Timing of events in overlapping cell reproductive sequences and their mutual interactions in the alga *Scenedesmus quadricauda*

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Summary

Synchronous populations of the chlorococcal alga *Scenedesmus quadricauda* were subjected to various irradiances, photoperiods, heterotrophic growth and chloramphenicol treatment in order to induce different cell cycle patterns. The courses of cellular and nuclear division, and of commitments to them, were followed to measure duration of growth and reproductive steps and to analyze their mutual interactions in overlapped cell reproductive sequences. Duration of the growth steps (precommitment periods) was inversely proportional to the irradiance applied. It was prolonged if the growth was retarded by chloramphenicol. It is argued that light has an exclusively trophic function in regulating growth steps and can be replaced by glucose. The number of growth steps, and consequently the number of reproductive sequences, at a given irradiance can be experimentally changed by the photoperiod. The reproductive steps (postcommitment periods) are triggered by the termination of corresponding growth steps. They do not require any supply of external energy, and can therefore be terminated in darkness or in the presence of chloramphenicol. By varying the number of growth steps and their duration, a different extent of overlapping consecutive reproductive steps can be obtained. This overlapping is the major factor affecting the duration of reproductive steps which are proportional to the extent of overlapping. Complex interactions between growth conditions and overlapped reproductive steps resulted in substantial variations in the duration of the whole cell cycle. The present findings do not support the operation of a timing mechanism such as a pacemaker (Zeitgeber) or circadian rhythms, either in regulating the duration of the cell cycle or in the duration of reproductive steps.

Key words: duration of cell cycle, duration of growth steps, duration of reproductive steps, irradiance, illumination regimes, chloramphenicol, heterotrophic growth, *Scenedesmus quadricauda*.

Introduction

The cell cycle is usually considered as the period between the birth of a cell and its division into new daughter cells. In the classical model of Howard and Pelc (1953), the cell cycle is subdivided into four, distinctly separated phases (G_1, S, G_2, M). Mitchison (1971) considered the cell cycle to encompass two independent but coordinated processes. One sequence of processes, termed 'growth', consists of activities which produce the bulk of new cytoplasm; the second is called 'DNA-division sequence' and comprises reproductive processes. This model takes account of the fact that synthetic and growth processes are not restricted only to the G_1 period but can run concomitantly with reproductive processes. At the time the model was developed, the concept of overlapping cell cycle steps was well-known only in prokaryotic cells (Cooper and Helmstetter, 1968; Helmstetter and Cooper, 1968; Helmstetter *et al.* 1968). Using algae dividing by multiple fission as model organisms, Šetlík *et al.* (1972) proposed a general concept of the cell cycle as overlapping cell reproductive sequences. It is a great advantage to use algae for the cell cycle studies because several cell reproductive sequences occur within one cell cycle. Furthermore, the number of these sequences and the extent of their overlapping can easily be regulated by the supply of light energy (Šetlík *et al.* 1972; Zachleder *et al.* 1975; Šetlík and Zachleder, 1983, 1984). Evidence that in general the same rules are valid for all eukaryotic cells comes from experiments in mammalian cells (Liskay *et al.* 1979; Liskay and Prescott, 1978), in *Ciliata* (Berger and Ching, 1988) and in yeasts (Singer and Johnston, 1981, 1985). It is therefore not surprising that a model very similar to that developed by Šetlík's group was formulated for eukaryotic cells dividing by binary fission (Cooper, 1979, 1984; Okuda and Cooper, 1989).

Bringing together the above mentioned findings in eukaryotic and algal cells (Šetlík *et al.* 1972; Zachleder *et al.* 1975; Zachleder and Šetlík, 1982), a unified concept of the cell cycle has been proposed (Šetlík and Zachleder, 1984). A doubling of cell mass and its organelles, duplication and separation of genetic information and corresponding structures occur in a complex sequence of several steps which can be called 'cell reproductive sequence'.

The first step in this sequence is the growth step in which the cell builds up functional structures and accumulates reserves of energy. The rate of progress

through the growth step depends on the supply of external energy. At the end of the growth step the cell becomes competent (committed) to proceed through the rest of the cell reproductive sequence without external energy supply.

The step following commitment is the DNA replication step. It includes those processes specifically required to prepare for DNA replication. Once initiated, replication will be completed even if the external energy supply is removed. This is also true of the last two steps of cell reproductive sequence, namely the nuclear division and cell division steps. Both these steps include the preparatory events which build up the mitotic and cytogenetic apparatus, and the division processes themselves.

If growth conditions allow it, a new cell reproductive sequence is initiated which overlaps with the preceding one. The extent of overlapping is determined by the duration of individual growth steps and is thus dictated by growth conditions. In cells dividing by binary fission, only rarely do more than two cell reproductive sequences overlap, whereas in algal cells several overlapped cell reproductive sequences usually occur in one cell cycle. The first one starts in the preceding cell cycle and the last one is terminated in the following cell cycle.

To explain cell cycle patterns in algal cells, the hypothesis of so called 'Zeitgeber' (pacemaker) was introduced (Lorenzen, 1957; Pirson and Lorenzen, 1958). According to this hypothesis, light functions as a pacemaker which induces the cells to terminate their cell cycles in a constant time period regardless of growth conditions (i.e. irradiance, photoperiod, temperature). This hypothesis has been perpetuated (Lorenzen and Hesse, 1974; Lorenzen, 1970, 1980) despite contradictory experimental results (Pirson and Lorenzen, 1966; Lorenzen and Albrodt, 1981; Tischner and Lorenzen, 1987).

Our earlier papers dealing with the cell cycles of algae (Šetlík *et al.* 1972; Zachleder *et al.* 1975; Šetlík and Zachleder, 1983, 1984) have indicated that the course of cell cycle processes is much more complex than hitherto thought, and that the overlapping reproductive processes and their mutual interactions must be taken in account.

In this paper, the effect of overlapping of individual cell reproductive sequences on both their own duration and the duration of the entire cell cycle was studied in synchronous populations of the chlorococcal alga *Scenedesmus quadricauda*. A wide range of cell cycle patterns, differing in a number of cell reproductive sequences and in an extent of their overlapping, was obtained by growing algal cells under different irradiances, different intervals of light, in the presence of chloramphenicol or under conditions of heterotrophic growth. The paper provides evidence that the regulation of the cell cycle in algae does not include any light-driven timing mechanisms, but is the result of the trophic function of light and of mutual relationships between overlapped cell reproductive sequences.

Materials and methods

Organism

The chlorococcal alga *Scenedesmus quadricauda* (Turp.) Bréb. strain Greifswald/15 was obtained from the Culture Collection of Autotrophic Organisms kept at the Institute of Botany, Třeboň, Czechoslovakia.

Culture conditions

Cultures were synchronized by alternating light (L) and dark (D) periods (L:D, 14:10 h) and were cultured in 1000 ml plate-parallel vessels (18 mm in width) at 30 °C, illuminated from one side by incandescent lamps (500 W). Irradiance at the surface of the culture vessels was approximately 180 W m^{-2} of photosynthetically active radiation (400 to 720 nm wavelength). Carbon dioxide concentration in the aerating gas mixture was 2 % (v/v). The inorganic nutrient solution was as described by Zachleder and Šetlík (1982). Cell concentration at the beginning of the cell cycle was about 10^6 cells ml^{-1}. Continuously diluted cultures were used for experiments. Various dilution rates were chosen to obtain cultures of different growth rate. Details of the culture equipment and conditions were the same as those described by Doucha (1979).

Assessment of commitment curves

Samples were withdrawn from synchronous populations at one- or two-hourly intervals and incubated under aeration in the dark at 30 °C. At the end of the cell cycle, the dark incubated samples were fixed by iodine kalium–iodide solution and the percentage of binuclear cells, four-celled and eight-celled daughter coenobia and the fraction of undivided mother cells were estimated. Also the content of DNA was assayed (as described by Zachleder and Šetlík, 1988) in the dark incubated samples.

The values obtained by the assay of dark samples were plotted against the time at which the samples had been darkened. The curves obtained are termed the 'commitment' curves. Note: in our earlier papers we have used the term 'induction' instead of the now more commonly used term 'commitment' (Šetlík *et al.* 1972; Zachleder *et al.* 1975). The significance of the term 'commitment' for various processes is explained and the method for its determination is described by Šetlík and Zachleder (1984, 1988).

Cell counting

Cells were counted in the Bürker counting chamber (produced by Meopta, Czechoslovakia).

Staining of nuclei

Nuclei were fluorochromed with acridine orange and observed through the fluorescent microscope. Details of the procedure were described by Zachleder *et al.* (1974).

Measurement of irradiance

In order to define light conditions in suspensions of varying density and irradiated by different incident irradiance, the mean irradiance (I) was calculated from the irradiance at the surface of the culture vessel (I_i) and the irradiance transmitted through the suspension (I_t) according to the formula:

$$I = (I_i - I_t)/\ln(I_i/I_t).$$

A non-selective device calibrated in energy units (W m^{-2}) was used to measure photosynthetically active radiation (400–720 nm) as described by Kubín (1971).

Results

Estimation of the duration of individual steps in cell reproductive sequences: commitment–division diagrams

The course of events in the cell cycle can be characterized by commitment–division diagrams. In these diagrams, the percentage of cells in synchronous populations which became committed to, or terminated after the first, second, third and further DNA replications, nuclear divisions or protroplast fissions, were recorded during the cell cycle.

Curves recording all these processes in one figure make the commitment–division diagrams rather complex, particularly at high growth rates where four extensively overlapped cell reproductive sequences occur (see Šetlík and Zachleder, 1983, 1984). For the purposes of this paper the commitment–division diagrams are substantially

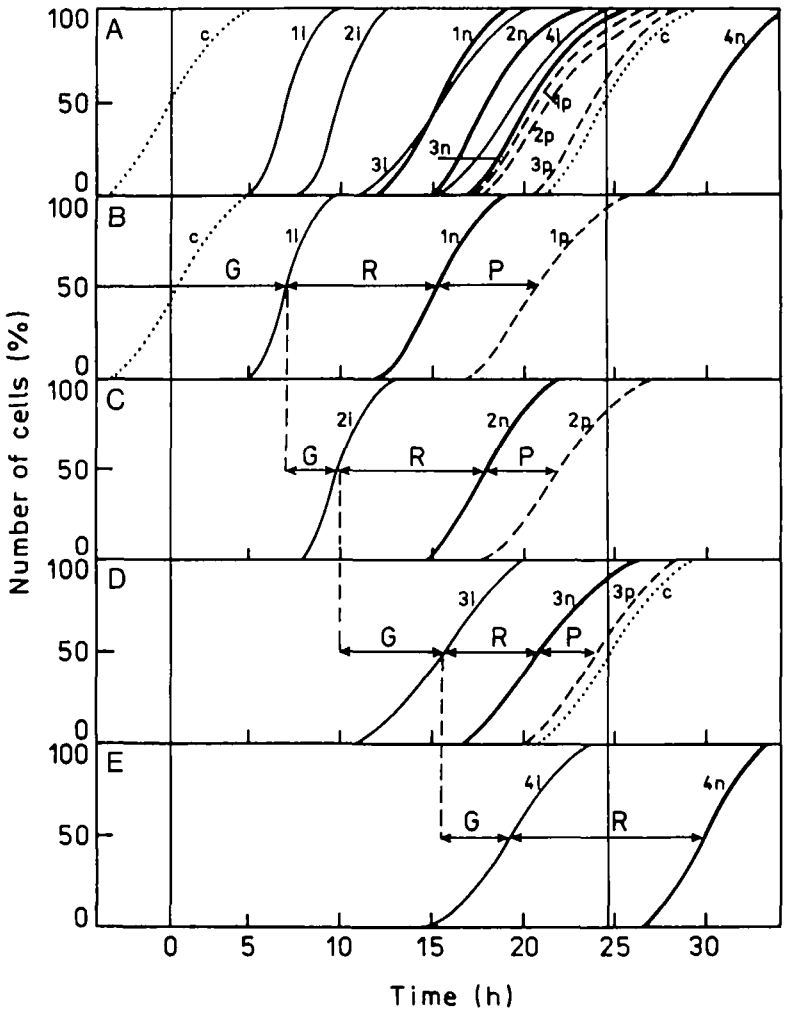

Fig. 1. Schematic illustration of commitment–division diagrams recording the course of nuclear, protoplast and cellular divisions and of commitments to these processes in synchronous populations of *Scenedesmus quadricauda* grown at high irradiances (A). The processes belonging to the first, second, third and fourth cell reproductive sequence are drawn separately in B, C, D and E, respectively. Horizontal abscissae between midpoints of consecutive commitment curves indicate the duration of growth steps (G); those between midpoints of commitment and corresponding nuclear curves indicate the duration of reproductive steps (summed DNA replication step plus nuclear division step) (R) and those between nuclear division and protoplast fission curves indicate the duration of the protoplast fission step (P). Vertical lines mark the boundary of the cell cycle between two division curves. Curves labelled 1i, 2i, 3i, 4i show the percentage of cells in the population which attained the first, second, third and fourth commitment to nuclear division, respectively. Curves labelled 1n, 2n, 3n, 4n show the percentage of cells in the population which terminated at the first, second, third and fourth nuclear division, respectively. Curves labelled c show the percentage of cells in the population which released their daughter cells.

simplified, and only nuclear and cellular divisions and commitments to them are illustrated.

Intervals (in h) between the midpoints of the curves recording the commitment to and nuclear division will be called reproductive steps (marked R in Fig. 1). Actually, the 'reproductive step' incorporates both the DNA replication step and the nuclear division step.

Protoplast division steps (marked P in Fig. 1) were determined by the intervals between nuclear division and the corresponding protoplast fission. The last protoplast fission was usually immediately followed by daughter cell release (Fig. 1); only the curves of daughter cell release were recorded in the subsequent figures (Figs 2–6). Duration of growth steps (marked G in Fig. 1) was measured as the distance between midpoints of consecutive commitment curves.

At high growth rates, synchronous populations were usually split by darkening into two subpopulations, which differed in the number of triggered cell reproductive sequences and consequently also in the extent of overlapping consecutive reproductive steps. These two subpopulations were, therefore, analyzed separately in commitment–division diagrams (Figs 3, 5, 6). The more advanced populations succeeded in attaining the commitment to the first reproductive sequence of the next cell cycle and binuclear daughter cells were formed. In the less advanced subpopulation, uninuclear daughter cells were released. In commitment–division diagrams it was assumed that the upper part of the curves belonged to those less advanced cells, and the lower part to more advanced cells. Different time schedules of individual steps in these two subpopulations were apparent as steps on the curves in commitment–division diagrams (see Figs 3, 5, 6).

Cell cycle patterns at different growth rate: the effect of irradiance

Slow growth rate. Scenedesmus cells grown at a low energy input (mean irradiances below 60 $W m^{-2}$) attained consecutively two commitments to nuclear division, and two cell reproductive sequences took place in one cell cycle. The mother cells yielded exclusively four daughter cells. Due to a low growth rate, growth steps were long and thus the reproductive steps of the first cell reproductive sequence had ended before the commitment to the next reproductive step was attained (Fig. 2A). With irradiance, a somewhat higher growth rate was observed and growth steps became shorter (see the intervals between two commitments in Fig. 2B). The beginning of the second reproductive step approached the first nuclear division (Fig. 2B), but no substantial overlapping occurred.

Under these conditions, the only variable parts of the cell cycle were the growth steps (compare the distances between the beginning of the cell cycle and the first commitment, as in curve 1i, or between consecutive commitments, as in curves 1i and 2i, in Fig. 2A,B; see also Table 1). The reproductive steps took place during fairly constant time periods regardless of growth rates (compare

Table 1. *Duration of growth and reproductive steps without overlap in the cell cycles of* Scenedesmus quadricauda *grown at different irradiances and growth rates*

Irradiance ($W m^{-2}$)	Growth rate (cell doublings h^{-1})	Duration of growth and reproductive steps (in h) in individual cell reproductive sequences					
		1st		2nd		3rd	
		G	R	G	R	G	R
25	0.070	11.4	6.0	10.8	4.4		
40	0.110	7.2	6.0	5.8	4.0		
75*	0.160			2.4	4.4	5.2	4.0
80*	0.160			2.0	3.8	4.0	4.0
75	0.200	2.0	6.0	2.0	4.7		

G: Duration of growth; R: reproductive steps.

distances between curve 1i and 1n or between 2i and 2n in Fig. 2A,B). With increasing irradiance, the length of the whole cell cycle shortened as a consequence of shortening growth steps (descending part of the curve 1, Fig. 4).

Moderate growth rate. At mean irradiances, between 60 and 80 $W m^{-2}$, overlapping consecutive reproductive steps occurred. These overlapped reproductive steps became longer than in cells where these steps did not overlap (compare the distances between 1i and 1n or 2i and 2n in Figs 2C, 3A with those in Fig. 2A,B). The extent of overlapping in the consecutive cell reproductive sequences increased with increasing irradiance. So, also, did the duration of nuclear division steps (Figs 2, 3A, Table 2).

This prolongation of reproductive steps compensated for the shortening of growth steps, and thus the length of the cell cycle remained fairly constant (horizontal part of the curve in Fig. 4) in the 60–80 $W m^{-2}$ range of irradiances (compare distances from the beginning of the light period to curve c in Figs 2B, 3A).

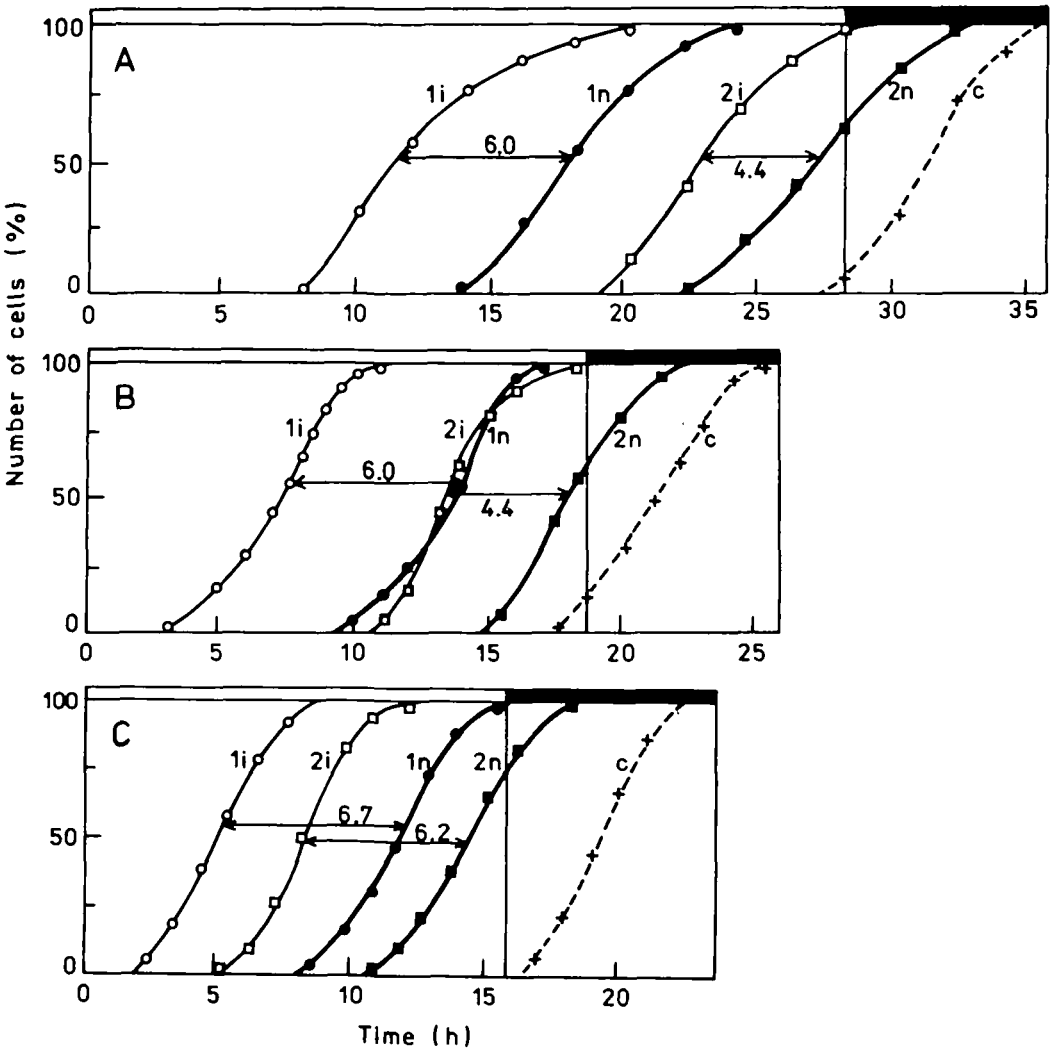

Fig. 2. Time courses of commitments to nuclear and cellular divisions and the termination of these processes in synchronous populations of *Scenedesmus quadricauda* grown at low irradiances. (A) mean irradiance=25 $W m^{-2}$, specific growth rate=0.07 cell doublings h^{-1}. (B) mean irradiance=40 $W m^{-2}$, specific growth rate=0.11 cell doublings h^{-1}. (C) mean irradiance=55 $W m^{-2}$, specific growth rate=0.15 cell doublings h^{-1}. For labelling of the curves and description of the figure see Fig. 3.

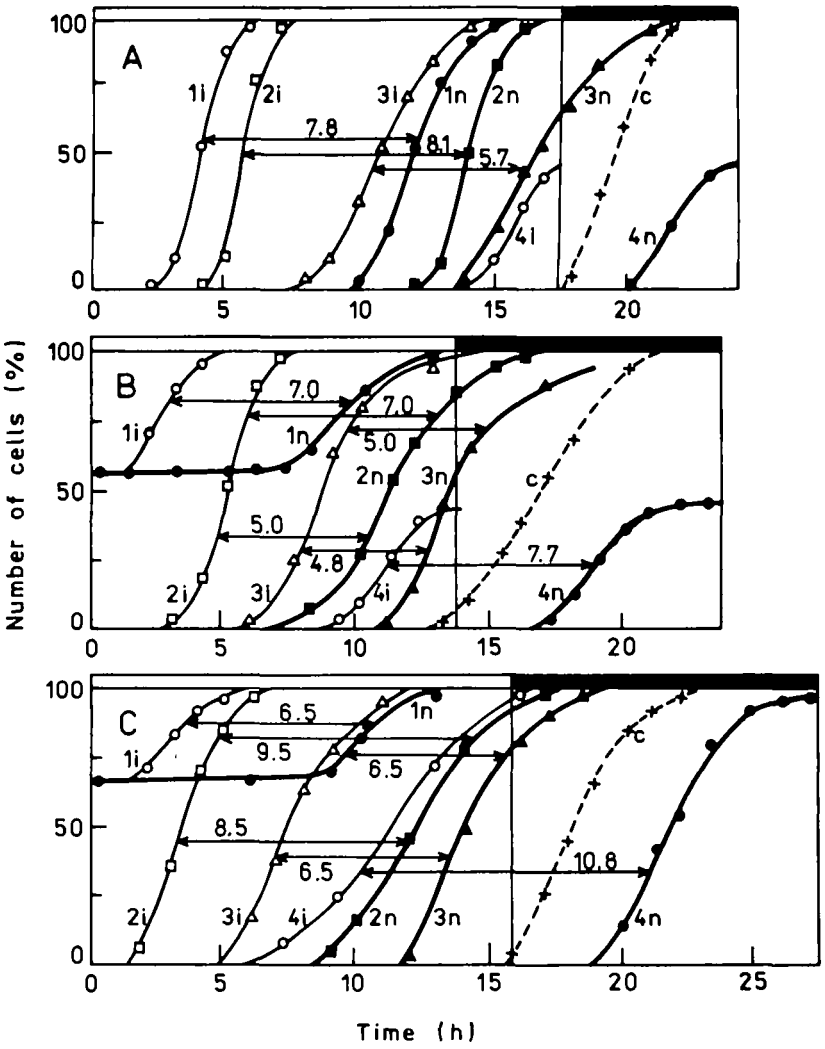

Fig. 3. Time courses of commitments to nuclear and cellular divisions and termination of these processes in synchronous populations of *Scenedesmus quadricauda* grown at moderate (A) and high (B,C) irradiances. (A) mean irradiance=65 W m^{-2}, specific growth rate=0.200 cell doublings h^{-1}. (B) mean irradiance=75 W m^{-2}, specific growth rate=0.211 cell doublings h^{-1}. (C) mean irradiance=85 W m^{-2}, specific growth rate=0.230 cell doublings h^{-1}. Curves 1i, 2i, 3i, 4i: percentage of the cells which attained commitment for the first, second, third and fourth nuclear division, respectively. Curves 1n, 2n, 3n, 4n: percentage of the cells in which the first, second, third and fourth nuclear division was terminated. Curves c: percentage of the cells which released their daughter cells. The intervals between commitment and corresponding nuclear division are indicated in h by the numbers above the abscissae connecting the corresponding curves (uninuclear and binuclear subpopulations were analyzed separately). Light and dark periods are indicated by white and black strips above panels and separated by vertical lines.

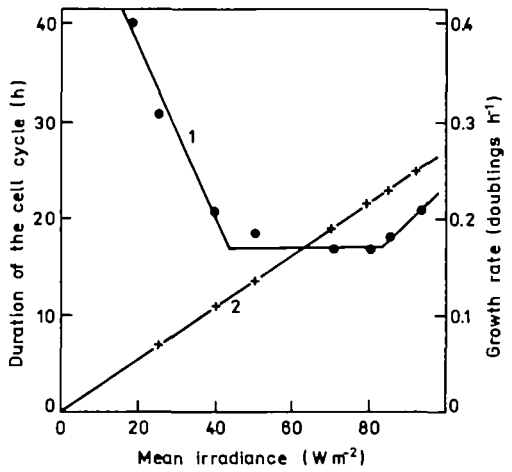

Fig. 4. Duration of the cell cycle (curve 1) and growth rate (curve 2) in synchronous populations of *Scenedesmus quadricauda* at various irradiances. Straight lines stress different variations in the duration of the cell cycle at low, moderate and high irradiances.

High growth rate. At mean irradiances above 80 W m^{-2}, the cells divided exclusively into eight daughter cells. Four overlapping cell reproductive sequences occurred in one cell cycle, and the extent of their overlap increased with increasing irradiance. This applied also to the fraction of cells which attained the commitment to the reproductive steps of the fourth cell reproductive sequence. This sequence was usually terminated after the daughter cells had been released (binuclear daughter cells entered the next cell cycle) and can therefore be counted as the first cell reproductive sequence of the following cell cycle. Duration of growth steps shortened if higher irradiance was applied (see distances between individual commitment curves in Fig. 3). This caused an increase in the extent of overlapping reproductive steps and therefore the duration of these steps was prolonged (compare distances between curves 2i and 2n or 3i and 3n in Figs 3B,C; see also Table 2).

This prolongation of reproductive steps at high irradiances was usually of such an extent that it caused prolongation (ascending part of the curve 1, Fig. 4) of the cell cycles in spite of shortening growth steps (compare Fig. 2A,B).

Table 2. *Duration of overlapped reproductive steps in the cell cycle of* Scenedesmus quadricauda

Extent of overlap %	Duration of reproductive steps (in h) in individual reproductive sequences			
	1st	2nd	3rd	4th
0	5–6	4–5	4	5
50	7	6–7	6–7	8
100	8	8.5	8	11

Cell cycle patterns in cells with different numbers of cell reproductive sequences: the effect of illumination regimes

The cells were grown at high irradiance, i.e. with a high growth rate, for light intervals of different length. By putting cells into the dark, further growth and the initiation of new cell reproductive sequences were stopped. Reproductive steps to which cells were committed were, however, completed.

The durations of individual growth steps which were allowed to realize during the light interval were the same as in a continuously illuminated culture at a given irradiance, but the extent of overlapping reproductive steps differed markedly (compare Fig. 3 and 5A,B).

When no or only slight overlapping occurred, the duration of the consecutive reproductive steps was the same as found in cells grown at low irradiance (see Figs 2 and 5A,B). By prolonging the light period, the number of cell reproductive sequences increased as well as the extent of their overlapping. Due to this overlapping, the duration of reproductive steps increased in the same way as had been found in cells grown at high irradiance (compare distances between curves 1i and 1n, 2i and 2n, 3i and 3n and 4i and 4n in Figs 5C and 3B,C).

The duration of reproductive steps is determined by the extent of their overlapping, and this rule is apparent also in the populations which were split into two subpopulations by darkening and which differed in the number of triggered cell reproductive sequences (Fig. 5A–C). These subpopulations also differed in the extent of overlapping of consecutive reproductive steps. Although the cells in both subpopulations grew under the same irradiance and the same illumination regime, the duration of their reproductive steps differed substantially (Fig. 5).

Cell cycle patterns under retarded or blocked photosynthetic growth: the effect of chloramphenicol and heterotrophic growth

In the experiments described above, duration of the growth steps and their number were changed by variations in light conditions (irradiance or the interval of illumination). It was desirable to know whether light could play any direct role in controlling the duration of the reproductive steps. Chloramphenicol, the inhibitor of chloroplast protein synthesis, applied at a concentration of 25 mg ml^{-1}, slowed down the growth rate of the cells.

In one experiment, chloramphenicol was added at the beginning of the light period and washed out 6 h later. In the presence of chloramphenicol, the growth of daughter cells was completely stopped (Fig. 6A). After chloramphenicol removal, the cells remained partially retarded and grew more slowly than those in an untreated culture (Fig. 3C). This prolongation of growth steps caused the reproductive steps of the successive cell reproduction sequence not to overlap. Duration of these steps (Fig. 6A) was much shorter than in cells in which extensive overlapping of consecutive reproductive steps occurred at

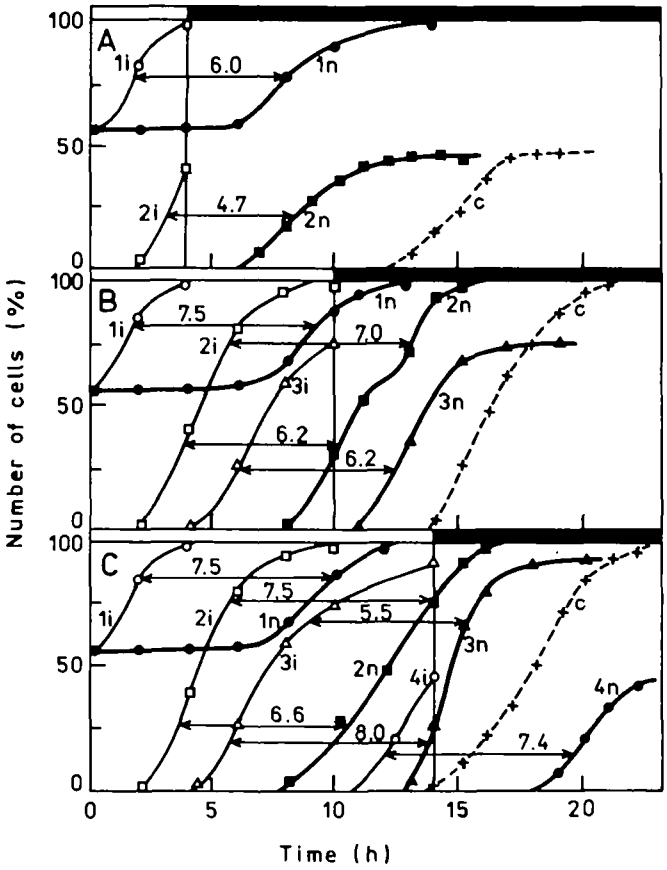

Fig. 5. Time courses of commitments to nuclear and cellular divisions and termination of these processes in successively darkened subpopulations derived from fast-grown synchronous populations of *Scenedesmus quadricauda*. Mean irradiance=75 W m^{-2}, specific growth rate=0.2 cell doublings h^{-1}. For labelling of the curves and description of the Figure see Fig. 3.

the same irradiance (compare Fig. 6 and Fig. 3 or Fig. 5C, see also Table 1). This experiment provided evidence that the light itself had no direct effect on the duration of reproductive steps.

In the second experiment, chloramphenicol (25 mg ml^{-1}) was added at the time when most of the cells of the population had attained the second commitment to divide (the 6th hour of light period). As can be seen in Fig. 6B, the uninuclear part of the population performed only two, but greatly overlapped, cell reproductive sequences. Duration of the reproductive steps in these cells was the same as in untreated cells which attained the same extent of overlapping. For comparison, see the commitment–division diagrams of dark treated cells (Figs 5B,C; 3A). Cells more advanced in the cell cycle (binuclear part of the population) succeeded in attaining the third cell reproductive sequence in presence of chloramphenicol (Fig. 6B). The extent of overlapping reproductive steps was, however, low and the reproductive steps became short, comparable with those found in cells in which the reproductive steps were separated either by dark treatment (Fig. 5A) or by growth at low irradiance (Fig. 2).

To exclude completely the effect of light on the cell cycle patterns, populations of daughter cells were allowed to grow in the presence of glucose, in darkness. Before the experiment, the cells were kept resting for several months in the dark at room temperature. After this treatment all

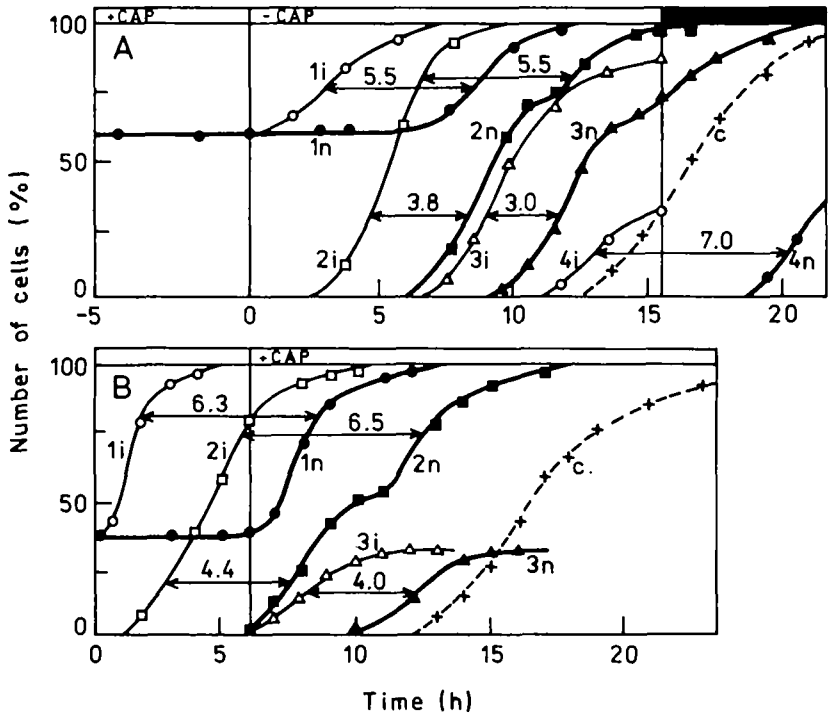

Fig. 6. Time courses of commitments to nuclear and cellular divisions and termination of these processes in synchronous populations of *Scenedesmus quadricauda* grown at high irradiances after chloramphenicol treatment. Panel A: mean irradiance=80 $W m^{-2}$. Chloramphenicol (25 mg ml^{-1}) was added at the beginning of illumination and washed off 6 h later. Specific growth rate was slowed down as a consequence of chloramphenicol treatment to the value 0.163 cell doublings h^{-1}. Panel B: mean irradiance=75 $W m^{-2}$, specific growth rate=0.2 cell doublings h^{-1}. Chloramphenicol was added (25 mg ml^{-1}) after 6 h of illumination and the culture grew further in continuous light. As a consequence of chloramphenicol the specific growth rate was slowed to the value of 0.162 cell doublings h^{-1}. Presence or absence of chloramphenicol is indicated by +CAP or −CAP, respectively, and corresponding time periods are separated by vertical lines. For labelling of the curves and description of the figure see Fig. 3.

possible timing effects of preceding light or circadian rhythms (if they existed) had probably disappeared. The cells grew under heterotrophic conditions at a rate comparable with those grown at low irradiances. Duration of the cell cycle was about 30 h, reproductive steps did not overlap and their duration was the same, as illustrated in Fig. 2. This experiment provided evidence that for the timing of events in the cell cycle of algae a light timer was not required as a pacemaker or inducer of circadian rhythms.

Discussion

The present findings indicate that running processes simultaneously in overlapping reproductive steps result in mutual interactions which affect their duration and consequently cell cycle patterns in the alga *Scenedesmus quadricauda*. One consequence of these interactions is that the overlapping reproductive steps become longer and their prolongation increases with the extent of their overlapping.

The present findings, as well as those published earlier on the regulation of the duration of the cell cycles in *Scenedesmus* (Šetlík *et al.* 1972; Zachleder and Šetlík, 1988; Zachleder *et al.* 1975) and in *Chlamydomonas* (Spudich and Sager, 1980; Donnan *et al.* 1983; Donnan and John, 1984, 1985; John, 1987), clearly rule out any endogenous oscillator, either circadian or pacemaker (Zeitgeger) in nature, that could regulate the duration of algal cell cycle or any of its stages. The role of light seems to be limited only to its trophic function in photosynthesis.

The main argument for the existence of Zeitgeber in *Chlorella* was the constant duration of the cell cycle under a relatively wide range of growth conditions (Lorenzen, 1957, 1980; Morimura, 1959). Actually, the duration of the *Scenedesmus* cell cycles can also be constant within a certain range of growth rates (irradiances), but this constancy occurs only when shortening growth steps caused by increasing irradiance are compensated by prolongation of reproductive steps resulting from their increasing overlap. The only difference is that the range of conditions under which it can happen is narrower in *Scenedesmus* than in *Chlorella* cells.

Additional support for this interpretation of the regulation of the length of the cell cycle and its various stages comes from the following findings of other authors.

Chlorella cells grown at sufficiently low irradiances (Wanka, 1959, 1967, 1968) had much longer cell cycles than when grown at high irradiances and their length was proportional to the irradiance used. We assumed that the length of the cell cycle was determined by the length of the precommitment period, which was in turn regulated by the level of light energy supply. Furthermore, the cell cycle of *Chlorella* cells grown at very high irradiances is prolonged (Lorenzen and Albrodt, 1981). Lorenzen and Albrodt (1981) tried to explain this finding, which disagreed with their hypothesis of 'Zeitgeber' damaging dividing cells by high irradiance. This conclusion, however, may be questioned since the irradiances used were harmless even for *Scenedesmus* cells, which are even more light sensitive than *Chlorella* cells. We assume that in this case the cell cycle prolongation was caused by prolongation of overlapped reproductive steps. The present findings, that even under these high irradiances the *Scenedesmus* cell cycles can be shortened if the extent of overlapping cell reproductive sequences is experimentally decreased, support this interpretation.

Similarly, *Chlorella* cells prolong their cell cycles with prolongation of the light period but to an even greater extent (Lorenzen and Albrodt, 1981). This finding clearly rules out the assumption that the beginning of the light period could act as a pacemaker and is in line with the assumed effect of overlapping reproductive processes on prolongation of the cell cycle. The authors' interpretation that the beginning of the dark period takes on the role of pacemaker seems to be too speculative.

The duration of reproductive steps (S, G_2 and M phases) was fairly constant in most of the organisms dividing by binary fission as well as in algae. We would like to stress

that this need not indicate that a timing mechanism can be found only under such conditions or in those organisms where only slight overlapping of cell reproductive sequences occurs. Under conditions of fast growth, where overlapping of two or more reproductive steps usually occurs, marked changes in the duration of reproductive steps can be observed. This has been demonstrated in *Scenedesmus* (this paper; Šetlík *et al.* 1972; Zachleder *et al.* 1975) and we assume that the same rules generally hold not only for the other chlorococcal and volvocal algae dividing by multiple fission but also for organisms dividing by binary fission.

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