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

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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.

The biflagellated green alga *Chlamydomonas* displays several behavioral responses, including positive and negative phototaxis (5, 23) and transient, light-induced reversal of the swimming direction (the "photophobic" response) (19, 21).

During the photophobic response, the two flagella coordinately change from a breast stroke-like, ciliary pattern of beating to an undulating, flagellar pattern (19, 21). This response is dependent upon extracellular Ca^{++} (21). It has been proposed that photostimulation of a specialized region of the plasma membrane overlying the eyespot (24) initiates transmembrane ion fluxes, leading to an elevated concentration of internal Ca^{++}; this in turn causes alteration of the flagellar waveform (21, 15). This is supported by observations that an increase in Ca^{++} concentration in the 10^{-6}–10^{-4} M range induces the transition from ciliary to flagellar waveform in isolated flagellar apparatuses (15) and in demembranated flagellar axonemes (1).

Ca^{++} also plays a crucial role in the phototaxis of *Chlamydomonas* (23), and it is presumed that phototaxis, like the photophobic response, involves stimulation of the photoreceptor followed by transmembrane ion fluxes and an increase in intracellular Ca^{++}, leading to the appropriate flagellar reaction. However, phototaxis in *Chlamydomonas* involves an abrupt change in the swimming direction with only a small change in swimming speed (2); this differs from the photophobic response in that, for such turning to occur, the two flagella must respond differentially to the events following photostimulation.

We show in this paper that a different pattern of beating occurs in the two axonemes of detergent-extracted models of *Chlamydomonas*, and that the balance of beating between the two axonemes is changed by varying the calcium concentra-

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tion at submicromolar levels. The responses of the axonemes to Ca^{++} appear to be sufficient to account for the behavior of living cells during turning and phototaxis. That the two axonemes respond differently to Ca^{++} 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 reinhardii* strain 137(+) was 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.[1] 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 dithiothreitol, 1 mM EGTA, 25 mM KCl, 0.5% polyethylene glycol (20,000 mol wt), 1% Ficoll (~400,000 mol wt), and 0.1% Nonidet P-40.[1] 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 dithiothreitol, 25 mM KCl, 2% polyethylene glycol [20,000 mol wt], 2 mM EGTA, and 1 mM ATP) or one of the Ca^{++}-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., Cambell, CA) was used in combination with a Strobex light source (Chadwick-Helmuth Co., Inc., Monrovia, CA). Kodak Tri-X 35-mm film was used at an effective speed of EI 3200 and developed in Perfection XR-1 developer (Perfection Photographic Products, Inc., Los Angeles, CA); Kodak Tri-X 16-mm film was exposed at EI 800 and processed commercially.

For electron microscopic examination, cells in demembranating solution were fixed by addition of an equal volume of cold 2% glutaraldehyde in demembranating solution. After 10 min, the suspension was centrifuged for 10 min at 12,000 *g*. The resulting pellet was further fixed in 1% glutaraldehyde in 100 mM sodium cacodylate, pH 7.2, for 50 min, followed by postfixation in 1% OsO_4 in 50 mM sodium cacodylate for 1 h. The pellets were then stained en bloc, dehydrated, and embedded as described elsewhere (12).

Quantification of Cis- or Trans-Axoneme Dominance: Reactivated cell models tended to rotate or swim in small circles against the surface of the microscope slide or coverslip. In each rotating or circling cell, the eyespot was facing toward or away from the center of rotation, depending on whether the *trans-* or *cis-*axoneme, respectively, was beating more effectively. In each experiment, approximately 50 rotating cells were scored as belonging to one of these two classes. Care was taken not to count cells rotating for trivial reasons, such as (*a*) one of the two axonemes was lost; (*b*) one of the two axonemes was partially disintegrated, as indicated by fraying or extrusion of the central pair from the tip (16); or (*c*) the tip of an axoneme was stuck to the glass surface. The proportion of cells rotating for these reasons varied from experiment to experiment, but amounted to as many as one-half of the total rotating cells. Cells were not scored if their eyespots were not readily apparent.

RESULTS

Appearance of Cell Models

When 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^{++} concentrations $\leq 10^{-6}$ 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^{++}), but a lower percentage were reactivated at 10^{-7} or 10^{-6} M Ca^{++} (see below). At 10^{-4} M Ca^{++}, 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^{++} were thus similar to those observed previously for isolated axonemes (1) or flagellar apparatuses (15). However, because of the Ca^{++}-dependent detachment of the axonemes, a model system composed of a cell body and two axonemes could be prepared only at low Ca^{++} 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 and 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

[1] More recently, we have found that inclusion of 4% sucrose in the wash solution protects the flagella from osmotic shock and prevents loss of flagella during washing, and that inclusion of 40% glycerol in the demembranating solution results in a cell model that can be reactivated after storage at −20°C for several days.

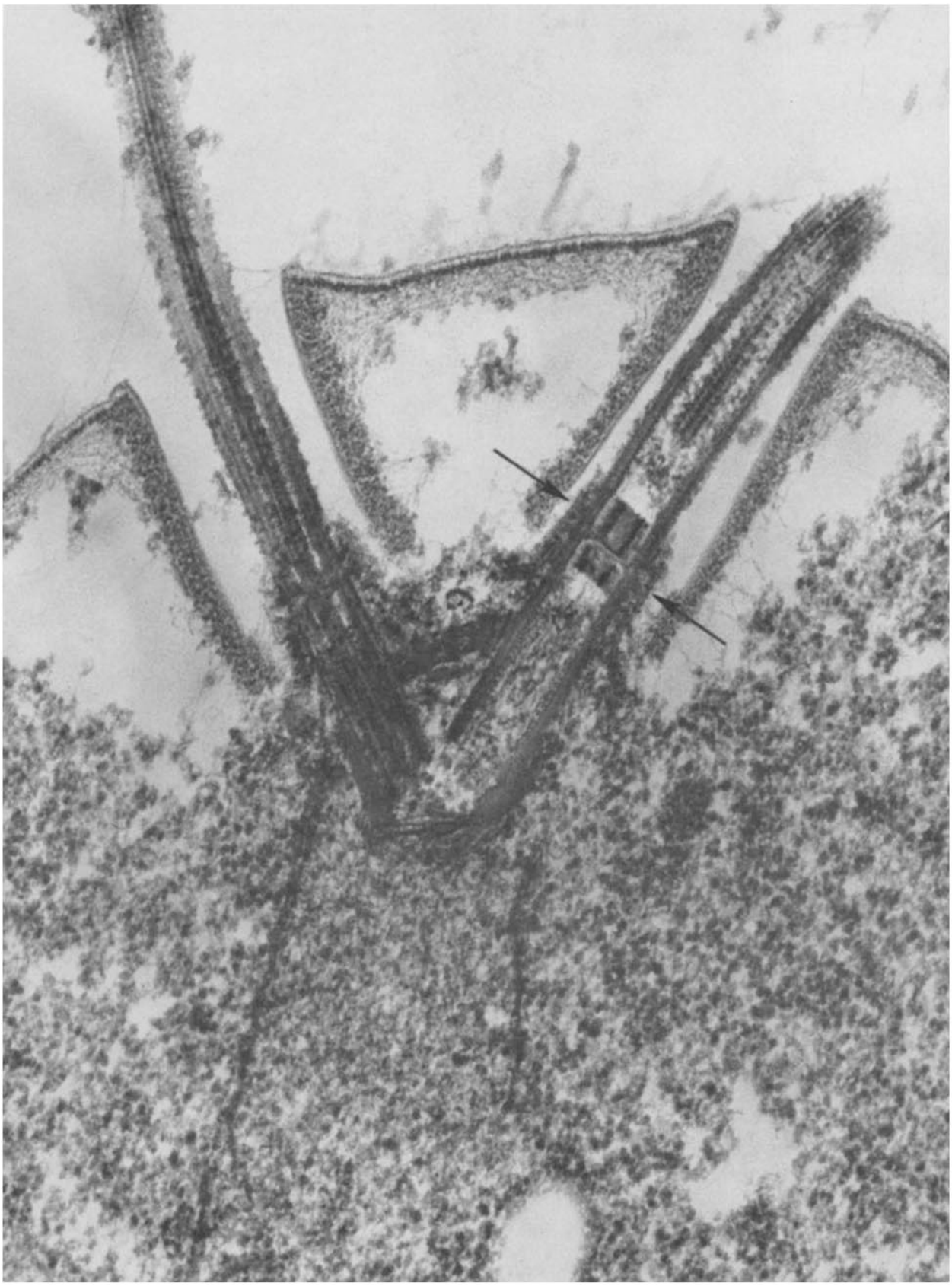

FIGURE 1 Electron micrograph of the flagella and basal bodies of a detergent-extracted *Chlamydomonas*. The plasma and flagellar membranes have been completely removed with the exception of a short cylinder of membrane (arrows) that often remains associated with the transition region of the flagellum. Proximal and distal striated fibers and basal body rootlets are still present after detergent treatment. × 88,000.

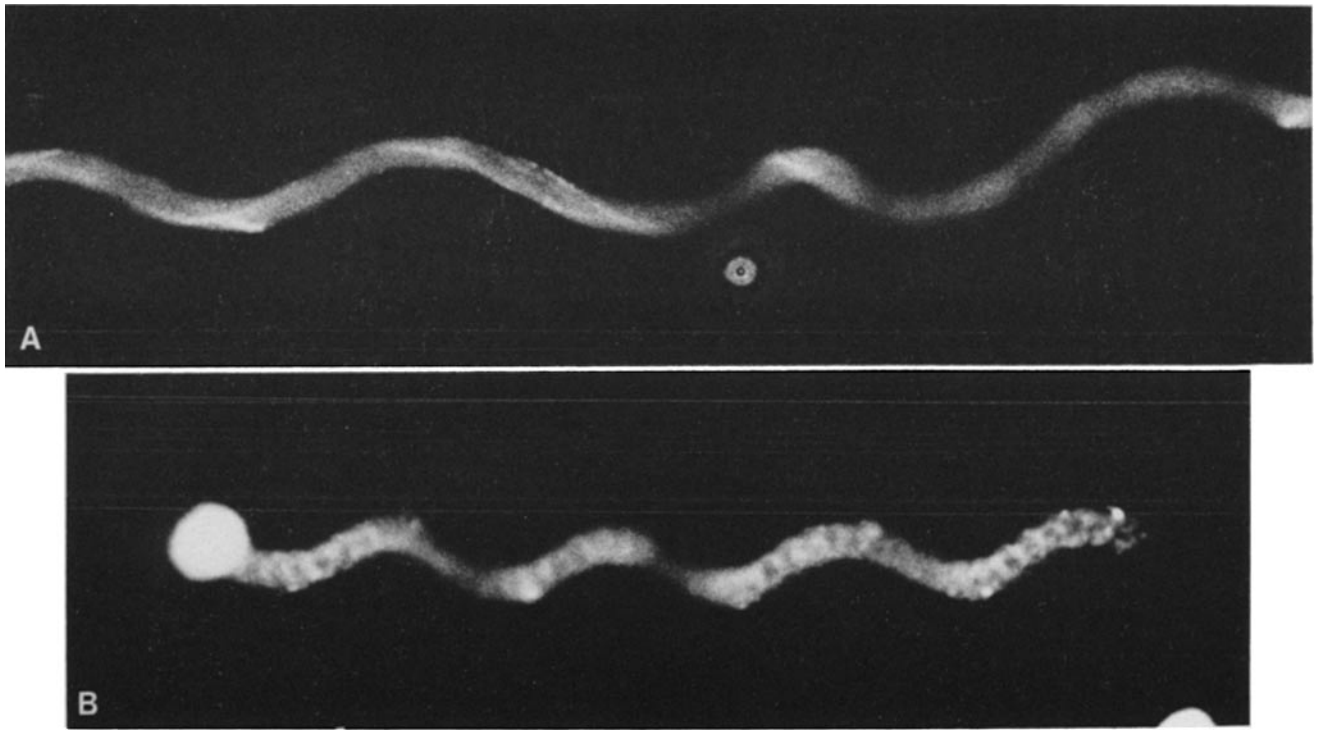

FIGURE 2 Dark-field micrographs showing typical helical swimming paths of (*A*) an intact cell in normal medium and (*B*) a demembranated cell model in a reactivation solution containing 10^{-9} M Ca^{++}. The micrographs are time exposures made using continuous illumination (*A*) or stroboscopic illumination at 10 Hz (*B*). × 710.

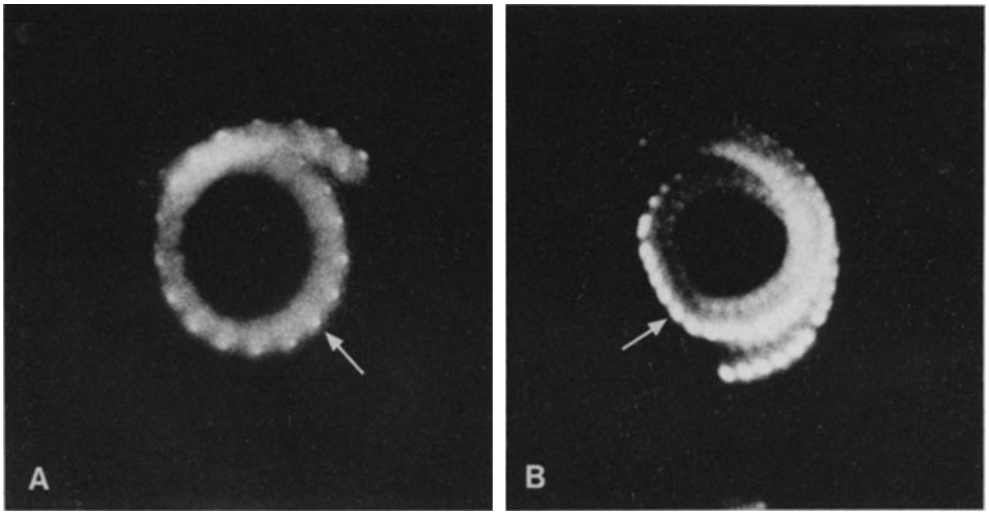

FIGURE 3 Dark-field micrographs showing tracks of demembranated cell models circling against the slide or coverslip in reactivation solution containing 10^{-9} M Ca^{++}. The eyespot is visible as a periodically repeating bright spot (arrow) along the outer edge of each track. Exposure conditions are the same as in Fig. 2*B*. × 890.

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[2]. 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*-

[2] We will use the terms "dominant," "most active," or "most effective" to refer to the flagellum or axoneme whose activity is most important in determining the direction of turning of the cell.

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^{-6} and 10^{-9} M. As in the standard conditions, cell models in reactivation solutions containing 10^{-6} 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^{-9} 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. 8*A*). Conversely, in 49 tracks recorded at 10^{-7} M Ca^{++},

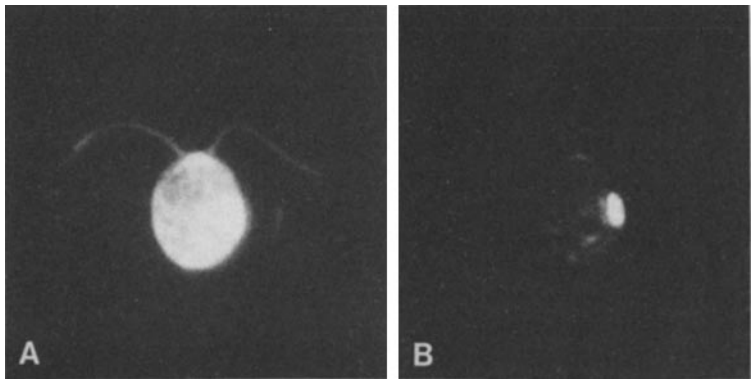

FIGURE 4 Single-flash dark-field micrograph of a demembranated cell model swimming in reactivation solution containing 10^{-9} M Ca^{++}. The micrograph was printed to show the axonemes (*A*) and the eyespot (*B*), which appears as a bright patch at the side of the cell. In this cell model the axoneme nearest to the eyespot (the *cis*-axoneme) was beating with a normal waveform, whereas the *trans*-axoneme was inactive and held in a circular arc. As a result, the cell was turning away from the eyespot. × 2,200.

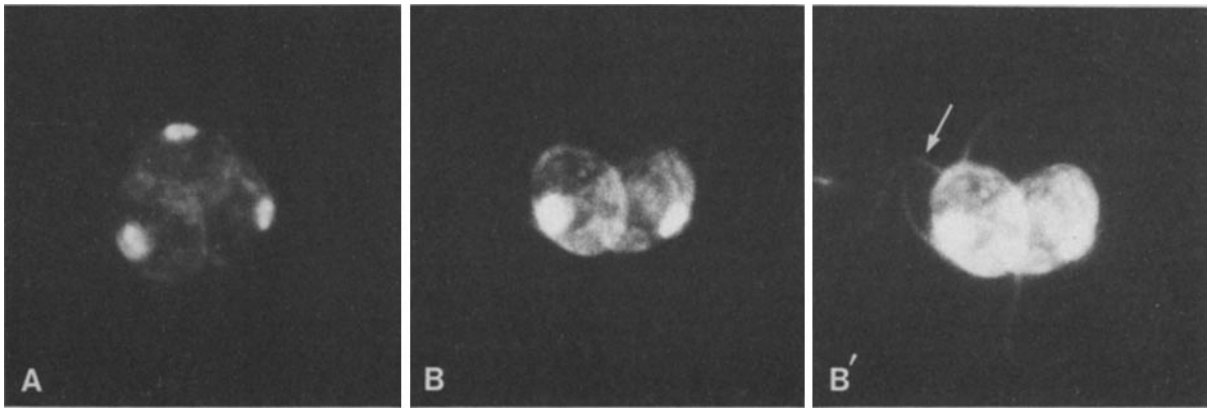

FIGURE 5 Triple (*A*) and double (*B* and *B'*) flash exposures of another cell model circling clockwise against the coverslip in reactivation solution containing 10^{-9} M Ca^{++}. *B* and *B'* were printed from the same negative to show the eyespot and axonemes, respectively. The *cis*-axoneme (arrow in *B'*) was beating; the *trans*-axoneme was inactive. Consequently, the side of the cell with the eyespot was farthest from the center of rotation. Flash rate, 3 Hz. × 2,000.

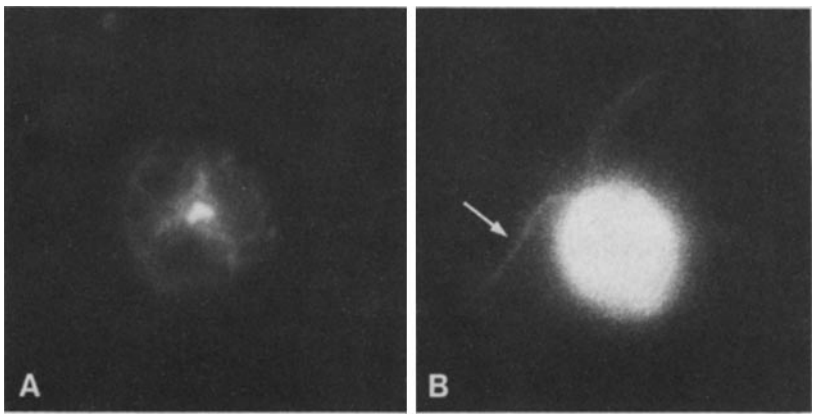

FIGURE 6 Triple (*A*) and single (*B*) flash exposures of a cell model circling clockwise against the coverslip in reactivation solution containing 10^{-7} M Ca^{++}. Under these conditions the *trans*-axoneme (arrow in *B*) was most active. In *A*, the three images of the eyespot are superimposed near the center of rotation. × 3,100.

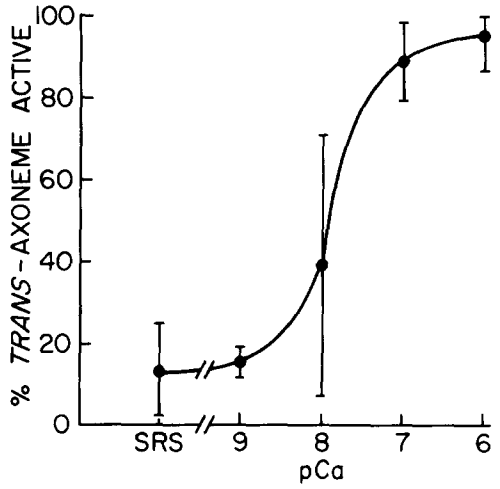

FIGURE 7 Graph of the percent of reactivated cell models in which the *trans*-axoneme was the most active one at the indicated concentrations of free Ca^{++}. Each data point represents the mean ± SD of about 10 experiments. *SRS*, standard reactivation solution (containing $<10^{-9}$ M free Ca^{++}).

67% of the models were oriented with their eyespots facing the inside of the helix (Fig. 8*B*). 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. 8*C*).

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 cinephotomicrography.

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 I). 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^{-6}–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

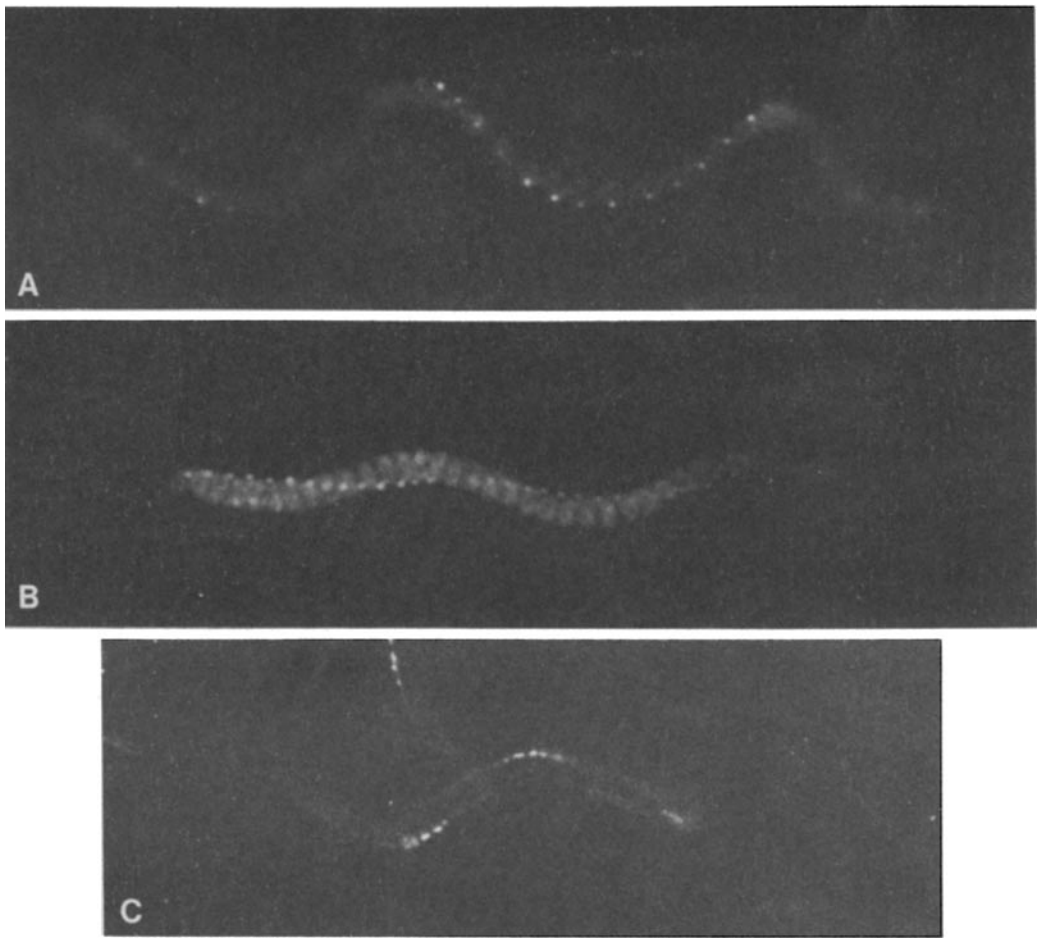

FIGURE 8 (*A* and *B*) Multiple exposure, dark-field micrographs showing the swimming paths of cell models reactivated at 10^{-9} M Ca^{++} (*A*) or 10^{-7} M Ca^{++} (*B*). The eyespot is visible as a periodic bright spot on the outer edge of the helix in *A* and on the inner edge of the helix in *B*. Flash rate, 10 Hz. × 630. (*C*) Swimming path of a living cell photographed with continuous illumination using a polarizing microscope. The eyespot is visible as a series of discrete birefringent spots along the outer edge of the helix. The small-scale discontinuities in the image arise from discontinuities in the rate of forward progression as the cell alternates between recovery and effective strokes (18). × 630.

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 $CaCl_2$[3]. The EGTA-induced quiescence of the *trans*-flagellum in the experimental cells was readily reversible; upon addition of 1 mM $CaCl_2$ to the HES solution, the cells quickly reverted to normal helical swimming. These observations indicated that reversible, highly selective, Ca^{++}-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[4] 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,

[3] *Chlamydomonas* was previously shown to swim and phototax normally in the presence of at least 10^{-2} M and 10^{-3} M extracellular Ca^{++}, respectively (21, 23). The passive influx of Ca^{++} under these conditions must therefore be sufficiently slow that the cell can maintain its normal intracellular Ca^{++} level, presumably by the action of calcium pumps in the cell or flagellar membrane.

[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.

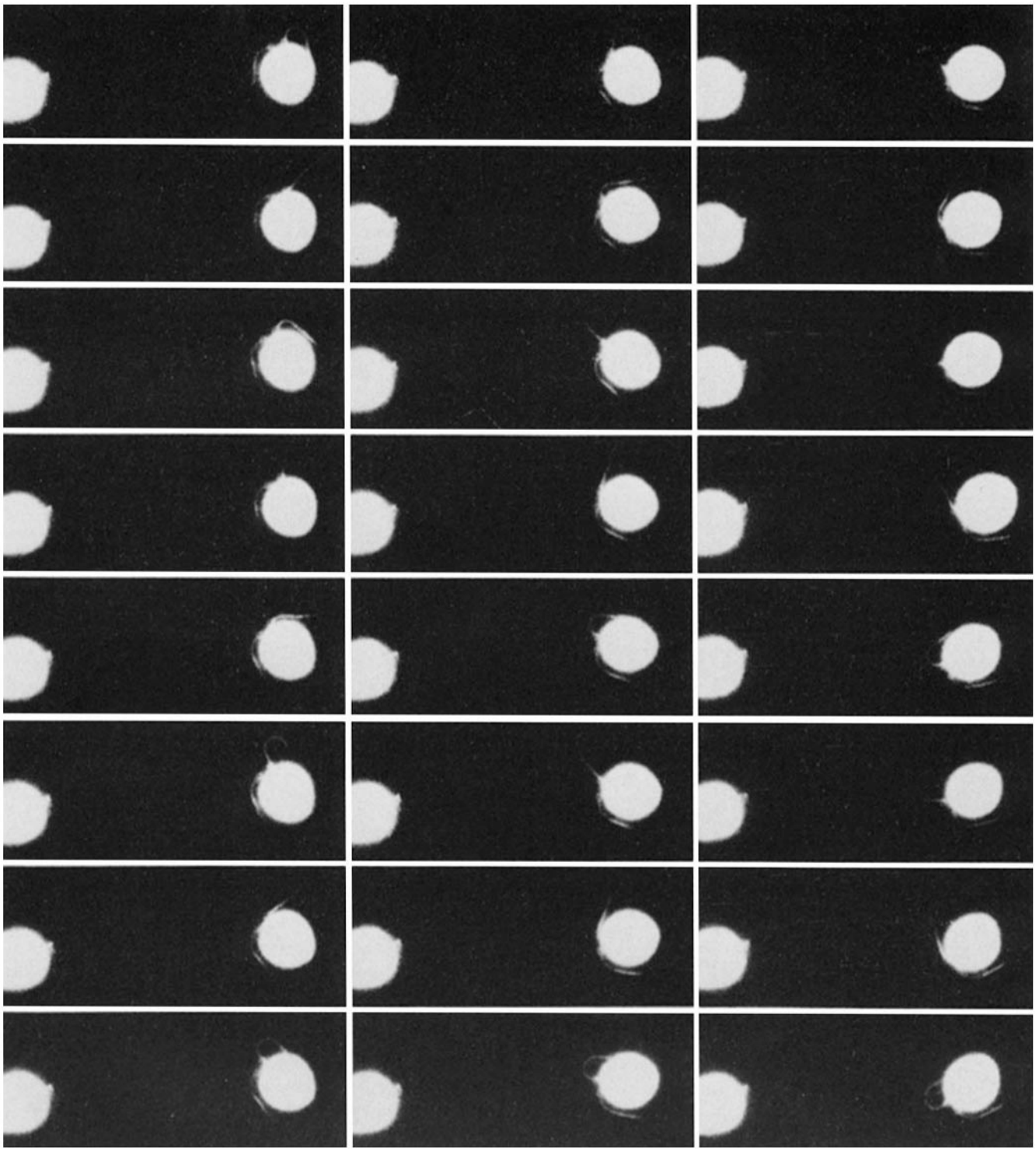

FIGURE 9 Film sequence of a cell model reactivated at 10^{-9} M Ca^{++}. One of the axonemes is beating with a normal waveform; the other is motionless and has a form resembling that of an axoneme at the beginning of its recovery stroke. Successive frames are arranged top to bottom beginning with the upper left frame. The frame rate was accelerating throughout the first column and thereafter was constant at 146 frames/s. × 1,400.

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. 8*C*, 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^{-6}–10^{-7} M Ca^{++} 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.

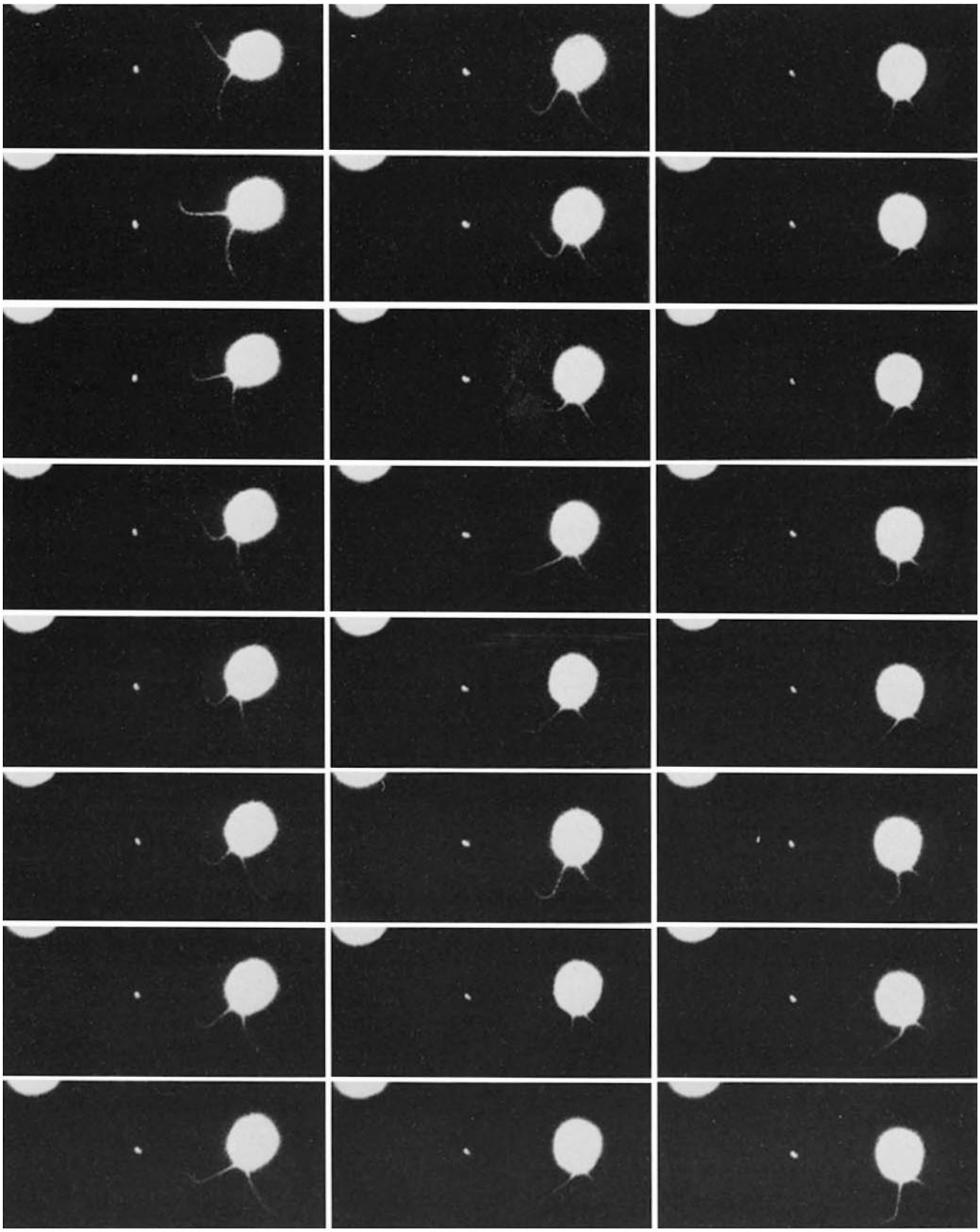

FIGURE 10 Film sequence of a cell model reactivated at 10^{-7} M Ca^{++}. One of the axonemes is beating with a normal waveform; the other is motionless and held in a smooth arc at an angle from the cell body. Consecutive frames run from top to bottom beginning in the upper left corner. Frame rate accelerating to ~150 frames/s. × 1,400.

TABLE I

Reversibility of Cis- and Trans-Axoneme Inactivation

	First solution		Second solution	
Exp	Ca^{++} concentration	Reactivated models with *trans*-axoneme dominant	Ca^{++} concentration	Reactivated models with *trans*-axoneme dominant
	M	%	*M*	%
1	10^{-7}	93	SRS*	39
2	10^{-7}	85	SRS	17
3	SRS	26	10^{-7}	92
4	SRS	0	10^{-7}	62
5	SRS	4	10^{-7}	65

Cell models were resuspended in a reactivation solution containing one concentration of Ca^{++} (the "first solution") and the reactivated cell models were scored for dominance of the *cis*- or *trans*- axoneme. The cells were then centrifuged and resuspended in the "second solution" containing a different concentration of Ca^{++}, and reactivated models were again scored for dominance of the *cis*- or *trans*-axoneme.

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

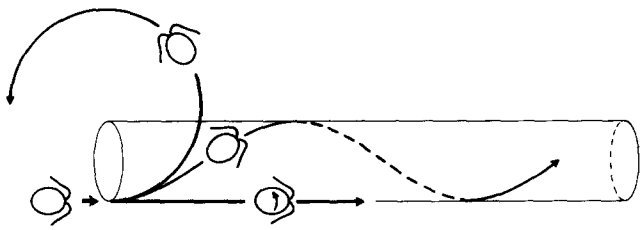

FIGURE 11 Diagram illustrating some of the patterns of swimming that could occur in *Chlamydomonas*. A cell that continuously yaws in the plane of the flagellar beat will swim in a circle (*left*). A cell that continuously rolls without yawing will swim in a straight line (*lower center*). A cell that 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*-axoneme of demembranated, reactivated *Chlamydomonas* respond differently to submicromolar levels of calcium: the *trans*-axoneme was 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 reactivatable 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 themselves, 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 *Chlamydomonas* axonemes that reactivated at various calcium concentrations was carefully quantitated, only ~50% of the axonemes were found to be active at 10^{-7} M Ca^{++} (1).

The calcium-dependent modulation of *cis*- or *trans*-axonemal 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 *Chlamydomonas* underlies a photosensitive region of the plasma membrane, and that together these structures form a directional 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 reference 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 $\leq 10^{-8}$ 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 decrease 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 inactivation 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^{-5} M Ca^{++} (1). Quiescence at this high concentration of Ca^{++} seems to be connected to the transient cessation of cell movement observed just before and after backwards swimming during the photophobic 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

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^{-8} 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.

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