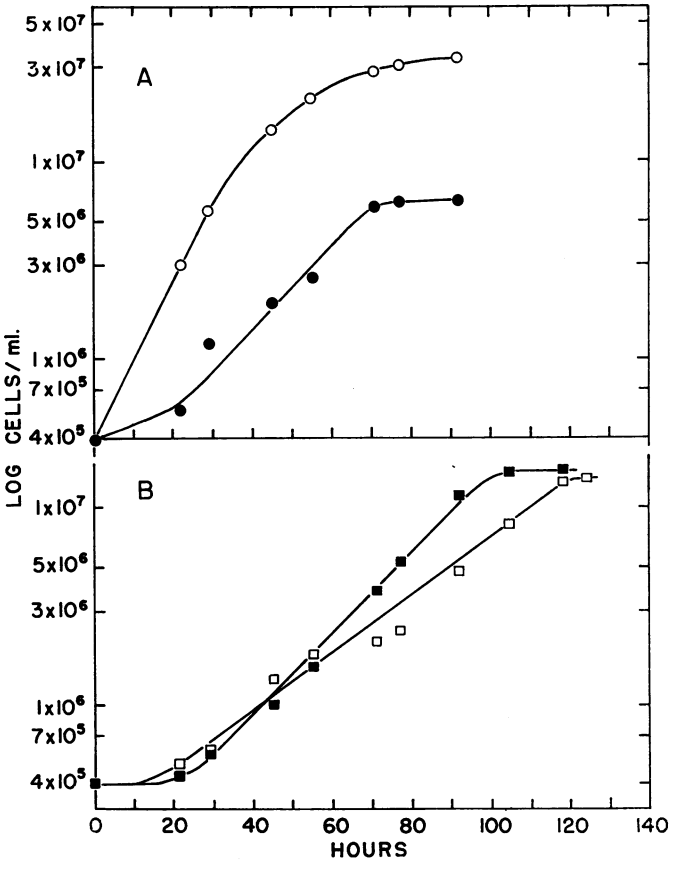


FIG. 1.—Growth of wild-type and *ac-21*. A. Growth of wild-type in the light (—○—) and in the dark (—●—). B. Growth of *ac-21* in the light (—□—) and in the dark (—■—).

for example, are the species *C. moewusii* and *C. eugametos.*[1] The wild type strain of *C. reinhardi* will grow in the dark if the minimal medium is supplemented with sodium acetate.[2]

Growth rates were measured in liquid medium in cylindrical separatory funnels 18 cm long and 5 cm in diameter. Each culture was aerated with five per cent carbon dioxide in air which also served as a source of agitation. The cultures were grown at 25°C and at a light intensity of 800 foot candles. For growth in the dark the funnels were covered with black masking tape and placed in the same

rack along with the funnels exposed to the light. Samples were withdrawn from the separatory funnels by way of the stopcock at the bottom and the measurements of growth were done by determining optical density at 750 mμ. The optical density values were converted to cells per ml according to a standard calibration curve previously obtained.

The results of a typical growth experiment are summarized in Figure 1. The growth rates, taken as the time for doubling of cell number during the logarithmic phase of growth, are as follows: wild-type (light), 8 hr; wild-type (dark), 14 hr; *ac-21* (light), 21 hr; and *ac-21* (dark), 18 hr.

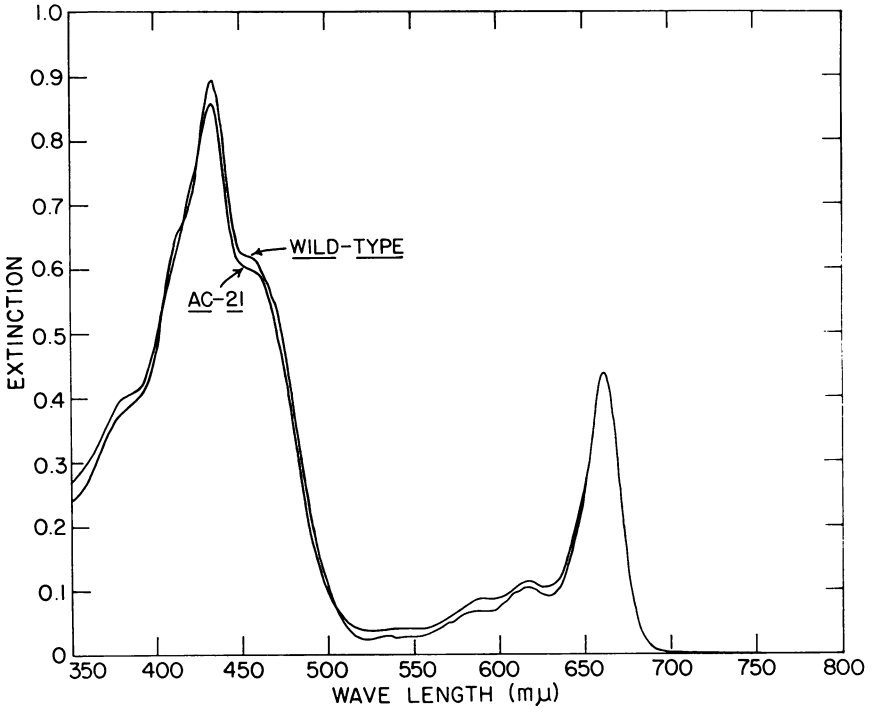


FIG. 2.—Absorption spectra of an 80 per cent acetone extract of the total pigments of wild-type and *ac-21*. The measurements were made with a Cary recording spectrophotometer and the maximum extinction in the red of *ac-21* was set equal to that of wild-type.

Unlike the growth of wild-type which is stimulated by light, the growth of *ac-21* in the light follows quite closely the curve of growth in the dark. For reasons not yet fully understood, wild-type grown in the dark reaches the stationary phase somewhat earlier than *ac-21*. This may be an artifact due to differences in cell size which would affect optical density readings, or may be due to a greater limiting effect of acetate concentration on the growth of wild-type in the dark.

*The Pigments of ac-21.*—The inability of *ac-21* to grow in the light in the absence of acetate might lie in an alteration of either the composition or concentration of its pigments. Numerous determinations were made of the amount of chlorophyll for both wild-type and *ac-21* using the method of Arnon.[3] Each chlorophyll determina-

tion was accompanied by a determination of cell number and the cells used were from log-phase cultures. The values for $\mu$g chlorophyll per $10^6$ cells in a series of four independent cultures of wild-type and of *ac-21* were as follows: wild-type—1.87, 1.40, 1.95, and 1.17; *ac-21*—1.69, 1.75, 1.71, and 1.07. The averages for wild-type and *ac-21* are, respectively, 1.68 and 1.62. There is, therefore, no marked quantitative difference between wild-type and *ac-21* with respect to the amount of chlorophyll per cell.

In addition, a comparison of the spectra from a total pigment extract reveals little, if any, qualitative variation between wild-type and *ac-21* (Fig. 2). Furthermore, electron micrographs have revealed that chloroplast structure is identical in wild-type and *ac-21*.[4]

*The Hill Reaction.*—Using parabenzoquinone as a hydrogen acceptor, the Hill reaction of whole cells was measured in phosphate buffer for both wild-type and *ac-21* which had been obtained from log-phase cultures. The measurements were carried out in duplicate for wild-type and *ac-21* in Warburg flasks in an atmosphere of nitrogen at 15°C and at a light intensity of 400 foot candles. The amount of chlorophyll per flask was 0.2 mg for both wild-type and *ac-21*. The $Q_{O_2}^{Chl}$ (calculated as microliters oxygen liberated per hr per mg chlorophyll) was 750 for wild-type and 546 for *ac-21*. These results were similar to those obtained in two additional experiments. Thus, under the conditions of the experiment described here, *ac-21* has a Hill reaction which is 73 per cent as effective as that of wild-type.

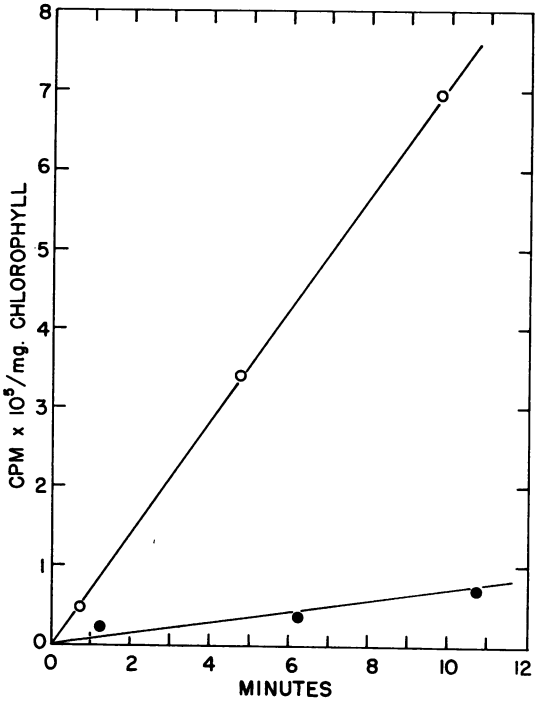


FIG. 3.—The rates of carbon dioxide fixation of wild-type (—○—) and *ac-21* (—●—). The values have been corrected for the amount of carbon dioxide fixation in the dark.

*The Rate of Carbon Dioxide Fixation.*—The rate of carbon dioxide fixation in whole cells of wild-type and *ac-21* was determined in a series of three experiments. The age of the cells and conditions of temperature and light were as for the measurement of the Hill reaction. The measurement of carbon dioxide fixation was carried out in the light and dark in 25 ml Erlenmeyer flasks stoppered with serum caps. For both wild-type and *ac-21* one flask was exposed to light while the other was covered with black masking tape. Washed cells, which were suspended in buffer, were allowed to equilibrate for 10 min before carbon-14 labeled sodium bicarbonate was added. The lights were turned on after the addition of the sodium bicarbonate. Samples were removed with a syringe at the times indicated in Table 1

TABLE 1

CARBON DIOXIDE FIXATION BY WILD-TYPE AND *ac-21*

| Wild-Type (cpm × $10^5$/mg Chl) | | | | *ac-21* (cpm × $10^5$/mg Chl) | | | |
|---|---|---|---|---|---|---|---|
| Time (min) | Light | Dark | Light minus dark | Time (min) | Light | Dark | Light minus dark |
| 0.75 | 0.59 | 0.11 | 0.48 | 1.25 | 0.36 | 0.13 | 0.23 |
| 4.75 | 3.60 | 0.20 | 3.40 | 6.25 | 0.58 | 0.21 | 0.37 |
| 9.75 | 7.23 | 0.26 | 6.97 | 10.75 | 0.97 | 0.26 | 0.71 |

Each reaction mixture contained either wild-type or *ac-21* (0.2 mg Chlorophyll), 4.7 $\mu$moles of $NaHC^{14}O_3$ (specific activity, 0.76 $\mu c/\mu$mole) and phosphate buffer (pH 6.8).

and Figure 3. Each sample was run into 0.06 ml of 0.5 *M* hydrochloric acid in order to stop fixation. Aliquots of each sample were plated in duplicate to weighed planchets, dried, reweighed, and then counted to determine the amount of radioactivity. The results from one of the experiments are shown in Table 1 and in Figure 3. They are the average for duplicate samples corrected for self-absorption, dilution, and background and are typical of those obtained in the other two experiments. It can be seen that the rate of carbon dioxide fixation in *ac-21* is some ten times less than it is in wild-type.

*The Carboxylation of Ribulose 1,5-Diphosphate.*—Fixation of carbon dioxide was measured in the dark in the presence of ribulose 1,5-diphosphate according to the method of Smillie and Fuller.[5] The results, which are summarized in Table 2,

TABLE 2

THE CARBOXYLATION OF RIBULOSE 1,5-DIPHOSPHATE (RuDP), BY WILD-TYPE AND *ac-21*

| | RuDP | $C^{14}O_2$ Fixed (cpm/mg Chl/hr) |
|---|---|---|
| Wild-type | + | $0.43 \times 10^5$ |
| | − | $0.14 \times 10^5$ |
| *ac-21* | + | $1.07 \times 10^5$ |
| | − | $0.11 \times 10^5$ |

The reaction mixture contained in addition to either wild-type or *ac-21* (0.2 mg of chlorophyll) which had been disrupted in a Mullard ultrasonicator, 1 $\mu$mole of ribulose 1,5-diphosphate, 4.7 $\mu$moles of $NaHC^{14}O_3$ (specific activity 0.76 $\mu c/\mu$mole), and phosphate buffer (pH 6.8).

show that both wild-type and *ac-21* possess an active ribulose 1,5-diphosphate carboxylase. Since the experiments as performed here are still in a very preliminary stage, it is difficult to draw conclusions regarding the significance of the difference that appears to exist between wild-type and *ac-21*. This difference, however, has been seen in four replicate experiments.

*Photosynthetic Phosphorylation.*—Since *ac-21* liberated oxygen as measured by the Hill reaction, the electron transfer system up to this stage of photosynthesis is intact. This suggested that the genetic block might be in the step of photosynthetic phosphorylation. The results of measurements of photosynthetic phosphorylation are given in Table 3. It can be seen that the rate of photosynthetic phosphorylation of the mutant is some twelve times less than that of the wild-type. However, the difference in phosphorylation shown by the mutant in the light and the dark is negligible when the counting error is taken into consideration. Therefore, it appears that the mutant has little, if any, photosynthetic phosphorylation.

*Discussion.*—The mutant, *ac-21*, is not capable of photosynthetic growth. It cannot grow unless the minimal medium has been supplemented with sodium acetate. Furthermore, unlike wild-type, its growth rates in the light and dark are

TABLE 3

PHOTOSYNTHETIC PHOSPHORYLATION OF WILD-TYPE AND *ac-21*

| | $P^{32}$ Esterified (cpm/mg Chl/hr) | | |
|---|---|---|---|
| | Light | Dark | Light minus dark |
| Wild-type | 133,846 | 52,548 | $8.13 \times 10^4$ |
| *ac-21* | 84,150 | 77,520 | $0.66 \times 10^4$ |

The reaction mixture contained either wild-type or *ac-21* which had been disrupted in a Mullard ultrasonicator and each of the following in $\mu$ molar amounts: Tris, 12; $MgCl_2$, 6; inorganic phosphate 2.4; ascorbate, 0.6; cysteine HCl, 0.6; adenosine diphosphate, 3; flavine mononucleotide, 0.06; and vitamin $K_3$, 0.0015. The reaction was carried out for 10 min in nitrogen at 15°C at a light intensity of 400 ft. c. The esterification of phosphate was measured according to the technique of Lindberg and Ernster.[8]

essentially identical. In addition, the rate of carbon dioxide fixation in *ac-21* is some ten times less than that of wild-type. This reduced rate of carbon dioxide fixation cannot be accounted for solely on the basis of a genetic block to electron transfer, since even though the rate of oxygen liberation is reduced in the mutant to 73 per cent of that found in wild-type, this reduction is insufficient to explain the tenfold decrease of carbon dioxide fixation. In addition, the preliminary experiments reported here have shown that both wild-type and *ac-21* can bring about the fixation of carbon dioxide in the dark in the presence of ribulose 1,5-diphosphate indicating the presence of a ribulose 1,5-diphosphate carboxylase such as is found in other photosynthetic organisms. The fact that the carboxylation reaction occurs in *ac-21* suggests that the genetic block does not lie in this dark reaction leading to carbon dioxide fixation.

It is clear, however, that *ac-21* has lost the ability to carry out photosynthetic phosphorylation. The loss of this phase of its photosynthesis, even though the light-dependent oxygen evolution is unaffected, suggests that the energy provided by photosynthetic phosphorylation is required for carbon dioxide fixation unless the appropriate carbon dioxide acceptor is supplied. This is in agreement with recent suggestions made by Arnon.[6]

It can be concluded that *ac-21* is a genetic alteration at a locus which controls the process of photosynthetic phosphorylation. A mutation at this locus may result in either a genetic uncoupling of photosynthetic phosphorylation or a disruption of electron transfer below the level of the release of molecular oxygen. An alternative still to be investigated is the possibility of a genetic block affecting acceptors of phosphate from adenosine triphosphate.[9]

The demonstration that at least one of the steps in photosynthesis is under genetic control opens the way to further investigations into the role of the nucleus in determining functions which occur in the chloroplast. Additional mutants which do not fix carbon dioxide have been obtained,[7] and they are now under investigation in order to determine the nature of their blocks to photosynthesis. The genetic analysis of the mutants is also under way. Both the genetic analyses and the functional tests of these mutants may shed light on the over-all organization of nuclear controls of photosynthesis.

The suggestions and criticisms of Drs. N. I. Krinsky, W. R. Sistrom, and J. A. Schiff during the course of this work are gratefully acknowledged. The ribulose 1, 5-diphosphate was kindly supplied by Dr. E. Racker.

* This work was supported by grants from the National Science Foundation and the U.S. Public Health Service.

[1] Lewin, J. C., *Science*, **112**, 652 (1950).

[2] In the experiments reported here, cultures were grown on minimal medium plus sodium acetate (0.2 gm per 100 ml). The minimal medium, described in Levine, R. P., and Ebersold, W. T., *Zeit. Vererbungs.*, **89**, 632 (1958), was altered by a fivefold increase in the concentration of Beijerinck's solution.

[3] Arnon, D. I., *Plant Physiol.*, **24**, 1 (1949).

[4] The author is indebted to Miss Jana Moravkova and Dr. Ian Gibbons for several electron micrographs of Chlamydomonas chloroplasts.

[5] Smillie, R. M., and Fuller, R. C., *Plant Physiol.*, **34**, 651 (1960).

[6] Arnon, D. I., *Nature*, **184**, 10 (1959).

[7] Levine, R. P., *Nature* (in press).

[8] Lindberg, O., and Ernster, L., in *Methods of Biochemical Analysis*, ed. D. Glick (New York: Interscience Publ., 1956), vol. 3, p. 1.

[9] Note added in proof: Subsequent experiments have shown that extracts of *ac-21* prepared by sonication are capable of carbon dioxide fixation in the light in the presence of adenosine triphosphate.

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## *THE FACTORIZATION OF CYCLIC REDUCED POWERS BY SECONDARY COHOMOLOGY OPERATIONS**

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Steenrod[1] has defined the cyclic reduced powers

$$P^j\colon H^m(X; Z_p) \to H^{m+2j(p-1)}(X; Z_p) \tag{1}$$

for any topological space $X$ and nonnegative integer $j$. The $P^j$ are linear mappings, stable under suspension and natural in $X$. Furthermore, the algebra $A$ of all stable cohomology operations over $Z_p$ is generated by linear combinations of the compositions of $P^j$ and $\Delta$, where the Bockstein

$$\Delta\colon H^m(X; Z_p) \to H^{m+1}(X; Z_p) \tag{2}$$

is associated with the exact coefficient sequence

$$0 \to Z_p \to Z_{p^2} \to Z_p \to 0.$$

Adem[2] and Cartan[3] have studied relations between products of $P^j$'s and $\Delta$'s. It turns out that if $j$ is not a power of $p$, $P^j$ can be expressed as a linear combination of compositions $P^{i_1} \ldots P^{i_n}$, where $i_\nu < j$, $\nu = 1, \ldots, n$. That is, in the Steenrod algebra $A$ the elements $P^j$, $j \neq p^r$, are decomposable.[2]

Associated with the Steenrod algebra $A$ over $Z_p$ are certain stable secondary cohomology operations.[4] We prove that there exist stable secondary cohomology operations $\mathfrak{R}$, $\Psi_k$ ($k = 1, 2, \ldots$) such that $\mathfrak{R}$ is defined on classes $u \in H^m(X; Z_p)$ with $\Delta u = 0$, $P^1 u = 0$, and $\mathfrak{R}(u)$ is a coset of the group

$$P^2 H^m(X; Z_p) + ({}^1/_2 \Delta P^1 - P^1\Delta) H^{m+2p-2}(X; Z_p) \tag{3}$$

in $H^{m+4(p-1)}(X; Z_p)$; the operations $\Psi_k$ ($k > 0$) are defined on classes $v \in H^m(X;$