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Phototaxis Mutants of *Chlamydomonas reinhardtii*

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A group of 31 mutants of *Chlamydomonas reinhardtii* with altered phototactic responses was isolated. Growth of all the mutants was normal and, with one exception, they had no gross morphological aberrations. One mutant produced supernumerary flagella. The mutants can be subdivided into five phenotypic classes on the basis of their abnormal responses: slow positive, no reponse, slow negative, fast negative, and variable. Random motility of the mutants was also characterized. About half were indistinguishable from wild type; the others exhibited "jerky" or "twirly" movements instead of relatively straight paths. These mutants can now be used for genetic and biochemical studies of the cellular basis of phototaxis.

Chlamydomonas reinhardtii is an attractive organism for use in studies of phototaxis, and investigations of this organism's response to light are being conducted in several laboratories. Quantitative assays have been devised for monitoring the response of algal populations to light (1, 3, 10, 15, 22), and considerable progress has been made in defining physiological and environmental factors that influence phototaxis (1, 4, 12-14, 21, 22).

There have also been reports describing the isolation of mutants of *C. reinhardtii* with qualitatively altered phototactic response. In one study a mutant that responds negatively to light was isolated, and a single gene locus *np* responsible for the phenotype was mapped in linkage group XIV (18; R. D. Smyth, Ph.D. thesis, University of California, Los Angeles, 1972). Three different negative phototaxis mutants were isolated in another study; each was found to result from mutation at a distinct locus (7).

There has not, as yet, been a comprehensive study combining the advantages of a quantitative assay system for comparing the responses of mutants to wild type with an extensive genetic and biochemical analysis of the mutants. This paper reports the first step in such a study, the isolation of a large group of mutants with altered phototactic responses.

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MATERIALS AND METHODS

Algal strains and culture conditions. All mutants are derived from the wild-type strain 21 gr which was obtained from R. Sager. The media and culture conditions for growth were described previously (22). For the qualitative phototaxis assay (see below), cultures were grown without shaking.

Quantitative phototaxis assay. Phototaxis was measured with an apparatus and procedures described previously (21, 22). In the procedure, the accumulation of cells in the actinically lighted region of an assay tube is measured spectrophotometrically. The rate of accumulation obtained (expressed as change in optical density at 800 nm per minute) is divided by the cell concentration (measured as optical density at 600 nm) to obtain a value called the phototaxis coefficient. This value reflects the directed motility of cells toward the light. Wild-type cultures have phototaxis coefficients of 1.0 to 1.8 under the specific conditions employed, and the cells swim toward the light (positive phototaxis).

Qualitative phototaxis assay. Large numbers of cultures were tested for phototaxis with a rapid screening procedure. Cultures were grown in tubes (13 by 100 mm) containing 5 to 6 ml of medium with black Kaput closures (Bellco Glass, Inc., Vineland, N.J.); illumination was provided from the bottom by fluorescent bulbs. After the cells had reached a density of 1×10^6 to 2×10^6/ml, the tubes were placed in solid black plastic racks as shown in Fig. 1. The racks were designed so that the tubes were suspended by their caps, thus preventing light from entering at the top of the tube. The horizontal slit in the rack (7 mm wide) permitted light to strike the middle of the culture tube. Illumination was provided by a 20-W cool white fluorescent lamp placed 3 cm from the slit. Five to ten minutes after the tubes were placed in the racks for qualitative screening, a dense green band was evident if the cells had responded normally (Fig. 1, right tube). In cultures with abnormal phototaxis, either no band was evident (Fig. 1, left tube) or an unusual distribution of cells was observed.

Motility assays. Motility of cell populations was characterized with the capillary tube assay described previously (22). The assay measures the ap-

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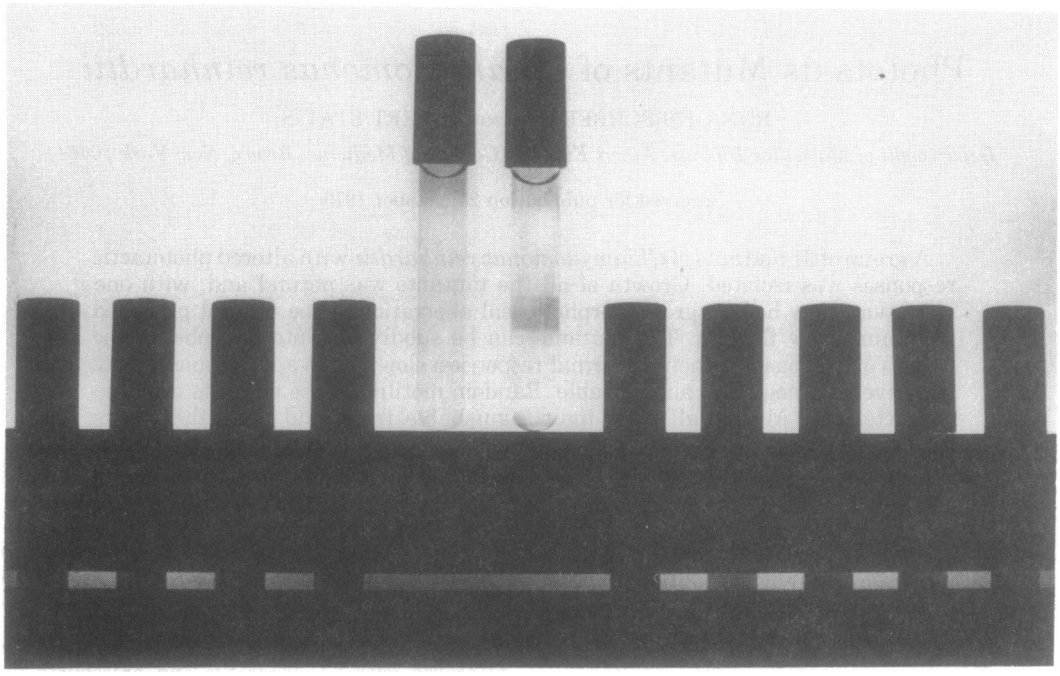

FIG. 1. *Qualitative screening procedure. The figure shows part of an assay rack at the end of a 5-min period of illumination. A normal (right) and an abnormal (left) culture have just been removed from the rack for observation of their phototactic responses.*

parent diffusion coefficient of a population of cells. The measure of motility obtained with this procedure is called a motility coefficient; wild-type cultures give values of 1.8 to 2.1 cm^2/h.

Information about the motility of individual cells was obtained with a photomicrographic velocity assay (21). The dark-field photomicrographs show tracks made by motile cells in a 1-s exposure. The tracks show both the velocity and changes in direction of the cells.

Mutagenesis procedures. Cells were treated with ultraviolet light (UV) or ethyl methane sulfonate (EMS) to produce 10 to 50% killing as determined by plate counts. For UV mutagenesis, cultures of exponentially growing cells (5×10^5 to 1×10^6 cells per ml) were either used directly or concentrated to a density of 10^7 to 10^8 cells per ml. Cells were stirred continuously in a small beaker 24 cm below a 15-W germicidal lamp for 5 to 8 min. The intensity of irradiation was approximately 8 J/m^2 per s. In a few cases, mutagenesis was conducted for 10 min at 50 cm from a 30-W lamp. In earlier experiments (from which mutants 1 through 11 were obtained), cells were diluted 150-fold immediately after UV treatment with minimal medium and grown in the light. In later work (mutants 14 through 37), mutagenized cells were held in the dark for 2 h to prevent photoreactivation and then diluted 50-fold with medium prior to growth in the light. Mutants 1, 2, 3, 4, 7, 8, 9, 10, 11, 16, 17, 18, 19, 20, 23, 24, 25, 26, 27, 29, 30, 31, 34, 35, and 37 were UV induced.

For EMS treatment, cells from an exponentially growing culture were concentrated to about 10^7 cells per ml, and 40 μl of EMS per ml of cells was added. After 10 to 12 min, an equal volume of 5% $Na_2S_2O_3$ was added. The mutagenized cells were collected by centrifugation, diluted 50-fold with medium, and grown in the light. Mutants 14, 22, 28, 32, 33, and 36 were isolated following EMS treatment.

Enrichment for abnormal phototaxis mutants. Following EMS or UV treatment, cultures were grown for 3 to 5 doublings to allow for phenotypic expression of mutations. Portions of the cultures (1 to 10^6 to 2×10^6 cells per ml) were then placed in sterile phototaxis assay tubes and exposed to the actinic light source (as in the quantitative phototaxis assay). After 20 to 30 min, about 1 ml of medium and cells was withdrawn from the unlighted region of the tube. This population is enriched 10 to 100 times for cells with abnormal phototaxis. Mutant clones were then obtained by one of the selection procedures described below.

Selection for abnormal phototaxis mutants. For the recycling method, an enriched cell population was diluted with medium, grown for 3 to 5 doublings, and again subjected to the enrichment procedure. At each cycle of growth and enrichment the response was monitored. When a substantially reduced response was observed (2 to 13 cycles in various experiments), colonies were isolated by plating and screened with the quantitative phototaxis assay. Those with abnormal phototaxis were recloned and saved. The recycling procedure was conducted on 4 independently mutagenized populations; 10 mutants, designated PT 1 to 11, were isolated.

For the bulk screening method, cells from the en-

richment procedure were diluted, and colonies were isolated by plating on minimal medium and 0.2% sodium acetate (for more rapid growth). Portions of the colonies were picked with sterile wooden applicator sticks and transferred to tubes (13 by 100 mm) containing minimal medium. When the cultures had reached a density of about 10^6 cells per ml, they were screened with the qualitative phototaxis assay. Cultures that appeared abnormal were subcultured and tested with the quantitative assay. After being recloned, the isolates were retained. The bulk screening procedure permitted a rapid, large-scale search for mutants and eliminated the problems associated with repeated selections. About 1 out of every 400 tube cultures screened gave an abnormal qualitative response. Of the 70 to 80 tentative mutants derived from the screening of more than 24,000 colonies, 22 had significantly altered phenotypes as determined by the quantitative assay to be retained; they are designated PT 14 to 37.

The procedures for mutant isolation are summarized in Fig. 2.

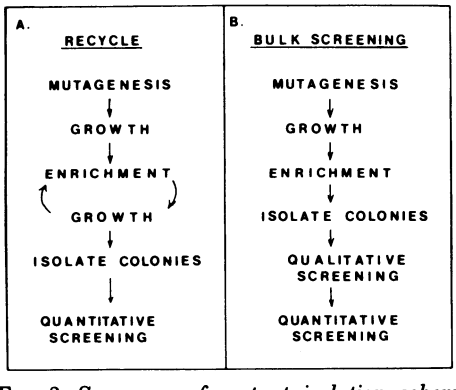

FIG. 2. *Summary of mutant isolation schemes. (A) Recycle procedure. (B) Bulk screening procedure.*

RESULTS

Characterization of phototaxis. Five classes of mutants of *C. reinhardtii* having abnormal phototactic responses were isolated (Fig. 3). Class I mutants (about half the isolates), such as PT 1, showed a positive response to light; however, both the rate of the response (measured by the phototaxis coefficient) and the final level of accumulation were considerably less than was observed for the wild type. Phototaxis coefficients for class I mutants ranged from 0.03 to 0.3 compared with wild-type values of about 1.0. Clones with phototaxis coefficients of 0.3 to 1.0 were found but not retained.

Class II mutants, such as PT 9, showed no response to light in our assay. Class III mutants (illustrated by PT 2) gave a slow negative response, resulting in a decrease in cell concentration in the illuminated area of the tube.

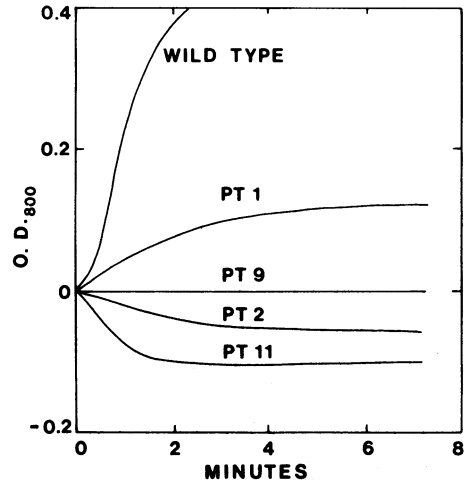

FIG. 3. *Phototactic response of strain 21 gr (wild type) and representative mutants. Cultures were assayed during exponential growth with the quantitative assay; the cell density in all cultures was approximately 1 × 10^6/ml.*

Class IV, which included only PT 11, exhibited behavior similar to that observed for class III mutants, but the negative response occurred very rapidly, and the magnitude of the response was greater. A fifth class of mutants was designated to include isolates that were inconsistent in their behavior. For example, PT 8 sometimes gave a slow positive response to light; at other times it responded negatively. However, the response was always very much slower in these mutants than in the wild type.

Characterization of motility. Since a phototactic response requires motility and hence, functional flagella, formaldehyde-killed samples of all the mutants were examined by phase contrast microscopy with respect to the appearance and arrangement of their flagella. With one exception (PT 34), all mutants had an apparently normal pair of flagella 10 to 12 μm in length. Cells of PT 34 are unusual in that they have a variable number of flagella ranging from 1 to 5, but usually they have 3 or 4. The uncoordinated movement of these supernumerary appendages may be related to this mutant's altered response to light. Other mutants of this type were described previously (19, 23).

Flagellar function was examined both by the capillary tube assay and by dark-field photomicroscopy. Based on their behavior in the tube assay, the mutants can be divided into two categories: (i) those with motility coefficients about the same as those in the wild type, and (ii) those with motility coefficients less than 40% of the normal. Mutants with normal motility coefficients also appeared normal in the sin-

gle-cell photomicrographic motility assay. The tracks appeared to have the same average velocity and frequency of turns as wild type (Fig. 4A, PT 9). Mutants with reduced motility coefficients also exhibited abnormal behavior when examined by dark-field photomicroscopy. Some

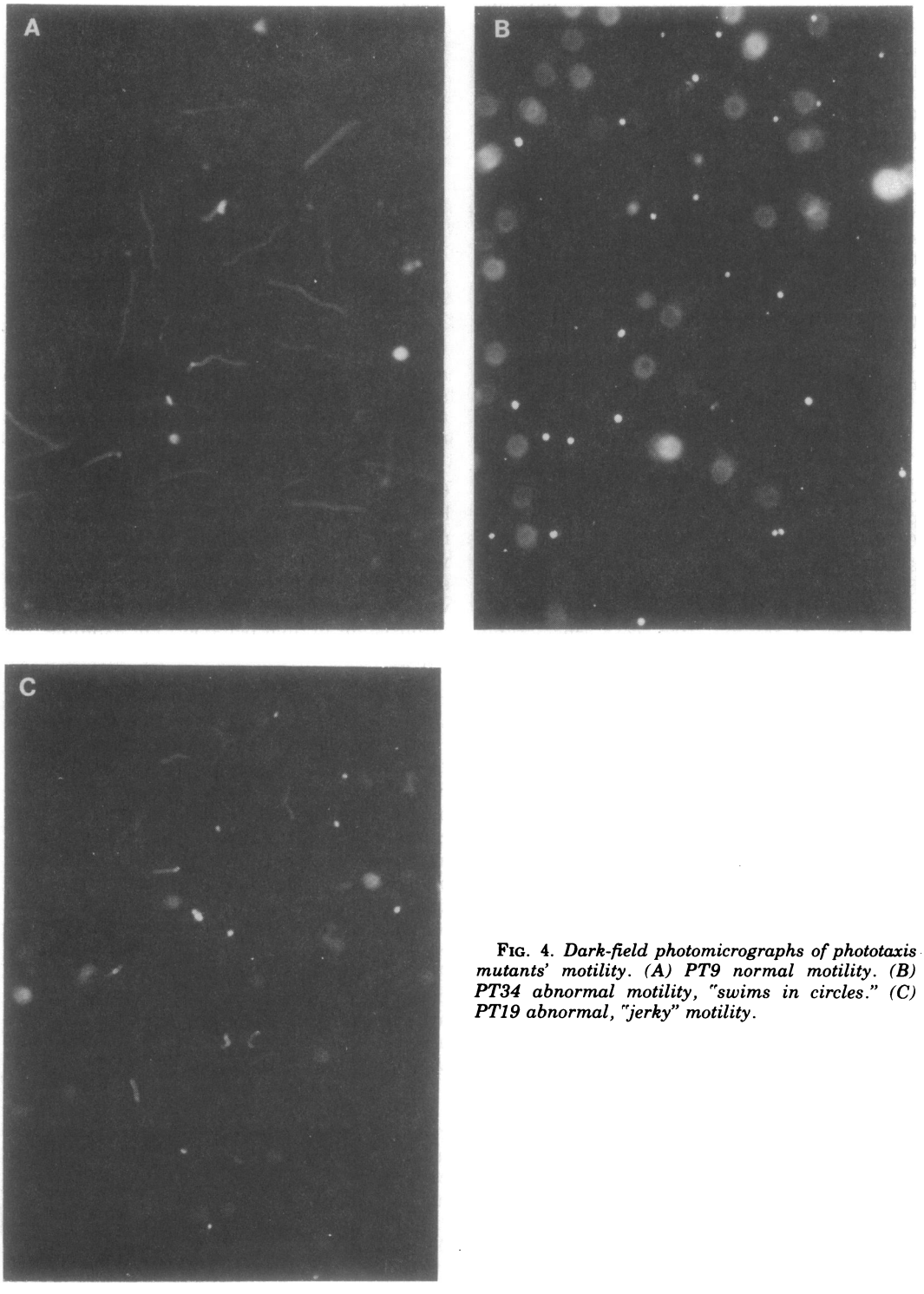

FIG. 4. *Dark-field photomicrographs of phototaxis mutants' motility. (A) PT9 normal motility. (B) PT34 abnormal motility, "swims in circles." (C) PT19 abnormal, "jerky" motility.*

(Fig. 4B, PT 34) twirled around rapidly, leaving blurred circles rather than relatively straight tracks; another (Fig. 4C, PT 19) made much more frequent stops than normal but without changing direction, resulting in short tracks. Some mutants seemed to exhibit both types of abnormal swimming.

The motility and phototaxis phenotypes of the 5 classes of mutants are summarized in Table 1. Since the two types of abnormal motility observed by photomicroscopy did not correlate with any particular phototaxis phenotypes, motility is classified only as normal or abnormal.

Other phenotypic characterization. All of the mutants grew normally under photosynthetic conditions with doubling times of 8 to 9 h, values equal to wild type. When examined by phase contrast microscopy, the cellular morphology of all the mutants, except PT 34, appeared normal.

DISCUSSION

The simplest model for phototaxis must have three components: a photoreceptor, a transmitter, and an effector. The first must be able to sense light and determine its direction; the photoreceptor must also determine the cells' orientation to the light (toward or away). Information must then be transmitted from the photoreceptor to the effector, the flagellar apparatus, where propulsion and change of direction actually occur.

Very little is known about the structure and function of the first two components of this system; however, the structure of the effector is fairly well defined. The flagellar apparatus consists of two external filaments that are anchored in the cytoplasm by a basal body (2, 5, 17, 18). The filaments contain the 9+2 arrangement of microtubules and associated dynein (the axoneme), which is embedded in a soluble matrix and surrounded by a membrane (6, 15, 16, 21). The filaments generate the power stroke for motility (15). The basal body contains several proteins beside tubulin (5); some of these may coordinate the beat of the two filaments and control the frequency of stops and turns (8). Mutations effecting the filaments lead to a variety of phenotypes (9, 11, 16, 24), including no external filaments, short filaments, long filaments, or incomplete axonemes (9+0). Most of these mutants are paralyzed and completely nonmotile.

TABLE 1. *Summary of mutant phototaxis and motility phenotypes*

Group	Mutants	Phototaxis	Motility
IA	1, 3, 10, 18, 25, 33	Slow positive	Normal
IB	16, 17, 22, 26, 28, 30, 31, 32, 36	Slow positive	Abnormal
IIA	9	No response	Normal
IIB	23, 24, 29, 34, 35, 37	No response	Abnormal
III	2, 14, 20	Slow negative	Normal
IV	11	Fast negative	Normal
VA	4, 7	Variable	Normal
VB	8, 19, 27	Variable	Abnormal

Mutations in basal body proteins would probably affect the coordination and pattern of swimming rather than lead to paralysis. Therefore, it is important to distinguish among three states: normal motility (with a characteristic velocity and frequency of stops and turns), abnormal motility (cells can move, but velocity or frequency of stops and/or turns is altered), and no motility (with or without external flagellar filaments). Mutation may lead to loss of motility or to loss of control of motility. Mutations could also affect the gene products required for photoreceptor and transmitter function.

This work demonstrates that a large group of phenotypically definable, stable phototaxis mutants can be isolated. Although precise definition of the mutants' defects will require further analysis, some general comments can be made and the basis of some mutant phenotypes can be tentatively assigned. First, these seem to be genuine phototaxis mutants, not metabolically or structurally defective strains with secondary effects on phototaxis. They grow normally and, with one exception, have apparently normal cell structure, including flagella. None of the mutants is completely paralyzed; all are capable of at least limited motility. This does not mean, of course, that subcellular structures and activities have not been altered, but only that the components affected seem to be involved specifically in phototaxis.

About half of our mutants (groups IB, IIIB, and VB) appear to have mutations that affect the effector component of the phototaxis system. Although they are motile, the cells swim abnormally; their abnormal phototactic behavior seems to be a direct result of this. Their mutations affect components that control motility; possibly basal body proteins are altered. Assuming these are single-site mutations, the other components of the phototaxis system (photoreceptor and transmitter) should be intact.

The remaining mutants, those with normal motility but altered phototaxis, presumably have defects in the photoreceptor or transmitter, but unequivocal assignment to one category or the other is not possible. The negative responding mutants (groups III and IV) all have normal motility. It is important to note that whereas others observe negative phototaxis in

wild-type strains in some situations (4, 12), we never do with strain 21 gr with the specific conditions of our assay. Therefore, we feel that these isolates are genetically altered and are not simply exhibiting an alternative phenotype. These mutants are not "blind" since they do respond to light, but their ability to perceive the direction of the stimulus or to orient toward it is altered. The rapid, negative response of PT 11 is particularly striking and has been partially characterized. Since one of the conditions that produces negative phototaxis is high light intensities (4), it seemed possible that PT 11 was just supersensitive to light and would respond positively with less light. Even at 1/64 the usual level of light, we found that this mutant still responded negatively; apparently increased sensitivity to light is not the basis of its altered response.

The remaining groups (IA, IIA and VA) are even more uncertain. The totally unresponsive but normally motile mutant PT 9 could be "blind," but the possibility of it having a defective transmitter cannot be eliminated. Similarly, the slow positive and erratic responders may have reduced amounts or less sensitive photoreceptors, but again a defective transmitter could be the problem. Although many of these mutants appear to have altered photoreceptor or effector systems, clarification of the precise nature of the mutations requires detailed biochemical and genetic analysis of the molecular basis of phototaxis.

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