Submicromolar Levels of Calcium Control the Balance of Beating between the Two Flagella in Demembranated Models of Chlamydomonas

R. KAMIYA and G. B. WITMAN
Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545.
Dr. Kamiya's permanent address is Institute of Molecular Biology, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464, Japan.

ABSTRACT
When detergent-extracted, demembranated cell models of Chlamydomonas were resuspended in reactivation solutions containing \(<10^{-8}\) M \(Ca^{++}\), many models initially swam in helical paths similar to those of intact cells; others swam in circles against the surface of the slide or coverslip. With increasing time after reactivation, fewer models swam in helices and more swam in circles. This transition from helical to circular swimming was the result of a progressive inactivation of one of the axonemes; in the extreme case, one axoneme was completely inactive whereas the other beat with a normal waveform. At these low \(Ca^{++}\) concentrations, the inactivated axoneme was the trans-axoneme (the one farthest from the eyespot) in 70-100% of the models. At \(10^{-7}\) or \(10^{-6}\) M \(Ca^{++}\), cell models also proceeded from helical to circular swimming as a result of inactivation of one of the axonemes; however, under these conditions the cis-axoneme was usually the one that was inactivated. At \(10^{-8}\) M \(Ca^{++}\), most cells continued helical swimming, indicating that both axonemes were remaining relatively active. The progressive, \(Ca^{++}\)-dependent inactivation of the trans- or cis-axoneme was reversed by switching the cell models to higher or lower \(Ca^{++}\) concentrations, respectively. A similar reversible, selective inactivation of the trans-flagellum occurred in intact cells swimming in medium containing 0.5 mM EGTA and no added \(Ca^{++}\). The results show that there are functional differences between the two axonemes of Chlamydomonas. The differential responses of the axonemes to submicromolar concentrations of \(Ca^{++}\) may form the basis for phototactic turning.
tion at submicromolar levels. The responses of the axonemes to Ca\(^{2+}\) appear to be sufficient to account for the behavior of living cells during turning and phototaxis. That the two axonemes respond differently to Ca\(^{2+}\) suggests either that there are inherent differences in their motile machinery, or that some aspects of flagellar activity are controlled directly by cell structures other than the axoneme.

**MATERIALS AND METHODS**

**Culture Conditions:** Chlamydomonas reinhardtii strain 137(+), grown synchronously at 24°C as previously described (26), 100 ml of fresh medium in a 250 ml Erlenmeyer flask was inoculated with 2 ml of a fully grown cell culture, and the new culture was used between 48 and 60 h after the inoculation.

**Demembranation and Reactivation:** For most of the experiments reported here, cells in 15 ml of culture medium were harvested by centrifugation at ~500 g for 3 min in a 15 ml conical plastic centrifuge tube, and washed three times with 10 mM HEPES (pH 7.4), 0.5 mM EGTA by repeated centrifugation and resuspension. Demembranation of the cells was carried out by adding to the cell pellet 5 vol of ice-chilled demembranating solution containing 30 mM HEPES (pH 7.4), 5 mM MgSO\(_4\), 1 mM diethyrotetrol, 1 mM EGTA, 25 mM KCl, 0.5% polyethylene glycol (20,000 mol wt), 1% Ficol (~400,000 mol wt), and 0.1% Nonidet P-40. In some experiments, the cell pellet was resuspended immediately in the demembranating solution. However, in most experiments, the cell pellet was overlaid with the demembranating solution and left standing for 5 min before being resuspended with a Pasteur pipette. The latter procedure permitted the pellet to soften somewhat so that the resuspension could be carried out more gently; this helped prevent the axonemes from coming off the cell body. All of the experiments reported here were carried out with cells stored in ice-cold demembranating solution for no more than 3 h; during this time there was no change in the results obtained upon reactivation.

For reactivation, about 0.1 ml of the cell suspension in demembranating solution was centrifuged at 1,500 g for 30 s in a 10 × 75 mm glass culture tube, and the supernate was carefully removed with a Pasteur pipette. The cell pellet was then gently resuspended in about 0.3 ml of standard reactivation solution (30 mM HEPES (pH 7.3), 5 mM MgSO\(_4\), 1 mM diethyrotetrol, 25 mM KCl, 2% polyethylene glycol (20,000 mol wt), 2 mM EGTA, and 1 mM ATP) or one of the Ca\(^{2+}\)-buffered reactivation solutions of Bessen et al. (1). All the procedures after the demembranation were performed at 0°C except for the microscope observations, which were carried out at room temperature.

**Microscope Observations:** Light microscope observation usually began immediately after the onset of reactivation. One drop of sample was placed on a microscope slide that had been pretreated with Surfasil® siliconizing agent (Pierce Chemical Co., Rockford, IL) to reduce sticking of the axonemes to the glass surface. The sample was then covered with a coverslip and sealed with Vaseline® petroleum jelly. In most cases, observations were carried out using a Zeiss Universal Microscope equipped with an Olympus DC dark-field condenser (NA 1.2-1.33) and Olympus 40X apochromatic oil immersion objective or Olympus 10X achromatic objective lenses. For recording images of moving cell models, a 35-mm still camera or a Locam Model 51 16-mm high-speed cine camera (Redlake Corp., Campbell, CA) was used in combination with a Strobex light source (Chadwick-Helmuth Co., Inc., Los Angeles, CA); Kodak Tri-X 16-mm film was used at an effective speed of El 800 and Kodak Tri-X 35-mm film was used at an effective speed of El 3200 and with a Strobex light source (Chadwick-Helmuth Co., Inc., Monrovia, CA). The beat patterns of the axonemes at low and high concentrations of Ca\(^{2+}\) were thus similar to those observed previously for isolated axonemes (1) or flagellar apparatuses (15). However, because of the Ca\(^{2+}\)-dependent detachment of the axonemes, a model system composed of a cell body and two axonemes could be prepared only at low Ca\(^{2+}\) concentrations.

These "complete" cell models were able to swim in the standard reactivation solution containing Ca\(^{2+}\) concentrations <10\(^{-6}\) M, their axonemes resumed beating with an asymmetrical, ciliary pattern that caused the cells to move through the medium. Greater than 90% of the cell models became motile in the standard reactivation solution (containing <10\(^{-9}\) M free Ca\(^{2+}\)), but a lower percentage were reactivated at 10\(^{-7}\) or 10\(^{-6}\) M Ca\(^{2+}\) (see below). At 10\(^{-4}\) M Ca\(^{2+}\), the axonemes detached from the cell bodies and began beating with a symmetrical waveform. The beat patterns of the axonemes at low and high concentrations of Ca\(^{2+}\) were thus similar to those observed previously for isolated axonemes (1) or flagellar apparatuses (15). However, because of the Ca\(^{2+}\)-dependent detachment of the axonemes, a model system composed of a cell body and two axonemes could be prepared only at low Ca\(^{2+}\) concentrations.

**RESULTS**

**Appearance of Cell Models**

When living cells were observed in the light microscope within 1 min after resuspension in demembranating solution, all cells had ceased active movement. The axonemes had various stiff forms resembling the waveforms seen at different phases of the flagellar beat cycle. The shapes of the axonemes were stable for at least several hours, indicating that the axonemes were in a rigor state similar to that observed in demembranated sea urchin sperm upon sudden depletion of ATP (8, 17).

Electron microscopic observations of cells fixed after 10 min in ice-cold demembranating solution revealed that the plasma and flagellar membranes were completely removed except for a narrow band surrounding the transition region (Fig. 1). Internal membranous organelles showed various degrees of disruption.

**General Behavior of Cell Models**

When the demembranated cells were transferred to a reactivation solution containing Ca\(^{2+}\) concentrations <10\(^{-6}\) M, their axonemes resumed beating with an asymmetrical, ciliary pattern that caused the cells to move through the medium. Greater than 90% of the cell models became motile in the standard reactivation solution (containing <10\(^{-9}\) M free Ca\(^{2+}\)), but a lower percentage were reactivated at 10\(^{-7}\) or 10\(^{-6}\) M Ca\(^{2+}\) (see below). At 10\(^{-4}\) M Ca\(^{2+}\), the axonemes detached from the cell bodies and began beating with a symmetrical waveform. The beat patterns of the axonemes at low and high concentrations of Ca\(^{2+}\) were thus similar to those observed previously for isolated axonemes (1) or flagellar apparatuses (15). However, because of the Ca\(^{2+}\)-dependent detachment of the axonemes, a model system composed of a cell body and two axonemes could be prepared only at low Ca\(^{2+}\) concentrations.

These "complete" cell models were able to swim in the standard reactivation solution with a maximum velocity of 30–50 μm/s. This is about one-third to one-half the velocity of living cells swimming in culture medium containing the same concentration of polyethylene glycol. Initially, many models swam in helical paths similar to those observed for living cells (cf. Fig. 2, A and B), except that the models swam more smoothly than living cells and did not respond to the illuminated field or change swimming direction as did living cells. Alternatively, some models swam in small circles (Fig. 3) or simply rotated at ~0.5–3 Hz against the surface of the slide or coverslip (Fig. 5). With time, the number of helically swimming models decreased, while more models began swimming in progressively smaller circles. In a typical experiment, the number of reactivated cell models swimming in helical paths was >70% when initially observed, and decreased to <5% after 10 min of observation under the microscope. Circling usually continued for at least an additional 10 min. Models swimming in contact with the surface of the
FIGURE 1  Electron micrograph of the flagella and basal bodies of a detergent-extracted Chlamydomonas. The plasma and flagellar membranes have been completely removed with the exception of a short cylinder of membrane (arrows) that often remains associated with the transition region of the flagellum. Proximal and distal striated fibers and basal body rootlets are still present after detergent treatment. × 88,000.
slide were circling predominantly (>90%) in a counterclockwise direction, whereas about the same proportion of those swimming against the coverslip were circling clockwise.

**Differential Activity of the Two Axonemes**

That a living cell or cell model swims in a helical path suggests that there is an imbalance in the forces exerted by the two flagella or axonemes, causing the cell to continuously turn to one side (see Discussion). Such an imbalance presumably occurs because one flagellum or axoneme is beating more rapidly or more forcefully than the other. Indeed, as will be shown below, in the extreme case of reactivated cell models rotating against the slide or coverslip, one axoneme appeared to beat normally whereas the other showed no movement or beat with only a very small amplitude.

To determine if the same axoneme was always dominant, we took advantage of the fact that the eyespot in *Chlamydomonas* is positioned to the side of the cell just out of a plane connecting the two basal bodies and therefore closer to one flagellum (the *cis*-flagellum) than the other (the *trans*-flagellum). In the strain 137(+) used in this study, the eyespot is visible as a red spot when viewed with bright-field optics or as a bright patch when viewed with dark-field optics (Fig. 4). By observing the position of the eyespot relative to the center of rotation of a circling cell, we could readily determine which axoneme was beating most effectively. A cell in which the *cis-
axoneme is most effective will turn away from the side having the eyespot; as a result, the eyespot will be on the side of the cell farthest from the center of rotation. Conversely, a cell in which the trans-flagellum is dominant will turn toward the side having the eyespot, and the eyespot will be on the side of the cell nearest the center of rotation. In the majority of cell models circling or rotating in standard reactivation solution, the eyespot was positioned far from the center of rotation (Figs. 3 and 5), indicating that the cis-axoneme was beating more effectively than the trans-axoneme. The proportion of rotating cells with the cis-axoneme dominant varied to some extent, but was almost always between 70 and 100% (Fig. 7). The cause and significance of this variability are unclear, although we found that the above percentage tended to be smaller when cells from older (denser) cultures were used.

Ca** Controls the Differential Activity of the Axonemes

Because Ca** is essential for phototaxis (23), it seemed possible that it might affect the pattern of beating of the axonemes. We therefore examined the effect of calcium over a concentration range between 10**4 and 10**7 M. As in the standard conditions, cell models in reactivation solutions containing 10**4 or 10**7 M free Ca** swam first in helical paths and then in circles of progressively smaller diameter. However, fewer models were reactivated than in the standard solution, and they tended to stop beating after only 5–10 min. Most importantly, in contrast to the situation in the standard solution, the eyespot was usually positioned on the side of the cell nearest the center of rotation (Fig. 6), indicating that under these conditions the trans-axoneme was most active. The predominant direction of rotation was the same as in the standard solution: in a survey of 30 cells rotating on the glass slide, 77% were circling counterclockwise.

By repeating observations at different concentrations of Ca**, we obtained a curve showing the Ca** dependence of cis- or trans-axonemal activity (Fig. 7). The trans-axoneme was most active in ~94 and ~90% of the cells at 10**-6 or 10**-7 M free Ca**, respectively, whereas at 10**-8 M Ca**, the cis-axoneme was dominant in ~84% of the cells. At 10**-8 M Ca**, a larger number of cells continued helical swimming than at higher or lower concentrations, indicating that both axonemes continued to be relatively active. Moreover, among the few cell models that were rotating against the slide, the percent having either the cis- or trans-axoneme dominant varied considerably from experiment to experiment.

As stated above, when cell models were first observed after resuspension in reactivation solution, many models were swimming in smooth helical paths. If the above described pattern of Ca**-dependent activation or inactivation of the cis- or trans-axoneme occurs in these helically swimming models, then at 10**-9 M Ca** the cis-axoneme will be most active and, for reasons described in the Discussion, the eyespot will be on the outside of the helix. At 10**-7 M Ca**, the opposite will be true. Indeed, this is exactly what we observed. In 76 randomly recorded tracks of cell models that were swimming helically at 10**-9 M Ca** and had identifiable eyespots, the eyespot was on the outside in >80% of the tracks (Fig. 8A). Conversely, in 49 tracks recorded at 10**-7 M Ca**,
Form of the Axonemes

FIGURE 7 Graph of the percent of reactivated cell models in which the trans-axoneme was the most active one at the indicated concentrations of free Ca++. Each data point represents the mean ± SD of about 10 experiments. SRS, standard reactivation solution (containing <10^-9 M free Ca++)

67% of the models were oriented with their eyespots facing the inside of the helix (Fig. 8B). These results indicate that in helically swimming cell models, as well as circling or rotating models, the balance of beating of the two axonemes is affected by the concentration of free Ca++ in the reactivation solutions. This pattern of activity had already been determined at the earliest times that we could make observations.

For comparison, intact cells swimming in normal medium are always oriented so that the eyespot faces the outside of the helix (Fig. 8C).

Form of the Axonemes

To more accurately assess the form and movement of the two axonemes at the different concentrations of Ca++, cell models rotating against the slide or coverslip were filmed by high speed cinemomicrography.

In cell models reactivated at 10^-9 M Ca++ (Fig. 9), the cis-axoneme beat with a waveform very similar to that of intact flagella of normal forward swimming cells (3, 15, 19). In contrast, the trans-axoneme showed no movement or only occasional, low amplitude bending movements; this axoneme appeared to be relatively rigid and was most commonly held in a smooth arc at various angles relative to the cell body.

Similarly, in cell models rotating at 10^-7 M Ca++ (Fig. 10), the trans-axoneme beat with a waveform similar to that of normal intact flagella, whereas the cis-axoneme was nonmotile. As at 10^-9 M Ca++, the nonmotile axoneme was rigid and usually held in an arc at a distance from the cell body.

Reversibility of Cis-Axoneme and Trans-Axoneme Inactivation

To determine if the selective, Ca++-dependent inactivation of either the cis- or the trans-axoneme was reversible, we reactivated cell models at various Ca++ concentrations and then switched them to a second reactivation solution containing a different Ca++ concentration. To assure reactivation in the first solution, a sample was brought to room temperature for 30 s. The sample was then chilled on ice and aliquots removed for microscopic observation to determine the direction of turning of the cells relative to their eyespots. After quantification of the eyespot position relative to the center of rotation, the remainder of the sample was centrifuged and resuspended in the second reactivation solution in the cold as in a usual experiment. During the exchange of medium, many cells lost their axonemes, probably due to mechanical or shearing forces during the centrifugation and resuspension. Nevertheless, observations on the remaining cells indicated that selective inactivation of both the cis- and the trans-axoneme was reversible (Table 1). When cell models were switched from standard reactivation solution to a solution containing 10^-7 M Ca++, the behavior of the cells changed from a pattern in which the cis-axoneme was usually dominant to one in which the trans-axoneme was most active in the majority of reactivated models. Conversely, the trans-axoneme was usually the most active one in models initially reactivated at 10^-7 M Ca++, but after the models were switched to standard reactivation solution, the cis-axoneme became most effective.

Selective Inactivation of Axonemes In Vivo

The fact that more and more cell models swam in progressively smaller circles at increasing times after reactivation in solutions containing 10^-9 M or 10^-8 - 10^-7 M Ca++ suggested that there was a progressive, Ca++-dependent inactivation of the trans- or cis-axoneme, respectively. To investigate whether such a process might also occur in the living cell, we examined intact cells after resuspension in HES solution containing 10 mM HEPES (pH 7.4), 0.5 mM EGTA, and 4% sucrose, with the expectation that this solution would result in a depletion of intracellular Ca++. Initially, these cells swam in helical paths similar to those of cells swimming in normal medium. However, with time, an increasing number of cells swam in

FIGURE 6 Triple (A) and single (B) flash exposures of a cell model circling clockwise against the coverslip in reactivation solution containing 10^-7 M Ca++. Under these conditions the trans-axoneme (arrow in B) was most active. In A, the three images of the eyespot are superimposed near the center of rotation. x 3,100.
circles against the slide or coverslip. After 1 h, ~70% of swimming cells circled in this way; in virtually all of these, the cis-flagellum was beating rapidly and the trans-flagellum was quiescent. In contrast, circling was observed in <10% of control cells swimming for the same length of time in HES + 1 mM CaCl₂. The EGTA-induced quiescence of the trans-flagellum in the experimental cells was readily reversible; upon addition of 1 mM CaCl₂ to the HES solution, the cells quickly reverted to normal helical swimming. These observations indicated that reversible, highly selective, Ca²⁺-modulated inactivation of at least the trans-axoneme could occur in vivo.

DISCUSSION

As seen in Fig. 2 A and 8 C, intact Chlamydomonas usually swim in helical paths. Gray (11) has discussed in detail the types of movements that result in helical and circular swimming in sea urchin sperm, and his analysis is generally applicable to Chlamydomonas (Fig. 11). If a forward swimming Chlamydomonas continuously turns to the left or right in the plane of the flagellar beat, then it will swim in a circular arc; such turning in the plane of the flagellar beat is termed “yawing”, and might occur if one flagellum is beating more rapidly or more forcefully than the other. If a forward swimming cell rolls about its longitudinal axis without turning to the left or right, it will swim in a straight line. Finally, if a forward-swimming cell both rolls and yaws, it will swim in a helical path. This will be a left-handed helix if the cell is rolling counterclockwise as viewed from behind, and a right-handed helix if the cell is rolling clockwise. A cell that yaws away from the side having the eyespot will swim in a circular or helical path with its eyespot facing out, regardless of the direction of roll.

There have been conflicting reports in the literature concerning whether Chlamydomonas reinhardii rolls as it swims, ⁴ In high speed movies of both wild-type Chlamydomonas (6; H. J. Hoops, J. Shapiro, and G. B. Witman, unpublished results) and a mutant with abnormal basal structure (13), one flagellum is frequently observed to beat faster than the other. On the basis of the findings reported here, the faster beating flagellum is probably the cis-flagellum.
with some investigators concluding that it does (2, 7) and some that it does not (18, 19). Under our conditions, rolling definitely occurs, as evidenced by tracks such as that shown in Fig. 8C, where the eyespot is always oriented toward the outside of the helix. Because the flagellar apparatus of Chlamydomonas has 180° rotational symmetry (12), such rolling probably results from the slight three-dimensional component of the flagellar waveform (1). However, rolling could also occur if the two flagella beat in different planes, even if their waveforms were not three-dimensional.

The majority of demembranated cell models that were reactivated at $10^{-9}$ M or $10^{-5}-10^{-7}$ M Ca$^{2+}$ first swam in helical paths similar to those of intact cells, but with time began to swim in circles or rotate against the slide or coverslip.
FIGURE 10  Film sequence of a cell model reactivated at $10^{-7}$ M Ca$^{2+}$. One of the axonemes is beating with a normal waveform; the other is motionless and held in a smooth arc at an angle from the cell body. Consecutive frames run from top to bottom beginning in the upper left corner. Frame rate accelerating to ~150 frames/s. × 1,400.
The cellswererollingcounterclockwiseasviewedfrombehind.

to theglasssurfacewillpreventitfromfurtherrolling,sothat

trappedagainsttheslideorcoverslip,wherecloseproximity

transitioninany onecell, itseemslikelythat asoneaxoneme

theaxonemes.Althoughwe havenotfollowedthecomplete

Thistransitionfromswimming ina helicalpathtocircling

presumablybecauseone axoneme was more effectivethan

notedthatthemodelsswam incirclesundersome conditions,

vatablecellmodelsusingproceduressimilartoourown,and

Goodenough (10)recentlyreportedthepreparationofreacti-

beendemonstratedforthetwo flagellaof Chlamydomonas.

medium .Thisisthefirsttimethatfunctionaldifferenceshave

Ca" concentrations.A similarunilateralinactivationof the

responddifferentlytosubmicromolarlevelsofcalcium:the

indicatesthatinitiallythehelicalpathwas left-handedand

viewedtowardthesurfaceover which theywere moving

circles.Thatthecellmodelscircledcounterclockwisewhen

stilllessactive, thetrappedcellswimsin smallerand smaller

increases,withtheresultthat thecellismore likelytobecome

increasesand the pitch of thehelicalswimming pathde-

FIGURE 11 Diagram illustrating some of the patterns of swimming

that could occur in Chlamydomonas. A cell that continuously yaws

in the plane of the flagellar beat will swim in a circle (left). A cell

that continuously rolls without yawing will swim in a straight line

(lower center). A cell that both yaws and rolls will swim in a helix

(center). (After Gray [11]).

This transition from swimming in a helical path to circling

against a surface involves a progressive inactivation of one of the

axonemes. Although we have not followed the complete transition in any one cell, it seems likely that as one axoneme

becomes less effective, the rate of turning (yawing) of the cell

increases and the pitch of the helical swimming path decreases,

with the result that the cell is more likely to become trapped against the slide or coverslip, where close proximity to the glass surface will prevent it from further rolling, so that it can only swim in a circle. As the affected axoneme becomes still less active, the trapped cell swims in smaller and smaller circles. That the cell models circled counterclockwise when viewed toward the surface over which they were moving indicates that initially the helical path was left-handed and the cells were rolling counterclockwise as viewed from behind.

Our results clearly show that the cis-axoneme and the trans-

axonemefordemembranated,reactivated Chlamydomonas

respond differently to submicromolar levels of calcium: the

trans-axonemewas less active at Ca\(^{++}\) concentrations below 10\(^{-8}\) M, whereas the cis-axoneme was less active at higher Ca\(^{++}\) concentrations. A similar unilateral inactivation of the trans-flagellum was observed in intact cells in calcium-free medium. This is the first time that functional differences have been demonstrated for the two flagella of Chlamydomonas. Goodenough (10) recently reported the preparation of reactiv-

able cell models using procedures similar to our own, and

noted that the models swam in circles under some conditions, presumably because one axoneme was more effective than

the other. However, no distinction was made between the

axonemes in that study.

The differential response of the axonemes to Ca\(^{++}\) may be
controlled by structural elements within the axonemes them-

selves, or modulated by extra-axonemal structures such as the

basal bodies or their associated fibers and rootlets. If the

former is true, then the two axonemes must be biochemically

different. In this context it is of interest that the cis- and trans-

flagella of Chlamydomonas differ in their development (14).

It may also be significant that when the percent of Chlamy-

domas axonemes that reactivated at various calcium concen-

trations was carefully quantitated, only ~50% of the axo-

nemes were found to be active at 10\(^{-7}\) M Ca\(^{++}\) (1).

The calcium-dependent modulation of cis- or trans-axon-

emal activity observed in the demembranated, reactivated

cell models is probably very important in the phototaxis of

intact cells. It has been proposed that the eyespot of Chla-

mydomonas underlies a photosensitive region of the plasma

membrane, and that together these structures form a direc-

tional antenna sensitive to light (7). As the cell swims in a

helical path, the antenna scans the environment over 360°
during every turn of the helix. When the intensity of light

striking the photosensitive region increases, the plasma

membrane is depolarized, leading to an influx of Ca\(^{++}\) from the

medium and a change in the flagellar beat pattern (see refer-

ence 7 for review). Because intact cells normally swim in

helical paths with the eyespot oriented toward the outside, the

intracellular concentration of Ca\(^{++}\) (or more accurately,

the concentration of Ca\(^{++}\) in the region of the Ca\(^{++}\)-sensitive

controlling element, which may be located entirely within the

flagellum) under these conditions is probably <10\(^{-8}\) M, the

concentration at which our models continued to swim in

helical paths. Based on our observations of the models, if the

Ca\(^{++}\) concentration increases from <10\(^{-8}\) to 10\(^{-7}\) or 10\(^{-6}\) M

upon photostimulation, then there will be a unilateral de-

crease in the activity of the cis-flagellum, causing the cell to

turn toward the side having the eyespot and hence toward

the source of the light. In the intact cell, the normal Ca\(^{++}\) levels

are presumably quickly restored, and the original balance of

beating is resumed. However, in our demembranated models

swimming in Ca\(^{++}\)-buffered solutions, the process that alters

the activity of the axonemes apparently continues until one of

the axonemes is completely inactivated.

The unilateral decrease in activity of the cis-axoneme as

the Ca\(^{++}\) concentration increases from 10\(^{-8}\) M to 10\(^{-7}\) or 10\(^{-6}\) M is therefore sufficient to account for the turning behavior of Chlamydomonas during positive phototaxis. During negative

phototaxis, photostimulation may result in a transient decrease in intracellular Ca\(^{++}\), leading to unilateral inactiva-

tion of the trans-axoneme and a turning away from the source

of the light. Interestingly, Smyth and Berg (22) have reported

that a pulse of light usually results in a transient decrease in

the beat frequency of a uniflagellate mutant having only the

trans-flagellum, and have suggested that this type of response

might occur in biflagellate cells during negative phototaxis.

Quiescence occurs in both axonemes of Chlamydomonas

when isolated, demembranated axonemes are resuspended in

a reactivation solution containing 10\(^{-7}\) M Ca\(^{++}\) (1). Quies-

cence at this high concentration of Ca\(^{++}\) seems to be con-
nected to the transient cessation of cell movement observed

just before and after backwards swimming during the pho-

tophobic response (2, 15, 21), and may or may not occur by a

mechanism related to that causing the selective inactivation

of the cis- or trans-axoneme. In any case, the patterns of

| Table I |

| Reversibility of Cis- and Trans-Axoneme Inactivation |

| Exp | Ca\(^{++}\) concentration | Reactivated models with trans-axoneme dominant | Ca\(^{++}\) concentration | Reactivated models with trans-axoneme dominant |
|-----|--------------------------|-----------------------------------------------|--------------------------|-----------------------------------------------|
| M   | % M                     | Ca\(^{++}\) concentration                   | % M                     | Ca\(^{++}\) concentration                   |
| 1   | 10\(^{-7}\)             | SRS                                        | 93                      | 10\(^{-7}\)                          |
| 2   | 10\(^{-7}\)             | SRS                                        | 85                      | SRS                                       |
| 3   | SRS                     | 10\(^{-7}\)                                 | 26                      | 10\(^{-7}\)                          |
| 4   | SRS                     | 10\(^{-7}\)                                 | 0                       | 10\(^{-7}\)                          |
| 5   | SRS                     | 10\(^{-7}\)                                 | 4                       | 10\(^{-7}\)                          |

* SRS, standard reactivation solution (containing 2mM EGTA and no added Ca\(^{++}\)).

TABLE I

Reversibility of Cis- and Trans-Axoneme Inactivation

| Exp | Ca\(^{++}\) concentration | Reactivated models with trans-axoneme dominant | Ca\(^{++}\) concentration | Reactivated models with trans-axoneme dominant |
|-----|--------------------------|-----------------------------------------------|--------------------------|-----------------------------------------------|
| M   | % M                     | Ca\(^{++}\) concentration                   | % M                     | Ca\(^{++}\) concentration                   |
| 1   | 10\(^{-7}\)             | SRS                                        | 93                      | 10\(^{-7}\)                          |
| 2   | 10\(^{-7}\)             | SRS                                        | 85                      | SRS                                       |
| 3   | SRS                     | 10\(^{-7}\)                                 | 26                      | 10\(^{-7}\)                          |
| 4   | SRS                     | 10\(^{-7}\)                                 | 0                       | 10\(^{-7}\)                          |
| 5   | SRS                     | 10\(^{-7}\)                                 | 4                       | 10\(^{-7}\)                          |

* SRS, standard reactivation solution (containing 2mM EGTA and no added Ca\(^{++}\)).
behavior seen in isolated axonemes and in demembranated cell models at various Ca** concentrations suggests that in the intact cell a turning response is induced by a relatively slight increase in intracellular Ca**, whereas a photophobic response is brought about by a much greater increase. The magnitude of the Ca** increase is probably related to the intensity of the stimulus; Boscov and Feinleib (2) reported that a more intense flash stimulus caused an increase in the number of photophobic responses relative to turn responses.

We do not yet know the mechanism by which the cis- or trans-axonemes are progressively and selectively inactivated. Inasmuch as the process occurs in at least the trans-axoneme of intact cells and is reversible in both intact cells and demembranated models, it could not be brought about by proteolysis. It seems more likely that the mechanism involves reversible modification of a polypeptide component, and that the equilibrium between the modified and unmodified states is shifted by changes in the Ca** concentration. Different mechanisms may operate in the two axonemes, or the cis- and trans-axonemes may respond differently to the same Ca**-dependent biochemical event.

The shift from dominance of the cis-axoneme to dominance of the trans-axoneme occurs as the free Ca** concentration increases from 10^-4 to 10^-7 M. Calmodulin is present in both the Chlamydomonas cell body and axoneme (9), and although soluble calmodulin binds little Ca** below 10^-7 M (4, 20), its affinity for Ca** could be considerably higher in the tightly bound state in which it exists in the axoneme (1, 25). Calmodulin could therefore be involved in the selective control of cis- or trans-axonemal activity. Alternatively, this control may be mediated by an as yet undescribed protein having a higher affinity for Ca** than does calmodulin.

We thank Dr. Richard Ray for Figs. 2A and 8C, Dr. John Aghajanian for expert assistance with the electron microscopy, and Dr. Harold Hoops for critically reviewing the manuscript.

This work was supported by U.S. Public Health Service grants GM 30626 and P30 12708.

Received for publication 7 July 1983, and in revised form 29 September 1983.

REFERENCES

1. Bessey, M., R. B. Fay, and G. B. Witman. 1980. Calcium control of waveform in isolated flagellar axonemes of Chlamydomonas. J. Cell Biol. 86:446-455.
2. Boscov, J. S., and M. E. Feinleib. 1979. Phototactic response of Chlamydomonas to flashes of light. I. Responsiveness of individual cells. Photobiol. Photobiol. 30:499-505.
3. Brokaw, C. J., D. J. L. Luck, and B. Huang. 1982. Analysis of the movement of Chlamydomonas flagella. The function of the radial spoke system is revealed by comparison of wild-type and mutant flagella. J. Cell Biol. 92:722-732.
4. Crouch, T. H., and C. B. Klee. 1980. Positive cooperative binding of calcium to bovine brain calmodulin. Biochemistry. 19:3692-3698.
5. Feinleib, M. E., and G. M. Curry. 1971. The relationship between stimulus intensity and oriented phototactic response (topotaxis) in Chlamydomonas. Physiol. Plant. 23:464-475.
6. Flagellar movement of Chlamydomonas [Motion picture]. Encyclopedia Cinematographic Japan Archives and Tokyo Cinema Inc., Tokyo; 1978. 16 mm. 5 min, 30 s.
7. Foster, K. W., and R. D. Snyth. 1980. Light antennae in phototactic algae. Microbiological Reviews. 44:572-680.
8. Gibbons, B. H., and J. R. Gibbons. 1974. Properties of flagellar "rigor waves" formed by abrupt removal of adenosine triphosphate from actively swimming sea urchin sperm. J. Cell Biol. 63:970-985.
9. Giteiman, S. E., and G. B. Witman. 1980. Purification of calmodulin from Chlamydomonas: calmodulin occurs in cell bodies and flagella. J. Cell Biol. 87:764-770.
10. Goodenough, U. W. 1983. Motile detergent-extracted cells of Tetrahymena and Chlamydomonas. J. Cell Biol. 96:1610-1621.
11. Gray, J. 1955. The movement of sea urchin spermatozoa. J. Exp. Biol. 77:325-353.
12. Hoops, H. J., and G. B. Witman. 1983. Outer double heterogeneity reveals structural polarity related to beat direction in Chlamydomonas flagella. J. Cell Biol. 97:902-908.
13. Hoops, H. J., R. L. Wright, J. W. Jarvis, and G. B. Witman. 1982. Characterization of flagellar activity in a Chlamydomonas mutant lacking normal striated fibers. J. Cell Biol. 95 (2 Pt. 2):3146 (Abstr.)
14. Huang, B., Z. Ramanis, S. K. Dutcher, and D. J. L. Luck. 1982. Unflagellated mutants of Chlamydomonas: evidence for the role of basal bodies in transmission of positional information. Cell 29:745-753.
15. Hyman, L. A., and G. G. Berry. 1978. Isolated flagellar apparatus of Chlamydomonas: characterization of forward swimming and alteration of waveform and reversal of motion by calcium ions in vitro. J. Cell Sci. 33:235-253.
16. Kamiya, R. 1982. Extrusion and rotation of the central-pair microtubules in detergent-treated Chlamydomonas flagella. Flagellar Motility. 1 (Suppl.) 169-173.
17. Okano, M., and Y. Hiramoto. 1979. Direct measurements of the stiffness of echinoderm sperm flagella. J. Exp. Zool. 20:77-87.
18. Racey, T. J., R. Hallett, and R. Nickel. 1981. A quasi-elastic light scattering and cinematographic investigation of motile Chlamydomonas reinhardtii. Biophys. J. 35:557-571.
19. Ringo, D. L. 1967. Flagellar motion and fine structure of the flagellar apparatus in Chlamydomonas: a cinematographic study. J. Cell Biol. 33:543-571.
20. Sasaki, O. 1983. Characterization of the role of cellular activation. Cell Calcium. 1:21-27.
21. Schmidt, J. A., and R. Eckert. 1976. Calcium couples flagellar reversal to photostimulation in Chlamydomonas reinhardtii. Nature ( Lond.). 262:713-715.
22. Snyth, R. D., and H. C. Berg. 1982. Change in flagellar beat frequency of Chlamydomonas in response to light. Cell Motility. 1 (Suppl.) 211-215.
23. Stark, R. L., and R. Hirschberg. 1973. Phototaxis in Chlamydomonas reinhardtii. J. Cell Biol. 59:367-377.
24. Waino, P. L., and H. J. Arnott. 1967. The comparative ultrastructure and possible function of eyespots. Euglena gracilis and Chlamydomonas eugametos. Plant Cell Physiol. (Bristol). 7:323-335.
25. Witman, G. B., and N. Minervini. 1980. Role of calmodulin in the flagellar axoneme: effect of phenothiazines on reactivated axonemes of Chlamydomonas. Cell Motility. 1 (Suppl.) 199-204.
26. Witman, G. B., R. B. Plummer, and G. Sander. 1978. Chlamydomonas flagellar mutants lacking radial spokes and central tubules. Structure, composition, and function of specific axonomal components. J. Cell Biol. 76:729-747.