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# Flagellar regeneration in the scaly green flagellate *Tetraselmis striata* (Prasinophyceae): regeneration kinetics and effect of inhibitors

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ABSTRACT: Flagellar regeneration after experimental amputation was studied in synchronized axenic cultures of the scaly green flagellate *Tetraselmis striata* (Prasinophyceae). After removal of flagella by mechanical shearing, 95 % of the cells regrow all four flagella (incl. the scaly covering) to nearly full length with a linear velocity of 50 nm/min under standard conditions. Flagellar regeneration is independent of photosynthesis (no effect of DCMU; the same regeneration rate in the light or in the dark), but depends on de novo protein synthesis: cycloheximide at a low concentration (0.35 µM) blocks flagellar regeneration reversibly. No pool of flagellar precursors appears to be present throughout the flagellated phase of the cell cycle. A transient pool of flagellar precursors, sufficient to generate 2.5 µm of flagellar length, however, develops during flagellar regeneration. Tunicamycin (2 µg/ml) inhibits flagellar regeneration only after a second flagellar amputation, when flagella reach only one third the length of the control. Flagellar regeneration in *T. striata* differs considerably from that of *Chlamydomonas reinhardtii* and represents an excellent model system for the study of synchronous Golgi apparatus (GA) activation, and transport and exocytosis of GA-derived macromolecules (scales).

## INTRODUCTION

In flagellated (ciliated) eukaryotic cells, flagella (cilia) readily regenerate after shedding or experimental amputation (e.g. Rosenbaum & Child, 1967; Rosenbaum et al., 1969). Flagellar regeneration has become an excellent model system to study the controlled development of a complex cell organelle and to analyse the mechanism and regulation of de novo flagellar protein synthesis (Guttman & Gorovsky, 1979; Lefebvre et al., 1980; Remillard & Witman, 1982; Brunke et al., 1982; L'Hernault & Rosenbaum, 1983; Baker et al., 1984; Schloss et al., 1984; Williams et al., 1986). Most of these studies were performed with *Chlamydomonas reinhardtii* which is very suitable for such studies because it is amenable to genetic analysis, and numerous flagellar mutants exist. In addition, the data base on the structure, biochemistry and function of the flagellar apparatus of *C. reinhardtii* is impressive (for a recent review see Huang, 1986). In contrast, information about flagellar regeneration in other algal systems is very limited. It has recently become clear, however, that significant differences in the pattern and kinetics of flagellar regeneration exist between *C. reinhardtii* and some other flagellated

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green algae (McFadden & Wetherbee, 1985; Melkonian et al., 1985a; Melkonian et al., 1985b; McFadden & Melkonian, 1986a; Coggin & Kochert, 1986; Melkonian et al., 1987a). Flagellar regeneration in scaly green flagellates (i.e. Prasinophyceae sensu Melkonian, 1987) is of particular interest because it involves a synchronous activation of the Golgi apparatus (GA) to assemble a whole new set of flagellar scales which are deposited on the regenerating flagella (review by Melkonian et al., 1986). It has been proposed to use flagellar regeneration in the prasinophytes *Tetraselmis* and *Scherffelia* as a model to study GA-activation and modus operandi in lower plants (Melkonian et al., 1987b). Toward this end it is necessary to learn more about the basic mechanisms of flagellar regeneration in these organisms. This paper describes the kinetics of flagellar regeneration and the effect of various inhibitors on flagellar regeneration in synchronized cultures of the scaly green flagellate *Tetraselmis striata.*

## MATERIALS AND METHODS

### Organism and culture conditions

*Tetraselmis striata* Butcher (Plymouth No. 443) was obtained from Dr. J. C. Green (Plymouth, England) and rendered axenic using the procedure of Surek & Melkonian (1980) except that cells were washed with 6 l of sterile seawater through a membrane filter of 8 µm pore size. Stock cultures were routinely screened for bacterial contamination with eleven different microbiological test media (Hoshaw & Rosowski, 1973: organic additives at 0.1 % w/v in 95 % seawater) made up either as liquid solutions or supplemented with 1 % agar. Incubations for sterility tests were made at two different temperatures (14 °C, 20 °C) and for varying periods of time (up to 6 months). Contamination was evaluated by light microscopy using phase contrast optics. Algae were cultured in an artificial seawater medium (ASP-2; McLachlan, 1973) which was modified by omitting the vitamin mix (Ricketts, 1974) and $Na_2SiO_3$, and by replacing $FeCl_3$ and Tris with Fe-EDTA and Hepes (McFadden & Melkonian, 1986b). Cultures were grown in 300-ml culture tubes in a light thermostat (Kniese; Marburg) at 20 °C, 25 watts/$m^2$ (Osram 40 W/25, warm-white) for 14 : 10 h light/dark cycle and bubbled with 6 l air per hour.

### Synchronized cultures

Cultures of *T. striata* were synchronized according to Ricketts (1979). One hour after the onset of the light regime the cells were diluted to a density of $0.45 \times 10^6$ cells/ml with fresh culture medium. To determine the degree of cell synchrony the following parameters were measured: cell density (using a hemacytometer with improved Neubauer ruling), % cell cycle stages (flagellated, non-flagellated, and dividing cells were counted separately in each sample), and DNA content (according to Ceriotti, 1952).

### Flagellar amputation and regeneration

Flagellar amputation was performed unless otherwise stated using cells from a synchronous culture two hours into the light period. 25 ml samples of cells were deflagellated using either the pH-shock method of Witman et al. (1972) or mechanical

shearing with a glass homogenizer (as described by McFadden & Melkonian, 1986a). Flagellar regeneration took place in the original culture medium under the same culture conditions and at the same cell density as in the synchronous culture. Aliquots were taken during different time intervals for determination of the percentage of flagellated cells and of flagellar length.

### Flagellar length measurements

For determination of flagellar length, cells were fixed and stained with Lugol's solution plus antipyrine/$FeCl_2$ according to Quader & Glas (1984). Flagellar lengths of 30 different cells from each sample were measured by light microscopy (Leitz, SM-lux) using a graduated ocular. Statistical evaluation involved determination of the average flagellar length plus standard deviation (s); for regeneration kinetics, regression curves were calculated and the correlation coefficient (r) determined. In addition, Student's t-test was used to compare the average flagellar lengths from different experiments. The flagellar length was measured from the opening of the apical flagellar groove to the flagellar tip and only on straight flagella.

### Use of inhibitors

Cycloheximide (Fluka, FRG) was used at a final concentration of 0.1 µg/ml (in culture medium); tunicamycin (2 µg/ml; Fluka, FRG) was applied to the algae according to Geetha-Habib & Bouck (1982). DCMU (3-[3,4-dichlorophenyl]-1,1-dimethylurea) was used at 4.3 µM (dissolved in ethanol), twice the concentration which completely blocked photosystem II-dependent oxygen evolution in *T. striata* as measured with an oxygen electrode (Lilley et al., 1975). Removal of an inhibitor during some experiments was performed by washing the cells without application of external pressure on a membrane filter (pore size: 1.2 µm) with 500 ml of sterile culture medium. The concentrated cells were then brought to the original cell density using filtered culture medium from a synchronous culture. Controls were treated in the same way.

## RESULTS

### Synchronous culture

Synchronous cultures of *T. striata* were established by diluting the culture each day to a density of $0.45 \times 10^6$ cells/ml one hour after the onset of the light regime. During the following 24 hours the cell density doubles and is again diluted to the original density (Fig. 1). *T. striata* reproduces by the formation of only two zoospores per cell irrespective of the growth conditions. Liberation of the zoospores from the parental thecae is not light dependent and takes place during the dark regime (Fig. 2). The degree of cell synchrony can be judged from the time interval required for cell number doubling (4 h; dividing cells were counted as two if two protoplasts were discernible; Fig. 1) and for DNA-synthesis (approximately 4 h; Fig. 1). DNA-content peaks at 10.5 hours (zero representing the onset of the light regime), cytokinesis starts at 10.5 hours and continues until the beginning of the dark regime (14.5 hours; Fig. 1). We have evaluated the relative

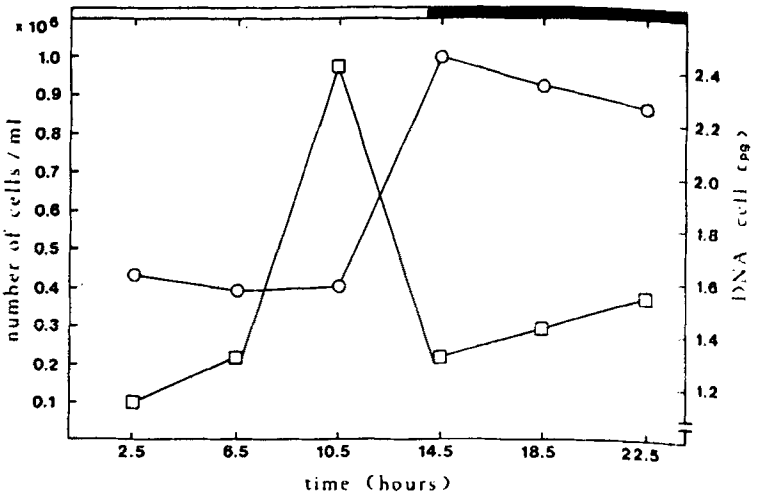



Fig. 1. Cell numbers (○) and DNA-content per cell (□) during a 24-h synchronous cell cycle of *Tetraselmis striata*

abundance of three distinct cell stages: flagellated cells, non-flagellated cells and cell division stages during a 24-h light/dark cycle in a synchronous culture (Fig. 2). It is evident that during the first 6 hours of the light regime the percentage of flagellated cells exceeds 80 % (Fig. 2). At the 6th hour the percentage of flagellated cells begins to decrease and at 10.5 hours less than 10 % of the cells are flagellated. We have determined that cells lose their flagella by shedding into the culture medium. At 10.5 hours more than 90 % of the cells are non-flagellated and apparently involved in DNA-synthesis (see

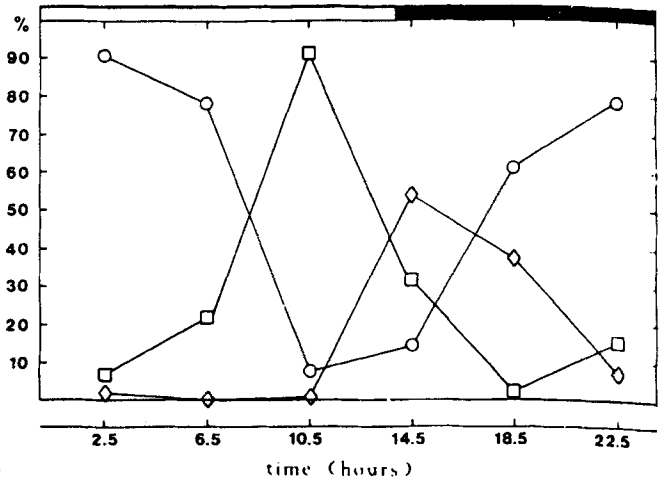



Fig. 2. Relative distribution (%) of different cell stages during a 24-h synchronous cell cycle. ○ = flagellated cells, □ = non-flagellated cells, ◇ = dividing cells (two daughter protoplasts within a parental theca)

above). Between 10.5 hours and 14.5 hours the percentage of non-flagellated cells decreases and correspondingly the number of division stages increases (Fig. 2). At 14.5 hours the percentage of flagellated cells increases again until the end of the dark regime. These flagellated cells all represent recently released zoospores. With the increase of the flagellated cells, the percentage of cell division stages decreases to zero until the end of the dark regime. A small percentage (10 %) of nonflagellated cells persists until the end of the dark regime (Fig. 2). Since there are always at least 5–10 % nonflagellated cells during the 24-h light/dark cycle, these might represent a non-dividing, "quiescent" population.

Although it should be possible to obtain an even better synchronization of *T. striata* using shorter light/dark cycles and more optimal growth conditions, the synchronization of a culture in a 24-h cycle is more convenient and the degree of synchronization was regarded as sufficient for the subsequent flagellar regeneration experiments.

## Flagellar length during the synchronous cell cycle

The four scaly flagella of *T. striata* are exclusively formed during the dark. This conclusion is based on measurements of flagellar lengths at different time points of the light/dark regime in a synchronous culture. In the interval between the 22nd hour and the 9th hour of the light regime no significant difference in the length of flagella (50 cells evaluated for each time point) was found. The mean flagellar length was 8.11 ± 0.52 µm. All four flagella of a cell have the same length. A small proximal portion of each flagellum is hidden in the apical flagellar groove. To determine the length of this portion, cells were deflagellated using a pH-shock (flagella always detach at the transition region) and flagellar lengths of isolated flagella were compared with flagellar lengths of whole cells. A difference of 0.9–1 µm was consistently found and therefore represents the length of the proximal portion of the flagellum retained inside the apical flagellar groove.

## Flagellar amputation and regeneration using pH-shock or glass homogenizer

Both methods of deflagellation usually lead to 98–100 % amputated cells. If deflagellation is performed around the 2nd hour of the light regime, usually more than 95 % of the deflagellated cells regenerate their four flagella (see below). Because the homogenizer-treatment yielded smaller standard deviations of flagellar lengths in regenerated flagella (10–20 %), all subsequent experiments were performed using cells deflagellated by homogenizer treatment.

## Kinetics of flagellar regeneration under standard conditions

Figure 3 shows the kinetics of flagellar regeneration of *T. striata* under standard conditions in a representative experiment. Flagella are regenerated (all four flagella with the same kinetics) to about 80–90 % of their original length within 180 min (Fig. 3, 4–7). No further flagellar growth occurs after 180 min and under no experimental conditions were flagella regenerated that reached the original flagellar length present before deflagellation. The velocity of flagellar regeneration is linear over the first 120 min (Fig. 3; regression curve: $y = 0.063x - 1.29$, $r = 0.9998$) with an average rate of 50 nm/min (n =

20; regression curve). Between 120 min and 180 min it decreases until flagella reach their final length. To determine whether a lag-phase before flagellar regeneration exists, we investigated the intersect of the flagellar regeneration curve with the abscissa assuming a linear velocity of regeneration during the first 60 min (Fig. 3). The curve intersects the

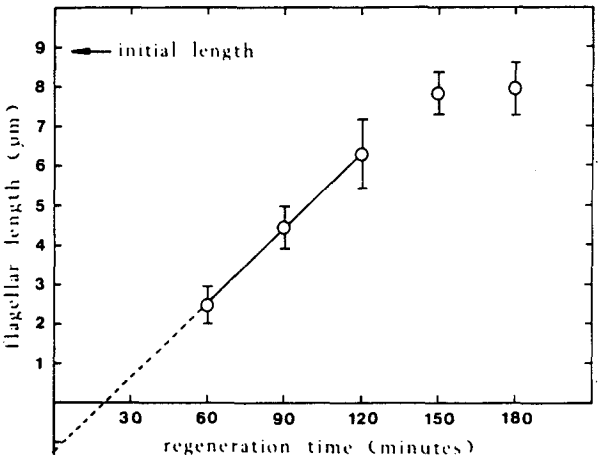
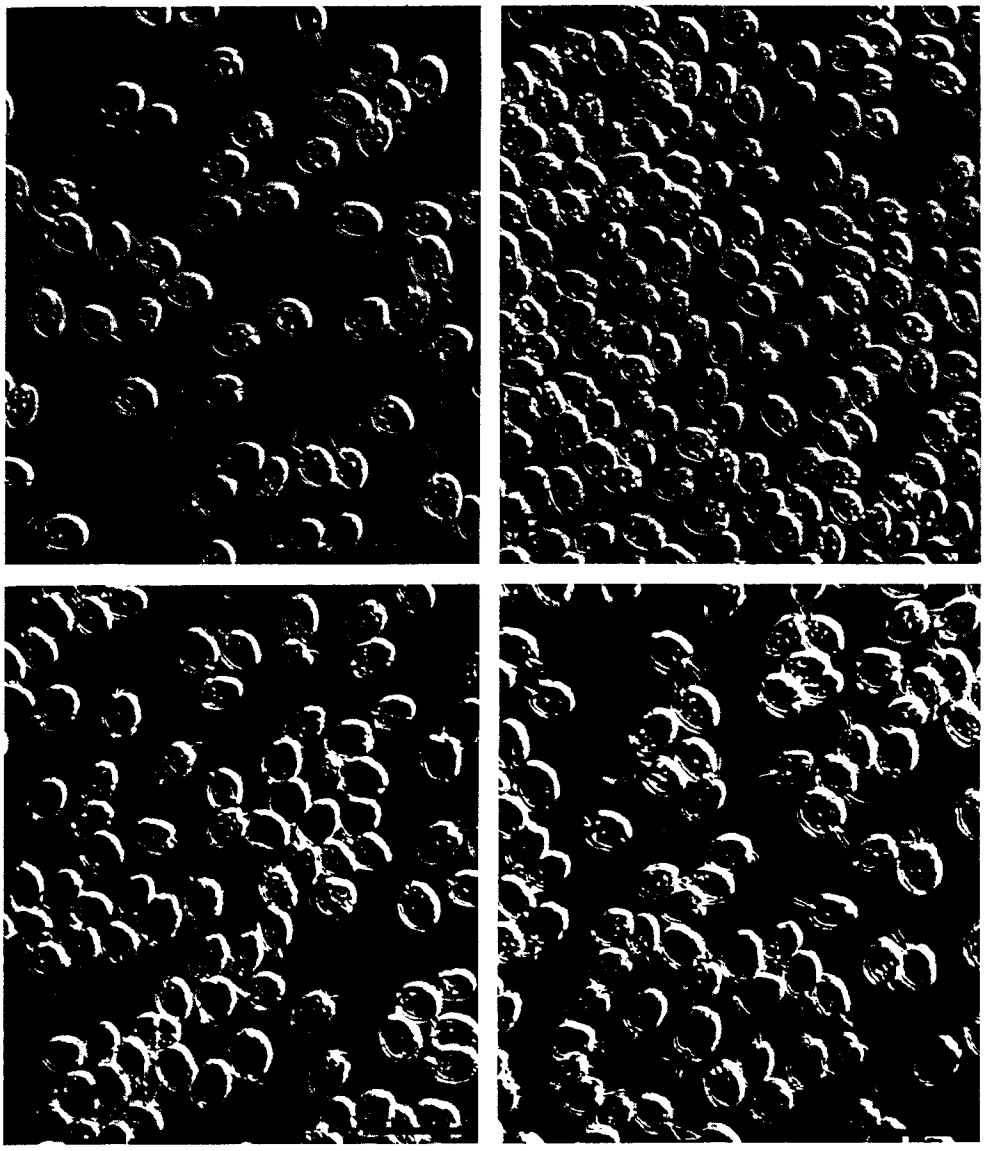



Fig. 3. Kinetics of flagellar regeneration of *Tetraselmis striata* under standard conditions (see "Materials and Methods"). ← = flagellar length before deflagellation (also in subsequent figures); bars represent the standard deviation at each time point during flagellar regeneration

abscissa at about −1.3 μm. Since the proximal portion of a flagellum retained inside the apical flagellar groove is about 0.9–1 μm (see above), only a short lag-phase (5–8 min) occurs before flagellar outgrowth commences. In accordance with this we note that newly formed mature flagellar scales have been seen inside the Golgi apparatus 10–15 min after deflagellation in the related *S. dubia* (McFadden & Melkonian, 1986a).

## Flagellar regeneration during the synchronous cell cycle

To determine whether the capacity for flagellar regeneration or the regeneration kinetics differ at different stages of the cell cycle, we investigated flagellar regeneration at four stages of the cell cycle (0.5 h, 3 h, 6 h, 9 h). Although the capacity for flagellar regeneration is greatly reduced in the 9th hour regenerations (only 45 % of the deflagellated cells regenerated their flagella in contrast to 98–100 % for the other time points), the kinetics of flagellar regeneration at the four time points were indistinguishable (not shown). The reduction in the percentage of cells regenerating flagella at the 9th hour presumably reflects the commitment of a proportion of the cells to cell division. The cells that started to regenerate flagella, however, proceeded with flagellar outgrowth for the following 2 hours even though they would have shed their flagella at this time point if they had not already been deflagellated.



Figs 4–7. Flagellar regeneration in *Tetraselmis striata* under standard conditions (see "Materials and Methods"). Magnification bar: 20 μm

Fig. 4. A population of cells from a synchronous culture (2 h into light period) before deflagellation

Fig. 5. Cells immediately after mechanical flagellar amputation (see "Material and Methods")

Fig. 6. Cells 90 min after deflagellation with partially regenerated flagella

Fig. 7. Cells 150 min after deflagellation

## Flagellar regeneration in the light and in the dark

Flagellar regeneration exhibited the same kinetics whether it was performed in the light or in the dark (regression curves of a representative experiment in the light: $y = 0.047x - 1.14$ [$r = 0.999$] and in the dark: $y = 0.055x - 1.56$ [$r = 0.998$]). This suggests that photosynthesis is not obligatory for flagellar regeneration of *T. striata*. To investigate this possibility further, we performed flagellar regeneration experiments in the light in the presence of DCMU which blocks photosynthetic electron transport through photosystem II. The kinetics of flagellar regeneration in the presence or absence of DCMU in the light are shown in Figure 8. DCMU had no effect on flagellar regeneration suggesting that photosynthetic electron transport through photosystem II is not involved in this process. The slightly prolonged lag-phase (ca 30 min.) for flagellar regeneration observed both in the presence or absence of DCMU (Fig. 8) might relate to DMSO (dimethylsulfoxide; 0.1 %) which was used as a solvent for DCMU. Flagellar regeneration being independent of light indicates that the breakdown of cellular reserves (mainly starch) could provide the energy required for flagellar growth. Accordingly we were able to induce four successive deflagellations and flagellar regenerations in the dark until no starch reserves were found after iodine staining (not shown).

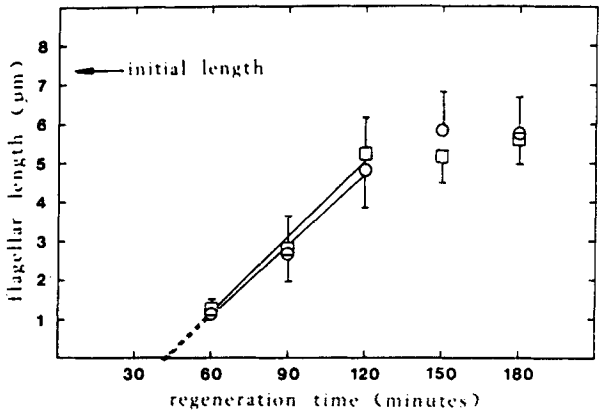



Fig. 8. Effect of DCMU on the kinetics of flagellar regeneration in the light. □ = 4.3 µM DCMU, ○ = control

## Effect of different temperatures on flagellar regeneration

Flagellar regenerations were performed at four different temperatures (10 °C, 15 °C, 20 °C, 25 °C). Regenerations at 20 °C and 25 °C had the same kinetics and were indistinguishable; higher temperatures (30 °C) were inhibitory with no flagellar regeneration observed. At 15 °C and 10 °C, the rates of flagellar regeneration were reduced (38 nm/min and 7 nm/min respectively), but still linear between 60 min and 120 min.

## Effect of cycloheximide on flagellar regeneration

To determine to what extent de novo protein synthesis is required for flagellar regeneration in *T. striata*, we tested the effect of cycloheximide (a protein synthesis

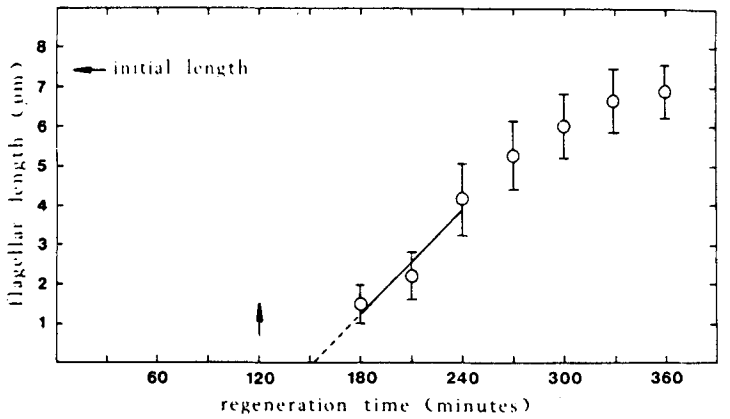



Fig. 9. Effect of cycloheximide (0.1 μg/ml) on flagellar regeneration. Cycloheximide was added immediately after deflagellation. 120 min after deflagellation cycloheximide was washed out (↑)

inhibitor at 80S ribosomes). Concentrations of cycloheximide as low as 0.35 μM completely blocked flagellar regeneration. This inhibition is fully reversible with no effect on the lag-phase, regeneration kinetics and final flagellar length or cell motility (Fig. 9). Higher concentrations of cycloheximide (> 1 μM) also blocked flagellar regeneration, but the effect was not fully reversible. We performed similar experiments at different stages of the cell cycle with the same results, suggesting that at times when there is no flagellar growth during the cell cycle there is no pool of flagellar precursors that can be used for flagellar regeneration in the absence of new protein synthesis. To determine whether a transient pool of flagellar precursors is formed during flagellar regeneration, we applied cycloheximide 60 min after deflagellation to regenerating flagella (Fig. 10). Interestingly, flagellar outgrowth is not immediately blocked by cycloheximide under these conditions but flagella grow for a further 2.5 μm with reduced velocity (21 nm/min) before no further growth takes place (Fig. 10). The same data were obtained when cycloheximide was added at 90 or 120 min after deflagellation (not shown). Once flagellar regeneration is

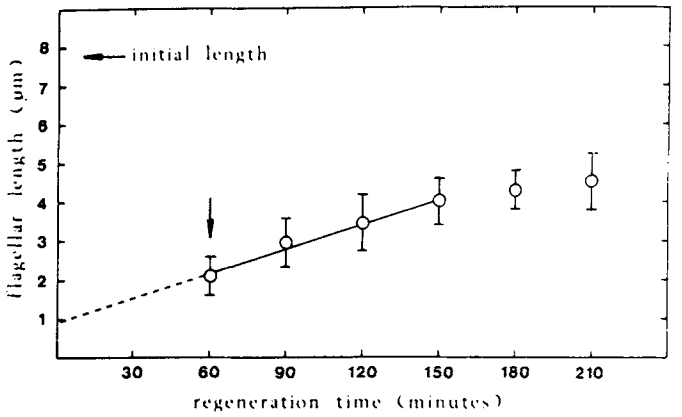



Fig. 10. Effect of cycloheximide (0.1 μg/ml) on flagellar regeneration. Cycloheximide was administered 60 min after deflagellation (↓)

completed (180 min after deflagellation), addition of cycloheximide after a new deflagellation again blocks flagellar outgrowth. These results suggest that only a transient pool of flagellar precursors is formed during flagellar regeneration and that this pool is depleted when flagella are fully regenerated.

## Effect of tunicamycin on flagellar regeneration

The effect of tunicamycin, an inhibitor of glycosyltransferase activity, on flagellar regeneration in *T. striata* is complex. If tunicamycin (2 µg/ml; a concentration found to block glycosylation in *Euglena;* Geetha-Habib & Bouck, 1982) is added immediately after deflagellation, its effect on the kinetics of regeneration and the final flagellar length is only slight (25 % inhibition of the rate of flagellar regeneration and of the final flagellar length; Fig. 11). After a second deflagellation in the presence of tunicamycin, however, flagella reach only 3 µm in length, a 60 % inhibition compared to the control. A 25 %

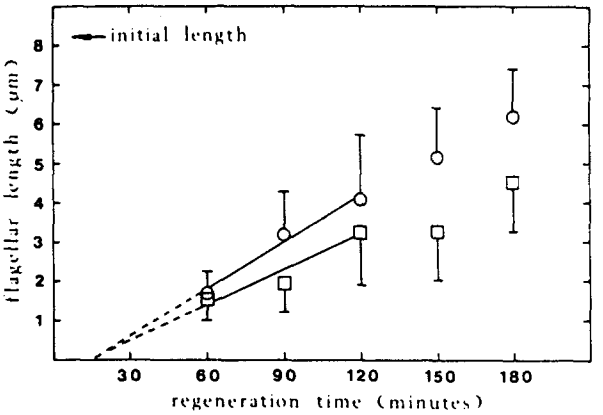



Fig. 11. Effect of tunicamycin (2 µg/ml) on flagellar regeneration. Tunicamycin was added immediately after deflagellation. □ = tunicamycin, ○ = control

inhibition of flagellar regeneration is also observed if tunicamycin is washed out 30 min after deflagellation (not shown), suggesting that the slight effect of tunicamycin is not due to a slow uptake into the cells. Thus, either glycoproteins are not required for flagellar regeneration (unlikely because glycoproteins have been detected, using PAS-staining after electrophoresis of flagellar proteins; unpublished results), or non-glycosylated proteins are added to the flagella (regenerated flagella, however, contain a full complement of structurally intact flagellar scales; McFadden & Melkonian, 1986a) and/or substantial pools of glycoproteins are available for flagellar regeneration. That the latter may be the case is also indicated by experiments in which we preincubated cells before deflagellation for various time periods in the presence of tunicamycin. A 180-min preincubation with tunicamycin reduced the final length of regenerated flagella by 50 % (compared to controls not preincubated in tunicamycin) irrespective of whether tunicamycin was present or absent during the actual flagellar regeneration period (Fig. 12). These data also suggested that the pool of flagellar glycoproteins is rapidly turned over. However, the renewed flagellar growth in the continuing presence of

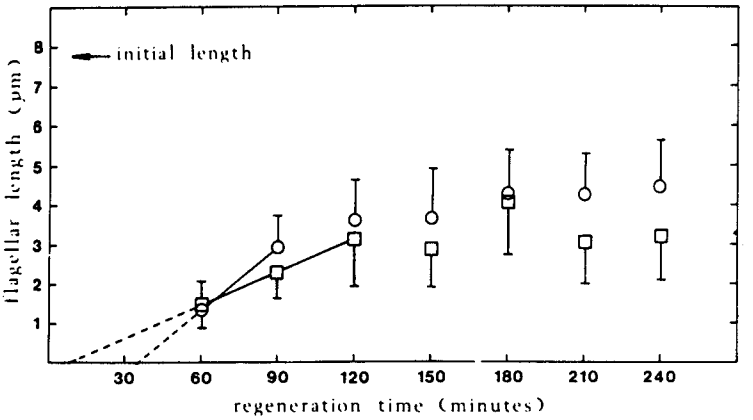



Fig. 12. Effect of a 180-min incubation in tunicamycin (2 μg/ml) prior to deflagellation on flagellar regeneration in the presence (□) or absence (○) of tunicamycin (2 μg/ml)

tunicamycin after the second deflagellation (see above) suggests that deflagellation possibly also induces de novo synthesis of glycosyltransferases which are inactivated by tunicamycin in a time-dependent process.

## DISCUSSION

Under adverse external conditions most flagellated green algae autotomize their flagella (Moestrup, 1982; Lewin et al., 1982). Flagellar shedding usually occurs at the distal end of the flagellar transition region (Rosenbaum et al., 1969; Lewin & Lee, 1985). The actual mechanism of flagellar shedding which, for example, requires localized breakage of axonemal microtubules and resealing of the membrane covering the flagellar stump is not yet understood. Recent studies suggested that external $Ca^{2+}$ is required for shedding (Huber et al., 1986; McFadden & Melkonian, 1986a) and an investigation using isolated, reactivated flagellar apparatuses of the green alga *Spermatozopsis similis* revealed that at $10^{-4}$ M $Ca^{2+}$ axonemes detached from the basal bodies and continued to beat with a flagella-type pattern (McFadden et al., 1987).

In contrast to the frequent occurrence of flagellar autotomy induced by external factors, regular autotomy of flagella during the cell cycle occurs only in a few green flagellates. Within the Prasinophyceae sensu Melkonian (Melkonian, 1987) only two taxa, *Tetraselmis* and *Scherffelia*, exhibit a regular autotomy of their flagella before the onset of cell division (this study and McFadden & Melkonian, 1986a). In general, green flagellates show three types of flagellar behaviour during the cell cycle: (a) flagella are autotomized before cell division (see above); (b) flagella are retained and cells remain motile during cell division (all other prasinophytes, and other "naked" taxa); (c) flagella are retracted into the cell body before cell division (most taxa with *Chlamydomonas*-type cell walls). It has been suggested that the presence of scales on the flagellar surface precludes retraction of flagella in the prasinophytes (Moestrup, 1982; McFadden & Wetherbee, 1985), but it is more likely that the retraction mechanism present in *Chlamydomonas*-type green algae evolved in this group only after the evolution of the chlamys and that the prasinophytes simply lack a mechanism for flagellar retraction.

Flagellar autotomy in green flagellates is usually followed by rapid regeneration of the flagella (Rosenbaum & Child, 1967; Rosenbaum et al., 1969). Flagellar regeneration has been most extensively studied in *Chlamydomonas reinhardtii* where it has become a model system for studies on gene expression and organelle morphogenesis (e.g. L'Hernault & Rosenbaum, 1983). Since flagellar regeneration in *Tetraselmis striata* differs in some important aspects from that of *C. reinhardtii*, it seems appropriate to discuss some of these differences in detail.

In *C. reinhardtii* and related organisms (e.g. *Volvox carteri*; Coggin & Kochert, 1986), a pool of flagellar precursors exists that can be used to regenerate at least part of the flagella in the absence of de novo protein synthesis. In wild-type *C. reinhardtii*, this precursor pool is sufficiently large to allow regeneration of half-length flagella (Rosenbaum et al., 1969; Lefebvre et al., 1978). Similar data were recently obtained using *Volvox carteri* (Coggin & Kochert, 1986). In other organisms, flagellar precursor pools also exist (Fulton & Kowit, 1975; Nelsen, 1975) and in the sea urchin embryo, flagella can be regenerated several times without the need for synthesizing additional flagellar protein (Auclair & Siegel, 1966). In *T. striata*, no flagellar precursor pool is present or can be used for flagellar regeneration in the absence of de novo protein synthesis. We used very low concentrations of cycloheximide (about 1/100 of the concentration used in the *Chlamydomonas* and *Volvox* experiments) and still obtained complete and fully reversible inhibition of flagellar regeneration. McFadden & Wetherbee (1985) also reported that in the prasinophyte *Pyramimonas gelidicola* cycloheximide blocks flagellar regeneration. It thus seems likely that scaly green flagellates lack a pool of flagellar precursors during their vegetative life history. Such a pool is present during flagellar development as our data indicate that following deflagellation a transient pool of flagellar precursors develops that is sufficient to grow about 2 µm of flagella in the absence of de novo protein synthesis. In accordance with this we found no mature flagellar scales during interphase in *S. dubia*, but abundant flagellar scales within the cell during flagellar regeneration (McFadden & Melkonian, 1986a). It may therefore be that the absence of a pool of flagellar precursors in prasinophytes is related to the lack of a pool of mature flagellar scales during interphase (see below). Our data using tunicamycin, however, suggest that a pool of flagellar glycoproteins sufficient to allow one complete flagellar regeneration in *T. striata* exists and that it is turned over relatively quickly. Similar observations have been made in other algae during flagellar regeneration (Bloodgood, 1982; Geetha-Habib & Bouck, 1982; McFadden & Wetherbee, 1985).

The flagellar regeneration kinetics also differ considerably between *C. reinhardtii* and *T. striata:* in *C. reinhardtii* flagellar regeneration proceeds with deceleratory kinetics and without a measurable lag-phase (Rosenbaum et al., 1969; Randall, 1969). The initial rate (conditions for regeneration similar to those used in this study) of flagellar regeneration in *C. reinhardtii* approaches 0.4 µm/min and nearly initial flagellar lengths are regained within 70–90 min (Rosenbaum et al., 1969). Then flagellar growth proceeds at a very slow rate of 0.0025–0.0033 µm/min for several hours. It is interesting in *C. reinhardtii* that flagella grow during interphase at about the same slow rate (Rosenbaum et al., 1969). In *T. striata*, however, no measurable growth of flagella was observed during the 14-h light period in synchronized cultures (see "Results"). Flagella are regenerated to only 80–90 % of the initial flagellar length before deflagellation in *T. striata;* a second deflagellation and flagellar regeneration lead to exactly the same final flagellar length

(see "Results"). It is possible that the lack of a basal slow rate of flagellar growth during interphase in *Tetraselmis* may be responsible for the reduced final length of regenerated flagella; alternatively the lack of a pool of flagellar precursors could be the reason for the shorter regenerated flagella.

These results also suggest that flagellar length control is regulated differently during flagellar regeneration after amputation compared to flagellar development during cell division, since we have not observed any significant differences of average flagellar lengths during several consecutive cell division cycles in synchronized cultures (unpublished observations). In *T. striata,* the rate of flagellar regeneration is linear over the first 120 min and is about 50 nm/min, i.e. only 12 % of the initial rate of flagellar regeneration in *C. reinhardtii.* Since, however, the rate of flagellar regeneration in *T. striata* is linear over the first 120 min, whereas it exhibits deceleratory kinetics in *C. reinhardtii* (Rosenbaum et al., 1969), flagellar regeneration in *T. striata* requires only about twice (2.3 times) the amount of time needed for flagellar regeneration in *C. reinhardtii.* If one takes into consideration that *T. striata* regenerates four flagella of 9 µm length compared to only two flagella of about 10 µm length in *C. reinhardtii,* the differences in the rate of flagellar synthesis/cell are further diminished (0.2 µm/min *[T. striata]* vs. 0.25 µm/min *[C. reinhardtii]* of total flagellar length formation/cell). Since it is known that the deceleratory kinetics of flagellar regeneration in *C. reinhardtii* are identical in the presence or absence of de novo protein synthesis (Rosenbaum et al., 1969), it appears likely that this type of kinetics results from the initial use of flagellar precursors from the pool, an assumption which is substantiated by other independent data (Rosenbaum et al., 1969).

Interestingly, in *Volvox carteri* (Coggin & Kochert, 1986), the initial rate of flagellar regeneration following amputation is at about 0.5 µm/min similar to that of *C. reinhardtii,* whereas during normal flagellar development this rate is about 60 nm/min, remains constant for 3 h and can be blocked by cycloheximide (Coggin & Kochert, 1986). It thus appears that in *V. carteri* too the higher initial rate and the deceleratory kinetics of flagellar regeneration following amputation are related to the presence of a pool of flagellar precursors, whereas during normal flagellar development only de novo protein synthesis contributes to the rate of flagellar growth. This rate is apparently very similar to the rate obtained in this study for flagellar regeneration in *T. striata* and in other studies using the freshwater prasinophytes *Scherffelia dubia* (60 nm/min; McFadden & Melkonian, 1986a) and *Nephroselmis olivacea* (52 nm/min; Melkonian et al., 1987a). However, McFadden & Wetherbee (1985) reported that flagellar regeneration in the scaly green flagellate *Pyramimonas gelidicola* took about 12–18 h and concluded that the "protracted flagellar regeneration is probably due to the requirement for the synthesis of a new complement of scales". Flagellar regeneration in this species was apparently performed at 15 °C. At this temperature, the rate of flagellar regeneration in *T. striata* is only 75 % of that at 20 °C (see "Results"). Taking the flagellar length (about 21 µm) into consideration, an average rate of flagellar regeneration for *P. gelidicola* under standard conditions can be calculated to be about 33 nm/min which is about 66 % of the rate of flagellar regeneration observed in this study. In conclusion, it appears that the rate and the kinetics of flagellar regeneration in scaly green flagellates are not significantly different from that of other green algae provided that only de novo flagellar protein synthesis occurs and no pool of flagellar precursors is available for regeneration. In addition, the

data suggest that the synthesis of flagellar scales in prasinophytes is presumably not the rate-limiting process during flagellar regeneration.

It has been found that de novo flagellar protein synthesis in *C. reinhardtii* is initiated 5–10 minutes after deflagellation (Lefebvre et al., 1978), a time interval that compares favourably with the lag-phase (5–8 min) after deflagellation before flagellar regeneration commences in *T. striata.* Since the formation of mature flagellar scales requires 10–15 minutes (McFadden & Melkonian, 1986a), the synthesis of flagellar proteins and flagellar scales appears to be tightly coordinated. Another interesting feature of flagellar regeneration in *T. striata* is that it occurs at the same rate and kinetics in light or darkness. Unfortunately, only few comparative data are available on the energy requirements for flagellar regeneration in green algae. Recently, Melkonian et al. (1985a) and Coggin & Kochert (1986) reported an absolute requirement for light and photosynthetic electron transport for normal flagellar development in *V. carteri* (Coggin & Kochert, 1986) and for flagellar regeneration in *Scourfieldia caeca* (only one of the two flagella; Melkonian et al., 1985a). In both organisms, inhibitors of photosynthetic electron transport (DCMU; atrazine) block light-dependent flagellar development. Surprisingly, no published data are available on flagellar regeneration of *C. reinhardtii* in the dark. In *Chlamydomonas moewusii,* Lewin (1953) observed that "when cells with shortened flagella are submerged in distilled water, in light or darkness, the flagella commence to elongate at once". It is, however, not known whether de novo flagellar protein synthesis in *Chlamydomonas* is light-dependent. In *T. striata* and other prasinophytes (e.g. *Nephroselmis olivacea, Scherffelia dubia;* unpublished observations), the rate of flagellar regeneration is independent of light, and in the light (if photosynthetic electron transport is blocked) or in darkness flagellar regeneration occurs at the expense of cellular reserves, primarily starch (see "Results"). These data may also explain why prasinophytes usually store excessive amounts of starch inside their plastids. These reserves enable the cell to develop flagella through several consecutive cycles in darkness (in *T. striata* up to four cycles; see "Results"). In *Chlamydomonas* and related organisms, the pool of soluble flagellar precursors is replenished during each flagellar regeneration (Rosenbaum et al., 1969) and can be used upon deflagellation to develop motile flagella in darkness/in the absence of de novo protein synthesis. Prasinophytes presumably cannot store sufficient amounts of mature flagellar scales for flagellar regeneration. The total number of flagellar scales needed for the development of flagella in *Tetraselmis* or *Scherffelia* is in the order of 50 000–70 000 (Melkonian, 1982; McFadden & Melkonian, 1986a). It thus seems likely that the lack of a pool of flagellar precursors in prasinophytes is related to the inability to store sufficient flagellar scales and that this inadequacy is overcome by excessive starch reserves that can be used for the development of flagella by de novo protein synthesis in the dark and perhaps also (at least in part) in the light. Conversely, one may speculate that the evolution of a significant pool of flagellar precursors in *Chlamydomonas*-type organisms was only advantageous once their flagellated ancestors lost/transformed their scales. This possibly enabled *Chlamydomonas*-type algae to reduce their starch reserves and increase the volume of the thylakoid system. This hypothesis should be further tested by comparative analysis of flagellar regeneration and development in additional flagellated green algae.

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