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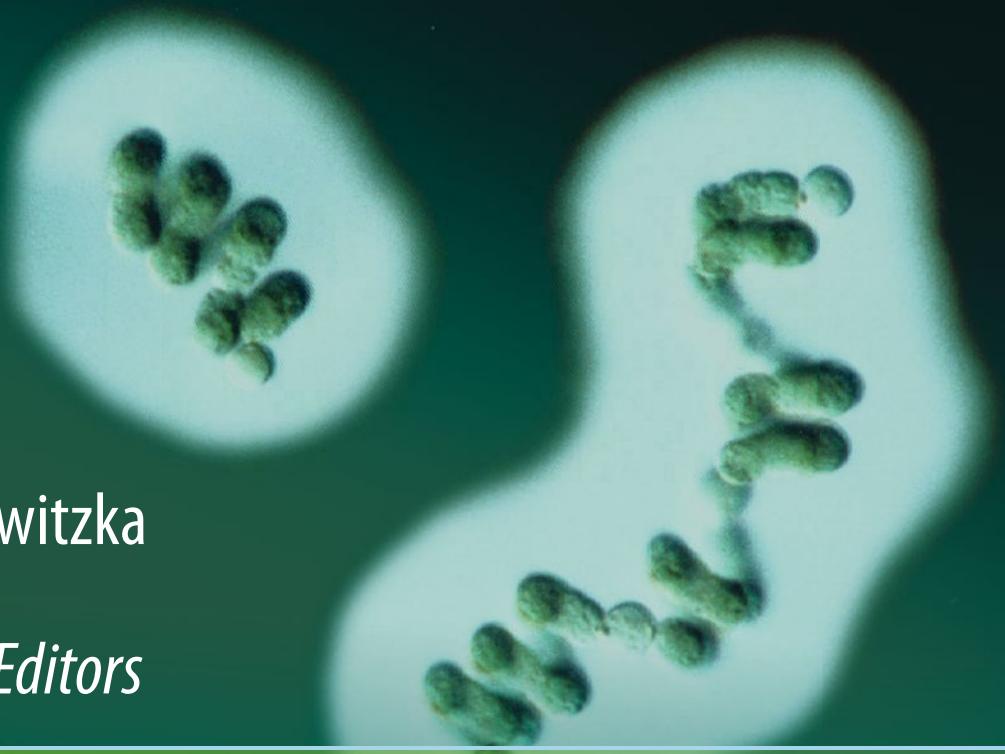


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Developments in Applied Phycology 6

Michael A. Borowitzka
John Beardall
John A. Raven *Editors*

The Physiology of Microalgae



Developments in Applied Phycology 6

Series editor

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The Physiology of Microalgae



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Preface

Algae play an enormously important role in ecology and, increasingly, in biotechnology. Microalgae in the world oceans, for instance, are responsible for nearly half of the CO₂ fixed (and O₂ released) by photosynthesis annually and form the basis of most marine and other aquatic food chains. With the potential of global warming and associated ocean acidification, the effects of these changes on phytoplankton communities and the flow-on effect on the marine ecosystems are of major interest. The impact of anthropogenic activities on aquatic environments, especially the effects of eutrophication and associated algal blooms and their mitigation, is of great importance. Through their application in wastewater treatment, microalgae are also part of the solution to reduce the detrimental effects of the discharge of wastewaters.

Microalgae are also of significant commercial importance. A number of species are important for the growing aquaculture industry, serving as critical food for larval fish and abalone and for shellfish. Since the early 1980s there has been a growing microalgal-based biotechnology industry, producing natural pigments such as β-carotene and astaxanthin and long-chain polyunsaturated fatty acids. More recently, microalgae have, once again, become the focus for the development of renewable biofuels, and this has also reinvigorated interest in the commercial production of other microalgal products and new applications of microalgae. A deep understanding of algal physiology is one of the most important factors in the development of new species and products for commercialisation.

In 1962 the first book to comprehensively review the research on the physiology and biochemistry of algae edited by Ralph Lewin was published (Lewin 1962), following on from the earlier small, but important, monograph on algal metabolism of Fogg (1953). Both of these books are still worth reading. The next major volume on this topic was *Algal Physiology and Biochemistry* edited by WDP Stewart published in 1974 (Stewart 1974). All of these books covered both the microalgae and the macroalgae.

Stewart in the preface to his volume noted:

Ten years ago it would have been possible to include in a book of this type, over 90 per cent of the relevant aspects of algal physiology and biochemistry but this is no longer the case.

It has now been 41 years later, and clearly it is impossible to include in a single book all relevant aspects of algal physiology, and it is therefore not surprising that since the publication of Stewart's book, no comprehensive book on algal physiology has been published, only reviews on particular topics and general chapters in a number of broader ranging books on algae. However, we strongly feel that there is a need for a reasonably comprehensive up-to-date reference work on algal physiology and biochemistry for the use of researchers in the field, both old and new. Such a reference work is probably now more important than ever, as few people have the time and capacity to keep up to date with the massive literature that has accumulated on algal metabolism and related topics. The days of generalist phycologists are past, and for a variety of reasons, researchers have needed to become more specialised. However, whatever the specific field of algal research, it is often important and instructive to consider one's work in a broader context.

Given the mass of knowledge on algae and their physiology and biochemistry that has been accumulated in the last 40 years, we had to make two decisions in the planning of this book. First, we decided to limit the scope to the microalgae, i.e. those algae one generally needs a microscope to see. Second, as it is impossible to cover all possible topics, we selected what we consider the major aspects of microalgal physiology. There are many important topics which are not covered, but we hope that these will be part of future volumes.

We invited a range of leading researchers to write authoritative review chapters on critical aspects of algal physiology and biochemistry. These range from the studies on the cell cycle and advances in our understanding of cell wall biosynthesis, through fundamental processes such as light harvesting and assimilation of carbon and other nutrients, to secondary metabolite production and large-scale cultures of microalgae and genomics. We also tried to ensure that all species names used were those currently accepted, and we have included a chapter which lists both the old and new names (as well as a plea to provide adequate information on strains used when publishing) to help researchers in finding all relevant literature on a particular species. The authors were given a relatively free hand to develop their topic, and we feel that the variety of approaches leads to a more interesting and useful book. We are very grateful to all those people we have cajoled into contributing to this enterprise and the many people who aided by reviewing particular chapters.

Our intention is that this book serves as a key reference work to all those working with microalgae, whether in the laboratory, in the field, or growing microalgae for commercial applications. The chapters are intended to be accessible to new entrants into the field (i.e. post-graduate students) as well as being a useful reference source for more experienced practitioners. We hope that the book thoroughly deals with the most critical physiological and biochemical processes governing algal growth and production and that any omissions do not disappoint too many readers. It is our hope that you find the information here as stimulating as we do – microalgae are exciting organisms to work with!

Murdoch, WA, Australia
Clayton, VIC, Australia
Dundee, UK
June 2015

Michael A. Borowitzka
John Beardall
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Part I

The Algae Cell

The Cell Cycle of Microalgae

Vilém Zachleder, Kateřina Bišová, and Milada Vítová

Abbreviations

CDK	cyclin-dependent kinase
chl-RNA	chloroplast ribosomal RNA
cyt-RNA	cytosolic ribosomal RNA
CKI	inhibitor of cyclin-dependent kinase
CP	commitment point
DP	dimerization partner
E2F	transcription factor
FdUrd	5-fluorodeoxyuridin, inhibitor of thymidylate synthase
NAL	nalidixic acid, an inhibitor of DNA gyrase, (1-ethyl-1,4-dihydro-7-methyl-1,4-oxo-1,8-naphthyridine-3-carboxylic acid)
nuc-DNA	nuclear DNA
pt-DNA	chloroplast (plastid) DNA
Rb	retinoblastoma protein

(microalgae) to multicellular ones resembling higher plants (macroalgae) with a very complex body shape built by morphologically distinct cells having various physiological roles. This section will deal only with the vegetative cell cycle of unicellular green algae, existing as single cells or gathered into coenobia (where daughter cells arising from a single mother cell stay connected together), colonies or filaments, but independent of each other. Although 60 years have passed since the first studies of the algal cell cycle (Tamiya et al. 1953), possible ways in which algae can still contribute to research into the biology of cell cycles are far from exhausted. The seemingly narrow range of these organisms provides such a broad variety of reproductive patterns that, in spite of extensive literature, they still represent a challenge for future researchers in cell cycle biology. The aim of this section is to summarize the significant progress made, from early historical findings up until the last few years, and to highlight the hidden potential of algae for the future.

1 Introduction

Algae are a unique group of organisms displaying a wide variety of reproductive patterns. Various division patterns can be found from simple division into two cells, similar to yeast (binary fission), to the formation of four and up to several thousand daughter cells in a single cell cycle in green algae dividing by multiple fission. In some algal species, both binary and multiple fission can be observed in the same organism, either under different growth conditions (Badour et al. 1977) or at different phases of the life cycle (van den Hoek et al. 1995). Furthermore, wide-ranging body organizational structures exist in algae, from unicellular organisms

About 60 years ago, chlorococcal algae of the genus *Chlorella* were among the first microorganisms to be successfully grown in synchronous cultures (Lorenzen 1957; Tamiya et al. 1953) and used for biochemical and physiological analyses of the cell cycle. The first experiments were therefore carried out at the same time that Howard and Pelc first separated the cell cycle into four phases G1, S, G2 and M (Howard and Pelc 1953). From the early years, other green algae, *Desmodesmus* (*Scenedesmus*) and *Chlamydomonas* also formed prominent cell cycle models (Lien and Knutsen 1979; Lorenzen 1980; Šetlík et al. 1972; Tamiya 1966). Their multiple fission reproductive patterns are, as is described below, rather different from the patterns terminated by binary fission that are characteristic of most eukaryotic cells. The multiple fission cell cycle and mechanisms governing its regulation are the most important contributions that algal cell cycle studies have made to the general field of cell cycle research.

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2 Types of Cell Cycle of Microalgae

2.1 Cycle Type C1

The purpose of the cell cycle is to consistently reproduce all cellular structures in order to produce a new daughter cell. Such a reproductive sequence normally comprises the following steps: growth, DNA replication, nuclear division, and cellular division or protoplast fission (Mitchison 1971). In the growth step, the cell builds up functional structures and accumulates reserves. At the end of this, the cell attains a critical size and content of essential constituents, including energy reserves; such a cell becomes competent to proceed through the reproductive sequence even in the absence of further growth. This is referred to as attainment of **commitment point** to divide. While the rate at which a cell attains commitment is tightly correlated with growth rate (in autotrophic algae, via photosynthesis), once the cell is committed, the two processes become independent. It is therefore convenient to divide the cell cycle of algae into **pre-commitment** and **post-commitment** periods. From now on, the term **DNA replication-division sequence** will be used for the sequence of processes and events that take place after the commitment point. Each step in the DNA replication-division sequence is comprised of a preparatory and an executive phase. The latter include DNA replication, and the morphologically well characterized stages of mitosis and cytokinesis. The events constituting the preparatory phases, in spite of intensive studies, are not yet completely characterized. Accumulation of deoxynucleotides in a pool, and of a sufficient number of molecules of a replicating enzyme, must precede actual DNA replication as a prerequisite for mitosis and cytokinesis. It is not difficult to establish the timing of the executive phases of individual events, however, exactly where and when the events of their corresponding preparatory phases are located and triggered is, in most cases, uncertain. The general impression is that the preparatory processes of DNA replication and nuclear and cellular division start soon after the commitment point and overlap with each other.

The classical cell cycle describes the basic organization of the cycle in cells dividing by binary fission (Howard and Pelc 1953); it is illustrated as a sequence of four phases: G1, S, G2 and M (Fig. 1a). This cell cycle organization, where the mother cell divides into two daughter cells, is common to most algae, particularly the filamentous ones (Fig. 2). For some algae, the mother cell can also divide into more than two daughter cells, in a process called multiple fission. Binary fission is denoted here as the C₁ cell cycle. This terminology is based on the fact that the cells can generally divide into 2ⁿ, where n is an integer. For binary fission, n=1, thus this cell cycle can be designated as C₁. The more general cell cycle pattern, C_n, or multiple fission, is described in

detail in the next section. The classical cell cycle (C₁) scheme can be modified in some organisms, like the budding yeast, where S and M phases overlap without an intervening G2 phase (Forsburg and Nurse 1991), or under some conditions such as in embryonic development, where rapid cell cycles consist of only alternating S and M phases without any gap phases (Hormanseder et al. 2013; Newport and Kirschner 1982, 1984). However, the basic rule of one mother cell giving rise to two daughters is always kept. Similarly to these organisms, cell cycle organization in green algae also requires additional features to be added to the classical cell cycle scheme (Fig. 1). The first novel characteristic is the commitment point (CP).

The existence of commitment points in algae became clear from experiments involving transfer into the dark. If algal cells are put into the dark at different time-points during their G1 phase, their behavior differs significantly. Cells darkened at early times stay the same, even after prolonged time periods. In contrast, at later time-points, the cells acquire the ability to divide in the dark without an external energy supply (John et al. 1973; Šetlík et al. 1972). The point (or stage) in the cell cycle when cells became competent to duplicate reproductive structures (DNA, nuclei) and to divide was, in early works, called variously the “point-of-no-return” (Moberg et al. 1968), “induction of division” (Šetlík et al. 1972) “transition point” (Spudich and Sager 1980) or “commitment point” (John 1984, 1987); recently only the last term has been generally accepted. Clearly, commitment point (CP) is of outmost importance for cell cycle progression and the algal cell cycle can be very simply split into pre- and post-commitment periods. The rules governing CP are similar to those found for Start in yeasts and the restriction point in mammalian cells (Fujikawa-Yamamoto 1983; Sherr 1996; Sherr and Roberts 1995). CP is thus considered a functional equivalent of both key decision points (John 1984).

The second typical feature of the algal cell cycle is directly related to the existence of CP. The “gap” phase following attainment of CP, prior to DNA replication, starts completely differently from the G1 phase preceding the commitment point. It corresponds to the preparatory phase for DNA replication. This phase also occurs in other organisms, (sometimes termed the late G1 phase), where its character, distinct from the preceding G1 phase, is well recognized. In cell cycle models illustrated in Fig. 1, this phase is termed a pre-replication phase (pS) (Zachleder et al. 1997). The main characteristic of this phase of the algal cell cycle (in contrast to the G1 phase) is that no growth processes or external energy supplies are required. Formation of the pre-replication protein complex in chromosomes, and the activation of S-phase CDKs (cyclin-dependent kinase), seems to be part of this phase in frogs and yeasts (Nasmyth 1996; Sherr 1995, 1996; Sherr and Roberts 1995). Maximum activities of

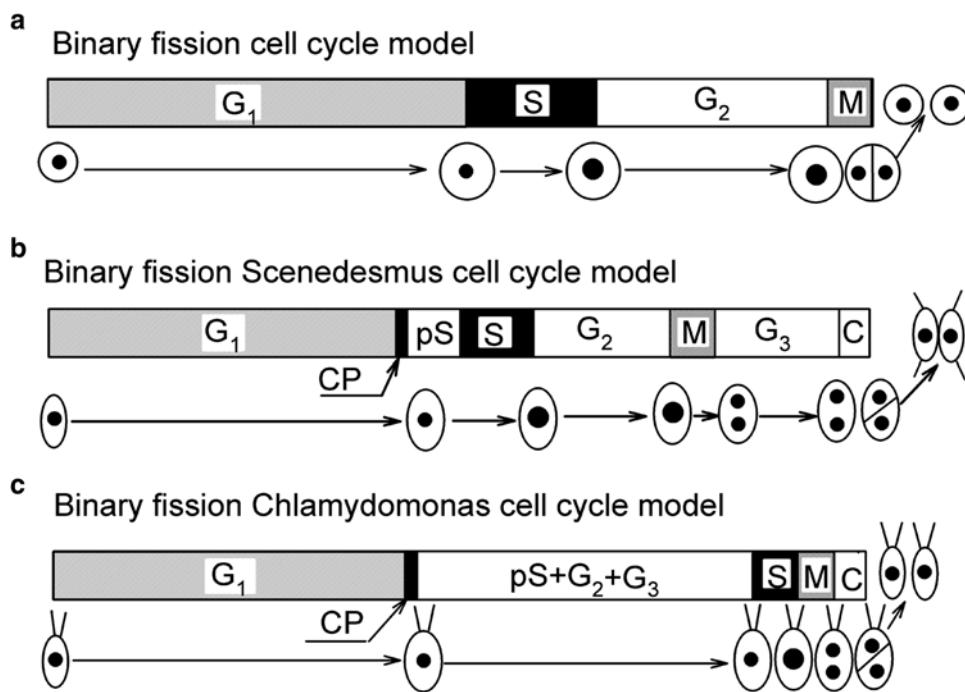


Fig. 1 Diagrams showing different types of cell cycle phases, including the classical cell cycle model and those found in *Desmodesmus* (formerly *Scenedesmus*) *quadricauda* and *Chlamydomonas*, which divide into two daughter cells. (a) Classical type cell cycle after Howard and Pelc (1953), (b) Scenedesmus-type cell cycle after Šetlík and Zachleder (1984), and (c) Chlamydomonas-type cell cycle after Zachleder and van den Ende (1992). Individual bars show the sequence of cell cycle phases and events during which growth and reproductive processes take place. Only one sequence of events leading to the duplication of cell structures occurs during the cycle of cells dividing into two daughter cells (Panels a, b, c). Thus all of the schemes correspond to a C₁ type of cell cycle (number of daughter cells is 2¹). Schematic pictures of the cells indicate their size during the cell cycle and the black circles inside illustrate the size and number of nuclei. Large black spots indicate a doubling of DNA. The lines at the terminal cells of *Desmodesmus* (*Scenedesmus*) coenobia represent spines typical for the species *D. quadricauda*. The lines at the top of the *Chlamydomonas* cells represent flagella, which are retracted by the cells before DNA replication begins. G1: the phase during which the threshold size of the

cell is attained. It can be called a **pre-commitment period** because it is terminated when the commitment point is reached. CP: the stage in the cell cycle at which the cell becomes committed to triggering and terminating the sequence of processes leading to the duplication of reproductive structures (**post-commitment period**), which consists of: pS: the pre-replication phase between the commitment point and the beginning of DNA replication. The processes required for the initiation of DNA replication are assumed to happen during this phase. S: the phase during which DNA replication takes place. G2: the phase between the termination of DNA replication and the start of mitosis. Processes leading to the initiation of mitosis are assumed to take place during this phase. M: the phase during which nuclear division occurs. G3: the phase between nuclear division and cell division. The processes leading to cellular division are assumed to take place during this phase. C: the phase during which cell cleavage and daughter cells formation occurs. In *Chlamydomonas*, apparent G2 and G3 phases are missing; it can, however, be assumed that all the required processes happen during the prolonged gap phase, which is thus denoted pS+G2+G3, for more details see text (Modified after Zachleder et al. 1997)

CDKs were also observed at commitment points in *Chlamydomonas reinhardtii* (Zachleder et al. 1997).

In some algae, there is a relatively long phase separating nuclear division and cleavage of the cells. This requires a third modification of the classical cell cycle. The term G3 phase seems to be an appropriate designation for this phase (Fig. 1b) (Zachleder et al. 1997).

Chlamydomonas has a very specific cell cycle, somehow resembling that of some embryos. It lacks apparent G2 and G3 phases since the S- and M-phases and cell cleavage occur nearly immediately after each other. However, all the preparatory processes for DNA replication, nuclear and cellular division must, by definition, precede the processes themselves. This is in line with the continuum concept of Cooper (1979, 1984), which is described in more detail below, stat-

ing that the preparatory processes do not necessarily immediately precede their respective phases but are performed continuously throughout the cell cycle, and the gap phase is only a manifestation of processes not yet completed. It can therefore be assumed that the processes from “missing” phases take place during the gap phase, between the time of commitment point attainment and the initiation of DNA replication. This phase has been designated as pS+G2+G3 (Fig. 1c) (Zachleder et al. 1997).

2.2 Cycle Type C_n

In the previous section, the C₁ cell cycle type was introduced, where the mother cell divides into two daughter cells; many

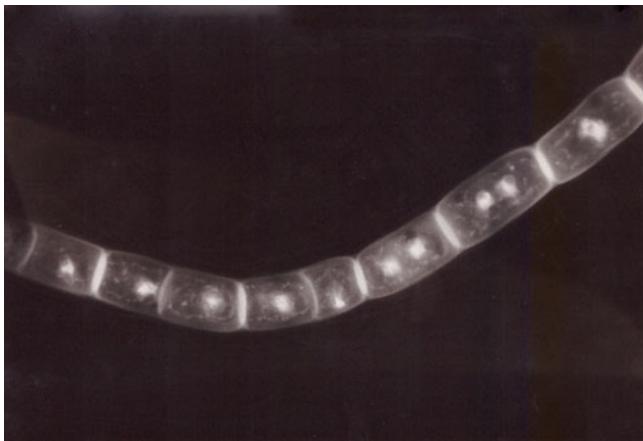


Fig. 2 Fluorescence photomicrographs of the green filamentous alga *Microspora* sp. (Ulotrichales) stained with DAPI. Different phases of the cell cycle and nuclear division can be seen in individual cells of the filament. Nucleoids are localized in chloroplasts along the cell wall (After Zachleder and Cepák 1987c)

algae divide into more than just two daughter cells in a modified cell cycle, denoted as the multiple fission cycle. Generally, any division will occur into 2^n daughter cells (cycle type C_n), where n is an integer from 1 to 15. The C_1 and C_n cell cycle types are, in some species, interchangeable and the one that will be used for division depends solely on growth rate. Cells grown under unfavorable growth conditions, with a low growth rate, will divide into only 2 ($n=1$, C_1) daughter cells while the same cells, when grown under optimal conditions, can divide into 8 ($n=3$, C_n) or 16 ($n=4$, C_n) daughter cells. Although C_n cell cycle types also occur in other organisms, their exclusive use for vegetative reproduction of cells in many taxonomic groups of algae is unique. C_n cycles are characteristic for most cells in the algal orders Chlorococcales and Volvocales, such as *Chlorella*, *Desmodesmus*, *Scenedesmus*, and *Chlamydomonas*. These algae became popular in cell cycle studies (Lorenzen 1957; Tamiya 1966) because they can be easily synchronized by alternating light and dark periods, a procedure that is considered natural and where induced synchrony is very high. Due to the presence of multiple DNA replications, nuclear and cellular division, the cycle is much more complex than the classical scheme, and has a number of modifications. Importantly, there is extensive overlapping of genome duplication by DNA replication, genome separation by nuclear division, and cell division. It is even more complex since cell cycle processes are coordinated with equivalent processes in both mitochondria and chloroplasts. It has become increasingly evident that the “classical” scheme, as originally proposed by Howard and Pelc (1953), is inadequate for interpretation of C_n cell cycles types. Interestingly, the C_n cell cycle shares some common features with the prokaryotic

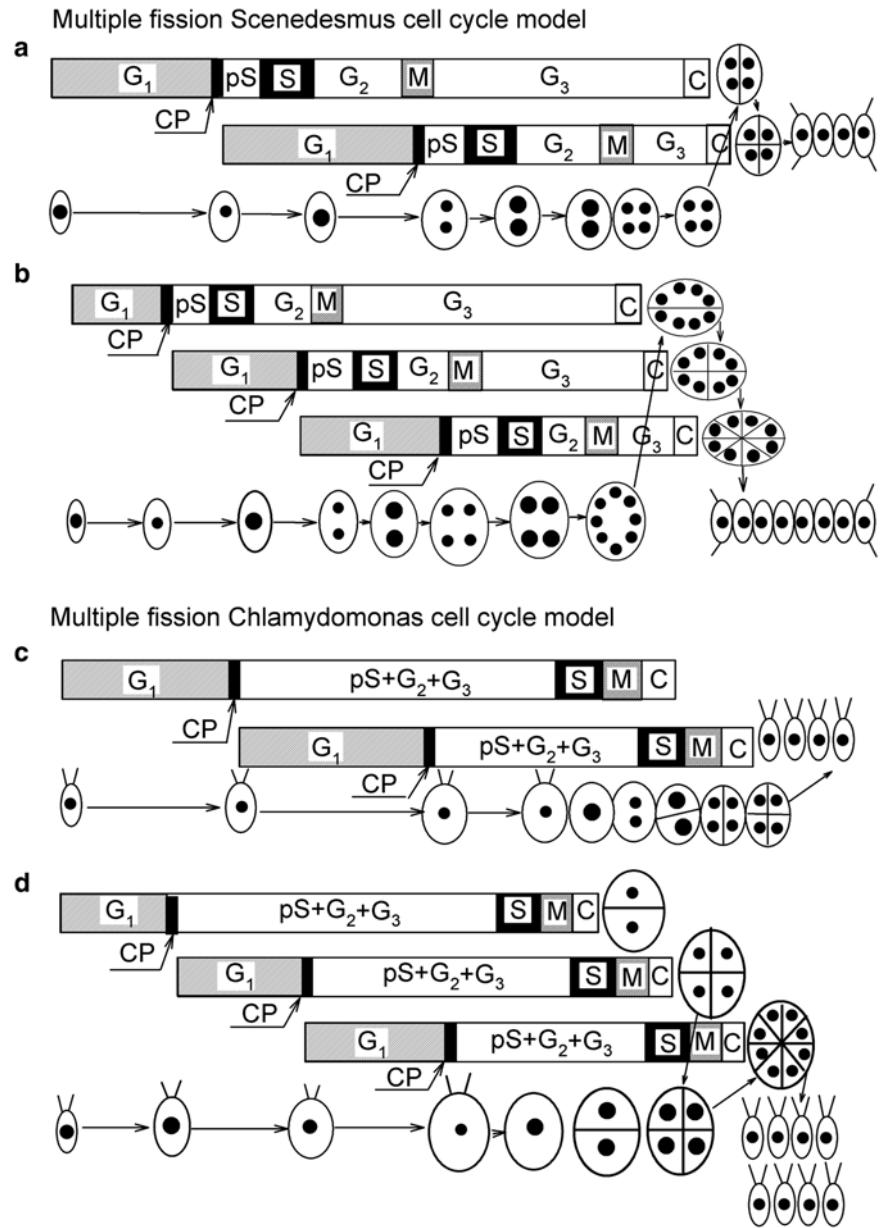
cell cycle (Šetlík et al. 1972). This notion was supported by Cooper who, based on extensive studies of bacterial and eukaryotic cell cycles (Cooper 1990; Cooper and Helmstetter 1968; Helmstetter and Cooper 1968; Helmstetter et al. 1968; Liskay et al. 1979, 1980; Singer and Johnston 1981), proposed a unifying concept that assumes some common principles in the control of eukaryotic and prokaryotic cell cycles (Cooper 1979, 1984). Similarly to the reproductive sequence concept introduced above, he argues that the cell cycle, generally perceived as a “cycle” since the same sequence of events happens in mother and daughter cells, is not a “cycle” but rather a sequence of events repeating themselves in each cell (Cooper 1979, 1984, 1987). Moreover, since it is not a “cycle” but rather a continuum, some of the events comprising each sequence may occur within the mother cell; this is particularly true of the growth step and all the preparatory phases of the DNA replication-division sequence. Research findings on the cell cycle of algae dividing by multiple fission fit well into Cooper’s unifying hypothesis. An understanding of cell cycle events as a sequence of processes not necessarily bound to some specific gap phases of a classical cell cycle, nor to the boundary of a single cell cycle, represents the best way for grasping mechanisms by which complex algal cell cycles are governed.

In line with Cooper’s predictions, the main difference between cell cycles of organisms dividing by binary or multiple fission is that in the latter case, multiple commitment points are attained during a single cell cycle. Each of the commitment points is preceded by growth to a threshold size (critical size), followed by a single DNA replication-division sequence. For each consecutive commitment point, a certain critical cell volume exists at which the commitment point is attained. A critical cell volume for a given commitment point is approximately twofold that of the previous one (Šetlík et al. 1972; Šetlík and Zachleder 1984). Growth is clearly a prerequisite for attaining consecutive commitment points. When a DNA replication-division sequence committed by the first commitment point attains a certain phase (preparation for protoplast fission), further commitment points cannot be attained and all committed reproductive sequences are terminated by the formation of daughter cells. However, until this phase, additional commitment points will be attained, provided that growth is sustained by continuous or prolonged illumination.

Obviously, to describe such a complex cell cycle in terms of the classical G1, S, G2, and M phases (Howard and Pelc 1953) will require major modifications (Fig. 3).

The gap phases, according to Cooper, are simply a manifestation of the fact that the preparatory processes for DNA replication (late G1 phase) and nuclear and cellular division (G2 phase) are not yet complete. Additionally, in many algal species or strains, particularly those with C_n type cycles,

Fig. 3 Diagrams showing different types of cell cycle phases found in *Desmodesmus* (*Scenedesmus*) and *Chlamydomonas* dividing by multiple fission (cell cycle type C_n). (a, b) Scenedesmus-type cell cycle after Šetlík and Zachleder (1984), and (c, d) Chlamydomonas-type cell cycle after Zachleder and van den Ende (1992). For description of figure characteristics see Fig. 1. Two (a, c) or three (b, d) partially overlapping sequences of growth and reproductive events occur within a single cycle in cells dividing into four daughter cells (a, b) or eight daughter cells (b, d) (Modified after Bišová and Zachleder 2014)



nuclear divisions are followed by additional “gap” phases, during which time, processes leading to cytokinesis (protoplast fission and daughter cell formation) occur, and are designated as G₃ phase (Zachleder et al. 1997). Various external or internal factors can stop further cell cycle progress during this phase, just after nuclear division is terminated, implying a control mechanism involved in the regulation of cell division. Also arising from Cooper’s concept of a continuum is the fact that if some of the gap phases are missing for a particular cell cycle type, it can be assumed that processes usually performed during these phases run concurrently with processes of other phases. For example, in organisms where cell division occurs immediately after mitosis, the processes leading to cell division can be assumed to take place during

G₂, together with the processes leading to mitosis. In algae dividing into more than two daughter cells, the cell cycle model must also be modified to take into account overlapping or parallel courses of entire phases of consecutive sequences of growth and reproductive events (Fig. 3).

In the C_n types of cell cycle, two distinct patterns of cell cycle phases can be distinguished:

One is typical for *Desmodesmus* and *Scenedesmus* and can be called a **consecutive pattern (Scenedesmus-type cell cycle)**. As presented schematically in Fig. 3, the cells replicate DNA shortly after attaining a commitment point, then nuclear division follows. If more than one commitment point is attained, several rounds of DNA replication and nuclear divisions occur consecutively during the cell cycle, and cells

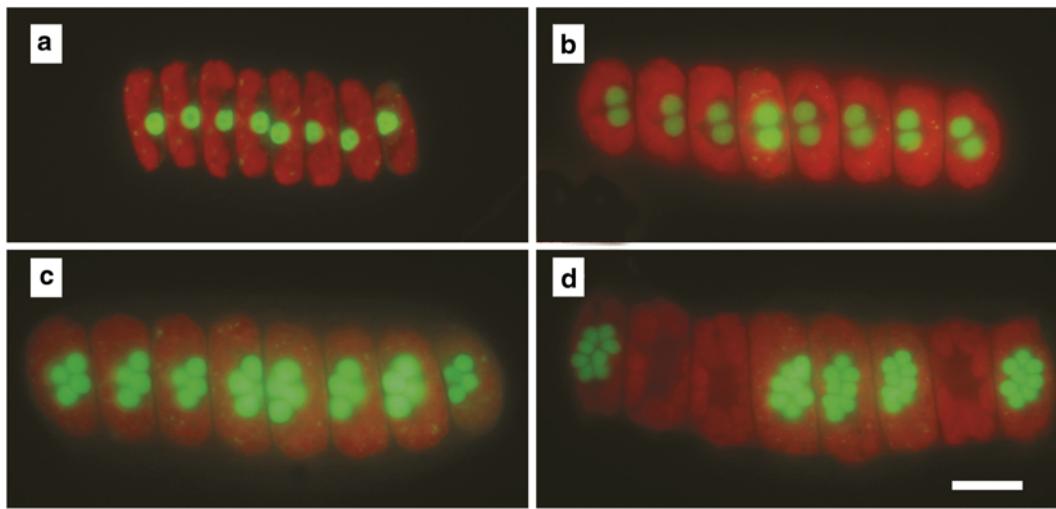


Fig. 4 Fluorescence photomicrographs of eight-celled coenobia of *Desmodesmus (Scenedesmus) quadricauda* during the cell cycle, stained with 0.3 % SYBR green I dye. Nuclei are visible as yellow-green spots. Chloroplasts are visible as a red color, which is autofluorescence of chlorophyll. (a) Uninuclear daughter coenobium, (b) binuclear coenobium, (c) tetranuclear coenobium, (d) Mother octanuclear coenobium. Cells already dividing protoplasts remained unstained. Scale bar = 10 µm (Modified after Vítová et al. 2005)

become polynuclear because mitoses follow, a relatively short time after the attainment of consecutive commitment points (Figs. 3a, b and 4). Then, during the cycle in which *Desmodesmus quadricauda*¹ cells divided into eight daughters, the nuclei are distributed in an octuplet coenobium. The uninuclear daughter cell (Fig. 4a) passed the first commitment point, quickly followed by the first committed mitosis, to become binuclear (Fig. 4b). It then consecutively attained another two commitment points and a second mitosis came about. The cell continued in the cycle as tetranuclear (Fig. 4c), with the third mitoses occurring after the preceding third commitment point (Fig. 4d), and octanuclear cells entered protoplast fission, forming an octuplet daughter coenobium.

The second cell cycle pattern is typical for *Chlamydomonas* and can be called a **clustered** pattern (**Chlamydomonas-type cell cycle**). As can be seen schematically in Fig. 3c, d and in the photos in Fig. 5, no nuclear division occurred until very late in the cell cycle (the same is true for DNA replication, see next section). However, similarly as in the Scenedesmus-type cell cycle, several commitment points can be attained during the cell cycle, leading to multiple

¹Wherever possible the currently accepted names for species are used. The name used in the paper cited is also indicated. For details of names see chapter “[Systematics, Taxonomy and Species Names: Do They Matter?](#)” of this book (Borowitzka 2016).

Concerning this chapter, genus *Scenedesmus* was re-assessed giving rise to two genera: *Scenedesmus* and *Desmodesmus* (An et al., 1999). Species formerly known as *Scenedesmus quadricauda* was re-classified as *Desmodesmus quadricauda*. The species has been for many years used as an important model organisms and has been referred mostly as *Scenedesmus quadricauda*. For the sake of clarity, the text referring to such publications states the current genus name *Desmodesmus* with the former name *Scenedesmus* in parentheses.

rounds of DNA replication, mitoses and protoplast fissions clustered at the very end of the cell cycle. In Fig. 3, the time course of three consecutive *Chlamydomonas* reproductive processes is shown. Photomicrographs of multiple clustered nuclear divisions, followed nearly immediately by daughter cell formation, are presented for the cell cycle where 4 commitment points were attained and nuclei divided four times, forming 16 daughter cells by the end of the cell cycle (Fig. 5).

Arising from the preceding text, progress in commitment point studies provides key information on regulation of the cell cycle. The principle of determination of commitment point in algal culture is based on the fact that attaining commitment point is dependent on light as an energy source while the post-commitment processes (DNA replication, nuclear and cellular division) are light-independent. Subcultures are exposed to light periods of increasing length and the average number of cells formed in successively darkened subpopulations is followed. This number depends on the light intensity and the length of illumination. Cells in the successively darkened samples do not start division immediately upon darkening since they must first undergo all the preparatory processes for cell reproduction. In samples withdrawn from the culture early in the cycle, it may take several hours before cell division sets in. But under physiological conditions, they will ultimately divide, i.e. they are committed to divide. The results collated from synchronized algal populations with time are called commitment diagrams or commitment curves (Fig. 6). To construct them, samples are withdrawn from a synchronously growing culture at regular intervals (as a rule, 1 or 2 h), and incubated in darkness under aeration at the temperature of the culture (Fig. 7). After a

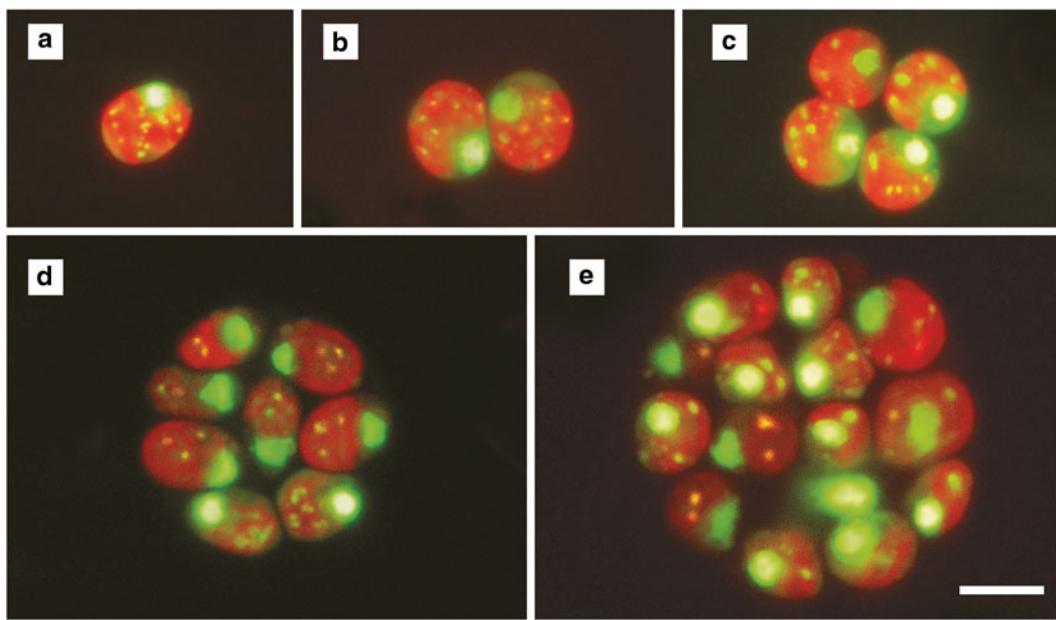


Fig. 5 Fluorescence photomicrographs of *Chlamydomonas reinhardtii* showing multiple division of protoplasts during the cell cycle. Stained with 0.3 % SYBR green I dye. (a) Uninuclear daughter cell. The nucleus is visible as a yellow-green spot and chloroplast nucleoids as tiny yellow-green dots. The chloroplast is visible in red color, which is due to autofluorescence of chlorophyll. (b) The first division of the pro-

toplasm; protoplast divided onward into two. (c) The second division of protoplasts; two protoplasts divided onward into four. (d) The third division of protoplasts; four protoplasts divided onward into eight. (e) The fourth division of protoplasts; eight protoplasts divided onward into 16 cells. Scale bar=10 µm (Modified after Vítová et al. 2005)

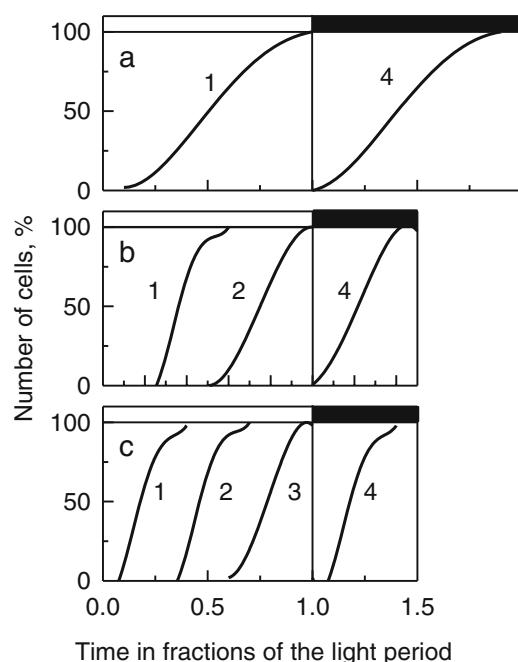


Fig. 6 Schematic drawing of commitment diagrams for three algal populations growing at low (a), medium (b), and high (c) growth rates. Detailed explanation in the text. Curves 1, 2, 3: percentage of cells in the population committed to division into two, four and eight daughter cells, respectively; curves 4: percentage of cells in the population which have released daughter cells. White and black strips above the panels indicate light and dark periods (After Šetlík and Zachleder 1983)

time period required to complete all committed processes (e.g. to finish all committed DNA replication-division sequences), they are examined under the microscope. The proportion of divided cells in the population is determined and, in doing so, mother cells that yielded different numbers of daughter cells are recorded separately. The numbers so obtained are plotted against the times at which the respective sample was darkened. In the case of coenobial species such as *Scenedesmus*, counting is very convenient since it can be done even in liquid medium; for other species, the cells are spread on a solid support (e.g. agar plates) and the resulting daughter cell microcolonies attached to the surface are counted. The resulting sigmoidal curves trace the increase in the percentage of committed cells with time. It is important to recognize that the shapes of the curves represent the variability in progress through the cell cycle among cells of the population, and thus characterize the degree of synchrony (Fig. 6).

The number of daughter cells (N_d) in most algae that divide into 2^n daughter cells is usually greater than 2 but the maximum number is rarely more than 32, usually $n=25$ (Figs. 8 and 9). The alga *Kentrosphaera* can produce about 2^{10} daughter cells, as illustrated in Fig. 10. There are, however, species such as coenobial algae of the family Hydrodictyaceae (*Hydrodictyon*) and colonial algae of the family Volvocaceae (*Volvox*) that may divide and produce up to several thousands of offsprings (Figs. 11 and 12). Species

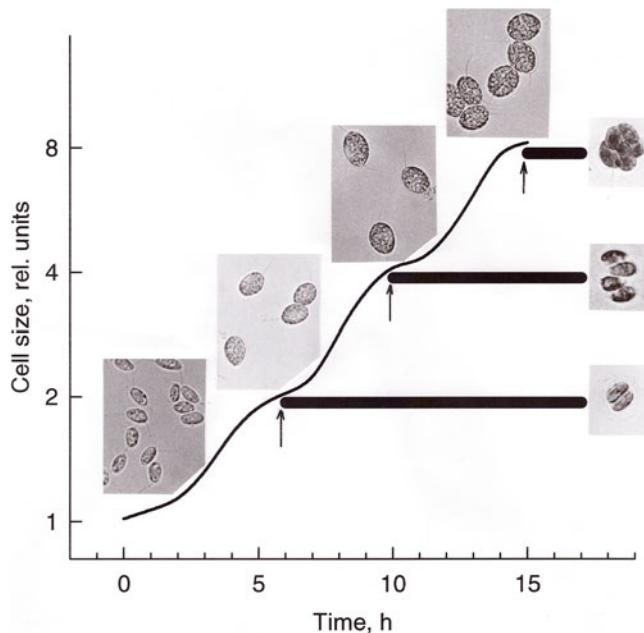


Fig. 7 Schematic illustration of the determination of commitment points to cellular division in synchronous populations of *Scenedesmus armatus*. The idealized curve represents the growth of cells in continuous light during the cell cycle; at the times marked by arrows, the subcultures were put into dark periods (indicated by horizontal black stripes); the microphotographs above the curve show typical cells from synchronized cultures at the time of transfer of subcultures into the dark; the vertical lane of photomicrographs illustrates (on agar plates) the micro-colonies of daughter cells that were released from one mother cell during the corresponding dark interval. The moments of transfer into the dark correspond to the attainment of the 1st (5 h of light), the 2nd (10 h of light) and the 3rd (15 h of light) commitment points; two, four, and eight daughter cells were released during the dark period, respectively (After Vítová and Zachleder 2005)

with cycle type C_n promise to provide significant results (Kirk 1998), although knowledge of their cell cycles is still limited. The cell cycle type for all algae in the family Hydrodictyaceae and Volvocaceae is of the C_n type. The value of n among members of families varies with growth conditions, but does not decrease below a certain lower limit; for the genus *Eudorina* $n=4\text{--}6$, for the genus *Volvox*, the values of n are between 8 and 14, and a similar range characterizes the genus *Hydrodictyon*. Of importance is the fact that algae closely related to these genera have a lower value of n under certain conditions, and can also divide into two daughter cells. Thus, over several related species, transition covers the whole range from $n=1$ to $n=14$. Related to the genus *Volvox*, there are genera for which typical colonies consist of 2 (*Didymochloris*), 4 (*Pascherina*), 8 or 16 (*Ulva*, *Spondylomorium*) cells and their closest relatives are *Gonium*, with 4–16 cells in the colony, *Pandora* with 8 or 16, and *Eudorina* with 1664. The genus *Pediastrum* belongs to the same family as the genus *Hydrodictyon*, whose cells divide into 2–128 daughter cells ($n=1\text{--}7$), and the genus *Sorastrum* with 8128 daughter cells ($n=3\text{--}7$). For comprehensive information on suborder Volvocinae algae, see the book “*Volvox*” (Kirk 1998).

3 Nuclear DNA Synthesis in the Cell Cycle

More than 60 years ago, analyses on the course of DNA synthesis in the synchronized chlorococcal alga *Chlorella ellipsoidea* (Iwamura and Myers 1959), and in volvocalean alga *Chlamydomonas reinhardtii* (Chiang and Sueoka 1967a, b) were first published. This was followed by studies on DNA replication in *Chlorella* (Wanka 1962, 1967; Wanka and Geraedts 1972; Wanka et al. 1972), *Desmodesmus* (*Scenedesmus*) *quadricauda* (Šetlík et al. 1972), and *Chlamydomonas reinhardtii* (Knutsen et al. 1974; Lien and Knutsen 1979).

The number of steps (rounds) of DNA replications is set by the number of commitment points attained and is determined by growth rate. In autotrophically growing cultures, it is light intensity-dependent; the higher the light intensity, the more DNA is synthesized (Donnan and John 1983; Iwamura 1955; Šetlík et al. 1988; Zachleder et al. 1988). While attaining a commitment point is light intensity-dependent, DNA replication itself is light intensity-independent. The ability of cells to replicate DNA can be assessed in dark samples taken from light grown cultures, where the committed DNA is replicated during sufficiently long dark intervals. If plotted against the time of darkening, “committed DNA” can be monitored. It was repeatedly found that rounds of DNA replication are committed in steps. A clear step-wise increase was observed not only in species with a *Scenedesmus*-type cell cycle but also in species with a *Chlamydomonas*-type cell cycle, such as *Chlamydomonas reinhardtii* (Donnan and John 1983), supporting the fact that DNA replication is indeed committed separately after each commitment point.

Based on published data, the course of DNA replication in synchronized populations of algae can be divided into two groups, consecutive and clustered.

3.1 Consecutive Rounds of DNA Replication

The increase in DNA content in synchronous populations begins to rise quite early in the cell cycle and has an apparent stepwise character with steps corresponding to consecutive DNA replication rounds (Fig. 13). This course is characteristic for algae with a *Scenedesmus*-type cell cycle (see preceding chapter) and it has been described in detail in synchronous cultures of *Desmodesmus* (*Scenedesmus*) *quadricauda* (Ballin et al. 1988; Šetlík et al. 1972; Zachleder et al. 1988, 2002; Zachleder and Šetlík 1988); it was also reported in some strains of *Chlorella*, e.g. *Chlorella vulgaris* v. *vulgaris* (Umlauf and Zachleder 1979) and the thermophilic strain of *Chlorella pyrenoidosa* (Vassef et al. 1973).

Fig. 8 Fluorescence photomicrographs of the yellow-green alga *Bumilleriopsis filiformis* (Mischococcales) stained with DAPI at different developmental stages of the cell cycle. (a) Binuclear and tetranuclear cells. (b) Multinuclear cells. Spherical arrangement of nucleoids in the individual chloroplasts can be seen. Scale bar = 20 µm (After Zachleder and Cepák 1987c)

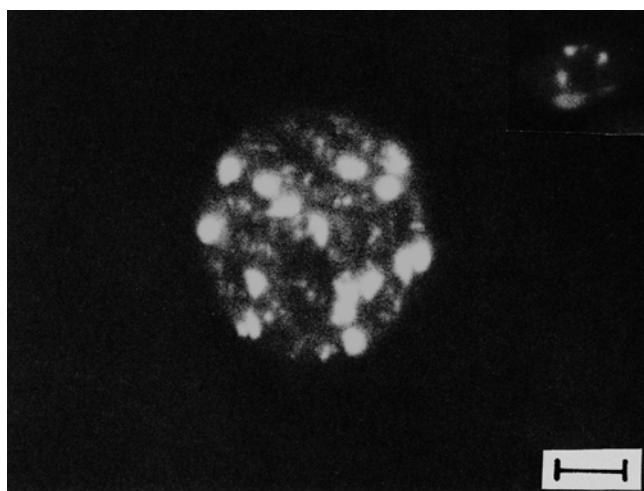
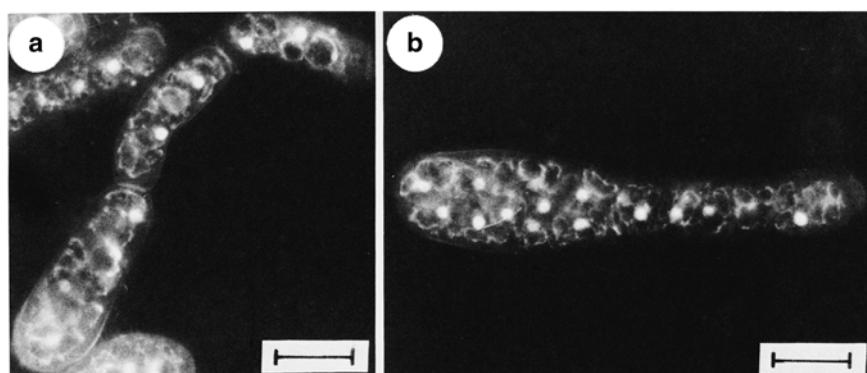


Fig. 9 Fluorescence photomicrographs of the green alga *Nautococcus piriformis* (Tetrasporales) stained with DAPI. A mature mother cell with 16 nuclei and numerous nucleoids. A young cell with one nucleus and four nucleoids is inserted in the top right-hand corner. Scale bar = 10 µm (After Zachleder and Cepák 1987c)

If DNA replication occurs in a stepwise mode, the consecutive DNA replications for each committed sequence are distinctly separated by time intervals during which it is assumed that extensive gene transcription occurs. Stepwise DNA replication is clearly connected with periodic fluctuations in the ratios of RNA:protein, and cell volume:DNA, since the ratios repeatedly rise to double values in the intervals between steps of DNA replication and decrease during DNA replication itself (Fig. 14) (Šetlík and Zachleder 1981).

In some synchronized cultures of different species, DNA content increased within a relatively lengthy phase of the cell cycle, and its progression was sigmoidal, with no apparent or only slight steps indicating changes in the rate of DNA replication. This course has been described in some species of *Chlorella* (Iwamura and Myers 1959; Senger and Bishop 1966, 1969) and of *Volvox* (Tucker and Darden 1972; Yates et al. 1975). Even in these cases, however, it cannot be excluded that in a single cell, rounds of DNA replication are separated by relatively long time intervals. Even in synchro-

nized populations, the lack of apparent separation between DNA replication cycles and the DNA content curve could be caused by high variability in cell generation times (Šetlík et al. 1972).

A very important result was that even in synchronized cultures, where the time course of DNA synthesis had a smooth sigmoidal shape without apparent steps, a stepwise increase in DNA content in cells incubated in the dark was found (Zachleder and Šetlík 1988); this was denoted as “committed” DNA synthesis (Fig. 15).

3.2 Clustered Rounds of DNA Replication

For this pattern of DNA synthesis, the time interval in which a single round of DNA synthesis takes place is not much longer than the time required for multiple replications corresponding to the number of duplications; it is characteristic of cells with a Chlamydomonas-type cell cycle (see preceding chapter). The DNA content in synchronized populations increases sharply, in one wave at the end of the cell cycle, to multiples corresponding to the number of daughter cells released by division.

The first publication on this type of DNA replication in the cell cycle of *Chlamydomonas reinhardtii* was in 1967 (Chiang and Sueoka 1967a, b). However, *Chlamydomonas reinhardtii*, belonging to cells with a C_n type of cell cycle, was grown in a synchronous culture under sub-optimal growth conditions that supported only a twofold increase in DNA and consequent division into two daughter cells (cell cycle type C_1). Nevertheless, one wave of DNA synthesis occurring at the end of the cell cycle (Fig. 16) is characteristic of all species with a Chlamydomonas C_n type of cell cycle, even with a much higher value of n .

The courses of multiple DNA replications in different strains and mutants of synchronized *Chlamydomonas reinhardtii*, as well as under phosphate limiting conditions, were described in several papers by Knutsen and Lien (1981), Knutsen et al. (1974), and Lien and Knutsen (1973, 1976, 1979); an example of the course of DNA replication multi-

Fig. 10 Fluorescence photomicrographs of the chlorococcal alga *Kentrosphaera* sp. stained with DAPI. (a) A giant mother cell with an enormous number of nuclei (showing only those seen in one focal plane). (b) Freshly released daughter cells from one mother cell

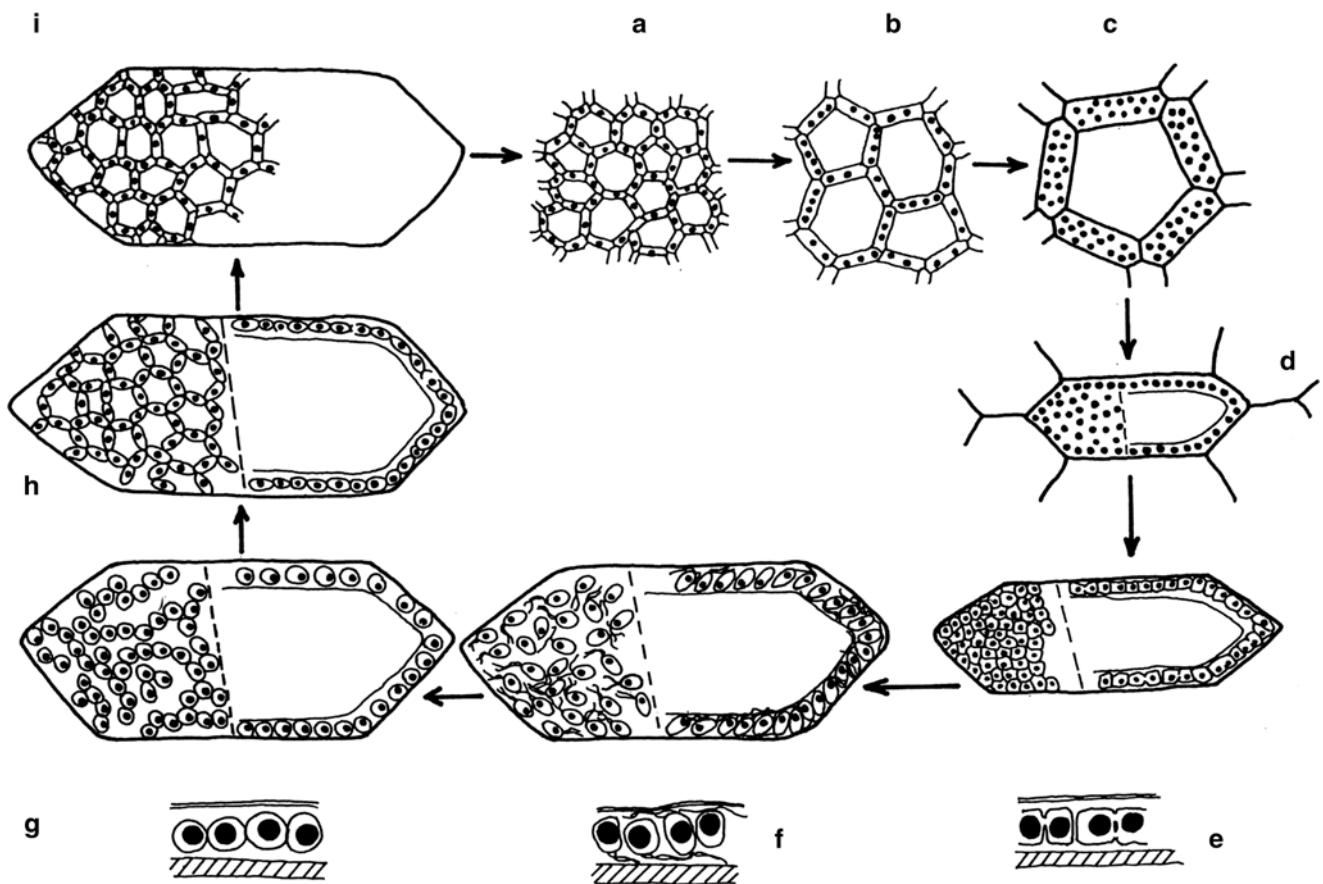
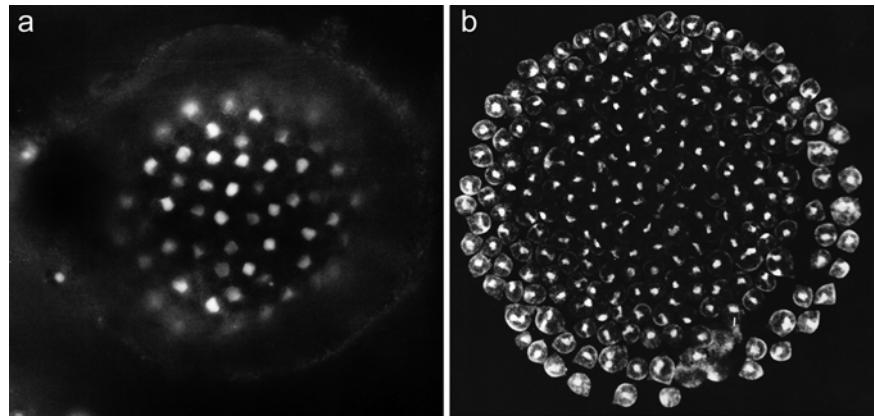


Fig. 11 *Hydrodictyon reticulatum* scheme of the cell cycle. (a) Uninuclear daughter cell released from mother cell wall, (b–d) multiple nuclear division in growing cells, (e) division into uninuclear protoplasts, (f) formation of biflagellar cell-wall-less zoospores, (g) conversion into zoospores without flagella, (h) forming of areolate coenobium (After Šetlák and Zachleder 1981)

plying to 16-fold ($n=4$) in a strain of *Chlamydomonas reinhardtii* is illustrated in Fig. 17. The level of DNA before replication was estimated to be 2×10^{-13} g cell $^{-1}$ and this amount remained constant for the first 89 h of the light phase. Thereafter, during the next 4 h, DNA/cell increased to the same extent as the increase in the average number of offspring, usually 16-fold (Lien and Knutsen 1979).

A similar time course of DNA replication was described not only in *Chlamydomonas reinhardtii* (Lien and Knutsen 1973, 1976) but also in the thermophilic species *Chlorella pyrenoidosa* (Hopkins et al. 1972) and in *Eudorina elegans* (Kemp and Lee 1975).

In all cases, the replication steps followed each other almost immediately and there was no time lag between them

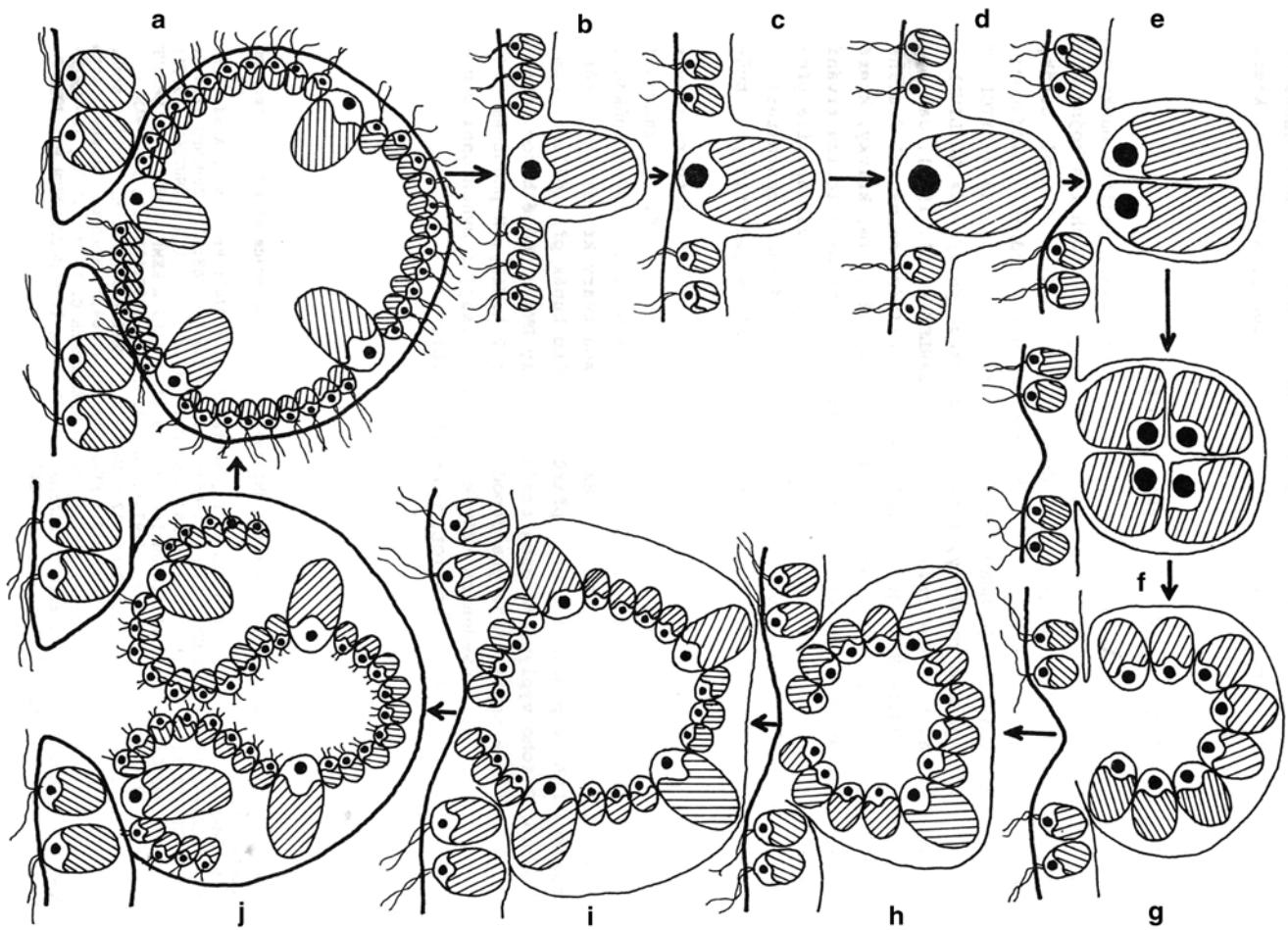


Fig. 12 *Volvox* scheme of the cell cycle. A diagram of the development of a new colony of gonidium for the genus *Volvox*. The young colony, which is released from the wall of the mother cell (**a**) nonflagellated gonidium, considerably larger than the other cells (**a–d**) and begins to divide (**e, f**). At one of early stages of synchronous division (**g**) unequal cells are formed. One type of cell does not divide more but grows in volume (gonidia), the other continues in division and remains small in

volume (vegetative cells). By division of vegetative cells inside the mother cell (**h, i**) the final number of cells and the future colony are attained. The colony is inverted (**j**) so that the internal poles of cells occurs on the surface and form flagella. The vegetative cells do not divide any more and will eventually die after colonies are released (After Šetlík and Zachleder 1981)

to allow for any other processes, including massive gene transcription.

Although the two patterns of DNA replication seem well separated, they can merge with each other under specific growth conditions. DNA synthesis in synchronous populations of *Chlamydomonas reinhardtii* growing in the absence of phosphorus occurs in several steps, as opposed to the standard increase in a single wave (Lien and Knutson 1973). On the other hand, the thermophilic species, *Chlorella vulgaris*, grown under a threshold temperature of 43 °C, has nuclear and cellular divisions blocked, but DNA replication occurs in steps (Šetlík et al. 1975).

4 Regulation of Cell Cycle of Algae

In general, the cell cycle consists of two distinct, but closely interacting, sequences of processes and events. These have been historically termed the “growth cycle” and the “DNA-division cycle” (Mitchison 1971, 1977). In the context of C_n cell cycle types, the “growth cycle” corresponds to a pre-commitment period and the DNA replication-division sequence to a post-commitment period (as already defined in preceding chapters). Most macromolecular syntheses occur during the pre-commitment period, which results in an increase in cell mass and the formation of cell structures.

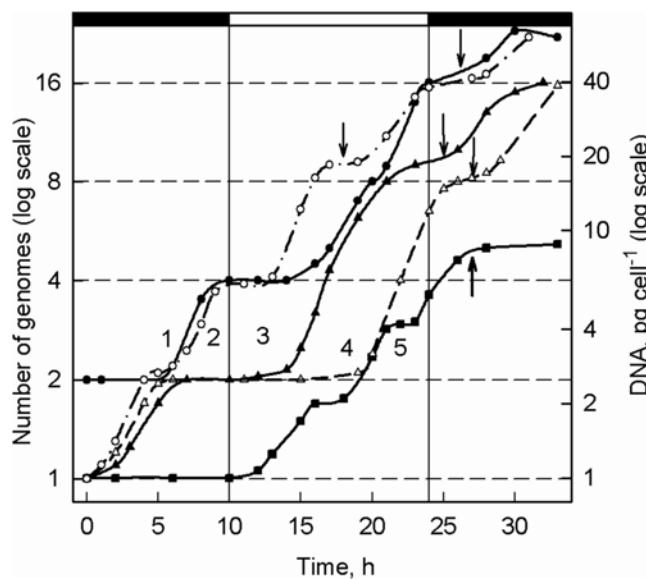


Fig. 13 The stepwise course of DNA replication under conditions of different growth rates and light-dark regimes in the cell cycle of *Desmodesmus (Scenedesmus) quadricauda*. Positions of the midpoints of cell divisions are indicated by arrows and the light-dark periods (for curves 2–5) are indicated by strips above the figure and by vertical lines. Curve 1: A synchronized culture grew in continuous light for two cell cycles (dark periods were omitted). The course of DNA synthesis in the second cycle is illustrated. Growth rate = 28 pg of protein $\text{cell}^{-1} \text{ h}^{-1}$. Curve 2: The population of the fastest growing (i.e. the biggest) cells was selected by sedimentation from the original strain and allowed to grow under alternating light-dark periods (14:10 h). Growth rate = 32 pg of protein $\text{cell}^{-1} \text{ h}^{-1}$. Curve 3: The same culture as illustrated by curve 1 grown under a light-dark regime (14:10 h). Growth rate = 24 pg of protein $\text{cell}^{-1} \text{ h}^{-1}$. Curve 4: The daughter cells were obtained from the culture darkened at the 6th h of light (6:8 h). Growth rate = 120 pg of protein $\text{cell}^{-1} \text{ h}^{-1}$. Curve 5: The culture grown under alternating light-dark (14:10). Growth rate = 20 pg of protein $\text{cell}^{-1} \text{ h}^{-1}$ (After Šetlík and Zachleder 1983)

The main events in the post-commitment period (DNA replication-division sequence) are: replication of DNA, nuclear division, and cytokinesis, including processes leading to their initiation (for more detail see Sect. 2). While the rate of growth processes depends primarily on the rate of energy supply and raw materials for synthetic processes from outside of the cell, reproductive processes are carried out under standard conditions at a strictly determined rate that is specific to a given organism and depends mostly on temperature (see below).

The main regulatory point separating sequences of pre- and post-commitment is the commitment point. In autotrophically grown algae, it is convenient to define the commitment point as a transition point when the cell becomes capable of division in the dark; more generally, in the absence of an external energy supply. This indicates that algae have a regulatory mechanism ensuring that the reproductive sequence is triggered only if the cell is capable of completing the whole sequence without any external source of energy.

However, it must be noted that commitment point is not a point but rather a short part of the cell cycle that consists of several segments: commitment point for DNA replication, commitment point for nuclear division and commitment point for cytokinesis. Usually, all these segments follow so close to each other that the difference is not noticeable. In some situations however, only one or two of them are committed and the cells become temporally arrested with polyploid (only DNA replication committed) nuclei or with multiple nuclei (DNA replication and nuclear but not cellular divisions committed).

The coordination of growth and DNA replication-division sequences appears to be controlled by the achievement of a threshold cell size necessary for the initiation of DNA replication (Nasmyth et al. 1979; Nasmyth 1979). Another cell size control is supposed to be a prerequisite for the onset of nuclear division (Fantes and Nurse 1977; Fantes 1977). It is, however, assumed that it is not the cell size itself, but some other more specific processes that can be coupled or coordinated with the increase in cell size. Synthesis of RNA and protein are the most important features of the growth cycle and both processes are considered to play a major role in the control of cellular reproductive processes via regulation at the commitment point (Alberghina and Sturani 1981; Darzynkiewicz et al. 1979a, b; Johnston and Singer 1978).

The importance of regulation at the commitment point is evident from the behavior of cells blocked in G1 phase due to limiting nutrients or energy supply. Algal cells taken from the stationary phase of asynchronous cultures (which are usually limited by light) are synchronized in G1 phase and thus are often used as inocula for synchronous cultures (Tamiya et al. 1953; Tamiya 1964). Synchronous populations of *Chlamydomonas reinhardtii* and chlorococcal algae grown from the beginning of the cell cycle in mineral medium deficient in nitrogen, sulfur or phosphorus are also blocked in G1 phase (Ballin et al. 1988; Lien and Knutson 1973; Šetlík et al. 1988; Tamiya 1966; Zachleder et al. 1988; Zachleder and Šetlík 1982, 1988, 1990). Diatoms can be arrested in G1 phase by a deficiency in silicon, which they need to build cell walls; consequently it is crucial for the start of DNA replication (Darley and Volcani 1969; Sullivan and Volcani 1973). Thus, as long as the critical size required for attaining commitment point is reached, no DNA replication-division sequence can take place.

The interdependency between growth processes and cell cycle progression can be assessed by studies of RNA and bulk protein synthesis in synchronized cultures. In control cultures of *Desmodesmus (Scenedesmus) quadricauda*, the RNA and protein content increased in several steps, each of them corresponding to a doubling of the preceding value (Šetlík et al. 1972; Šetlík and Zachleder 1984; Zachleder et al. 1975; Zachleder and Šetlík 1982, 1988). The number of stepwise increases in both RNA and protein matched the

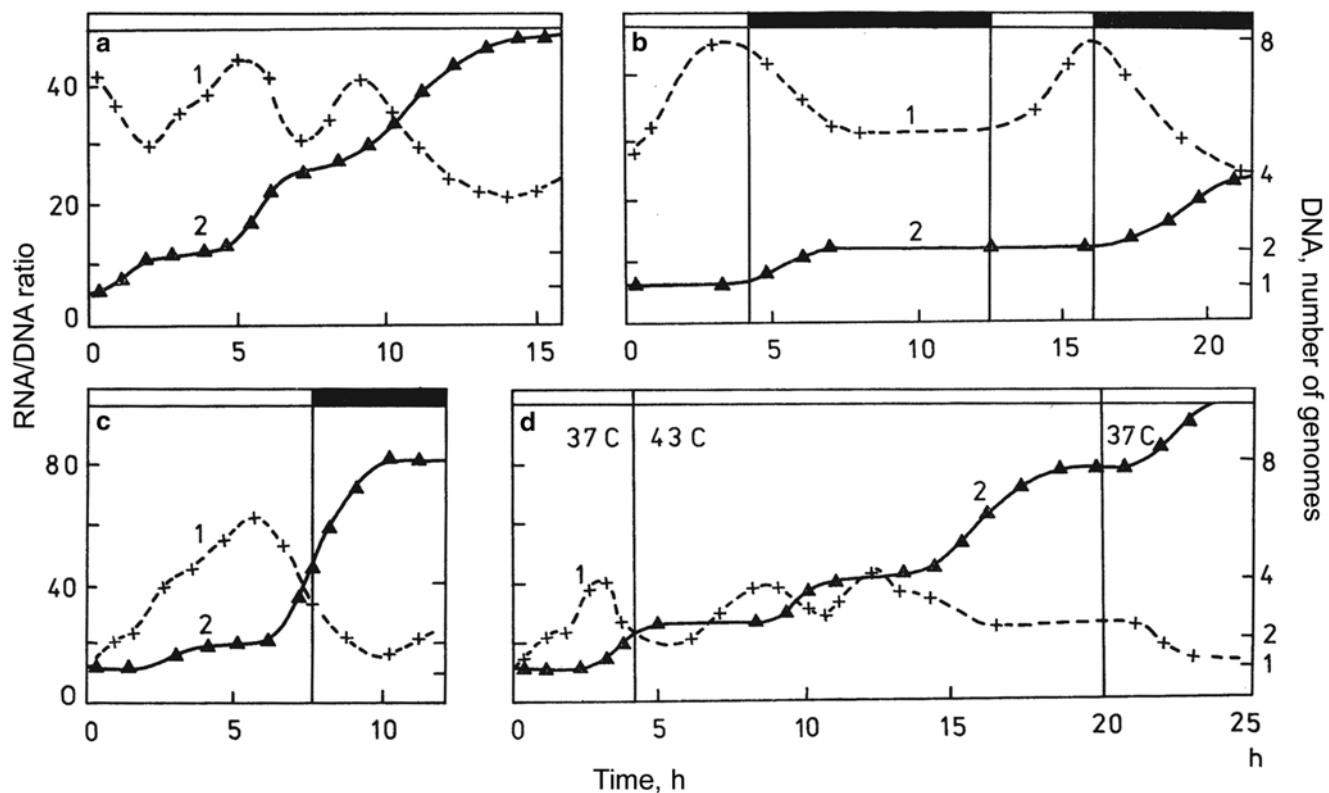


Fig. 14 Changes in RNA to DNA ratio in synchronized populations of *Desmodesmus (Scenedesmus) quadricauda*. (a) Continuous light (b) Inserted dark interval separated two growth steps. (c) Culture growing under alternating light and dark periods. (d) Inserted interval of supra-optimal temperature slowed down the DNA replication rate so that the

replication steps are well separated in time. Dark intervals are indicated by black stripes and separated by vertical lines. 1 the course of the ratio of RNA to DNA, 2 the course of DNA replication (After Šetlík and Zachleder 1981)

number of DNA replication-division sequences that were initiated (Figs. 18 and 19). For both RNA and protein, the maximum of each doubling precedes attaining the commitment point; this implies a threshold amount of both macromolecules has to be reached prior to the cell attaining commitment point.

Similarly, stepwise accumulation of RNA was shown to occur in *Chlamydomonas reinhardtii* (Knutsen and Lien 1981; Lien and Knutsen 1979). The number of steps of RNA accumulation affects the number of DNA replication rounds. Each of these steps, representing an approximate doubling of RNA, is followed shortly thereafter either by a corresponding replication of DNA, as in *Desmodesmus (Scenedesmus) quadricauda* (Ballin et al. 1988; Šetlík et al. 1988; Zachleder et al. 1988; Zachleder and Šetlík 1982, 1988, 1990) or multiple replication rounds at the end of the cell cycle corresponding to the number of RNA accumulation steps, as in *Chlamydomonas reinhardtii* (Knutsen and Lien 1981; Lien and Knutsen 1979). So the initiation of the DNA replication-division sequence, e.g. DNA replication, nuclear division and cell division, as well as their number, is tightly controlled by growth processes, i.e. by RNA and protein synthesis.

It was mentioned above that the entire DNA replication-division sequence is not always committed and completed so

the cells remain undivided with polyplloid or have multiple nuclei. How does this occur? Usually in a growth sequence, RNA synthesis precedes protein synthesis for different time intervals. RNA synthesis starts earlier and, in contrast to bulk protein synthesis, can be performed for some time in the dark. By an appropriate choice of cultivation conditions, the two processes can be uncoupled (Fig. 20). It is clear that DNA replication rounds are completed in proportion to the amount of RNA, while nuclei divide in proportion to the amount of protein (Zachleder and Šetlík 1988). Thus, *Desmodesmus quadricauda* requires a longer growth period for the commitment point to nuclear division than for the commitment point to DNA replication.

Is this growth-cell cycle relationship specific for algae? Not at all. A threshold RNA amount is required for DNA replication in mammalian cells (Adam et al. 1983; Baserga 1990; Darzynkiewicz et al. 1979a, b, 1980; Fujikawa-Yamamoto 1982, 1983; Johnston and Singer 1978) and blocking of RNA synthesis prevents DNA replication in both mammals (Baserga et al. 1965; Lieberman et al. 1963) and yeast (Bedard et al. 1980; Lieberman 1995; Singer and Johnston 1979, 1981). This suggests a more general mechanism governing the coordination between growth and cell cycle progression.

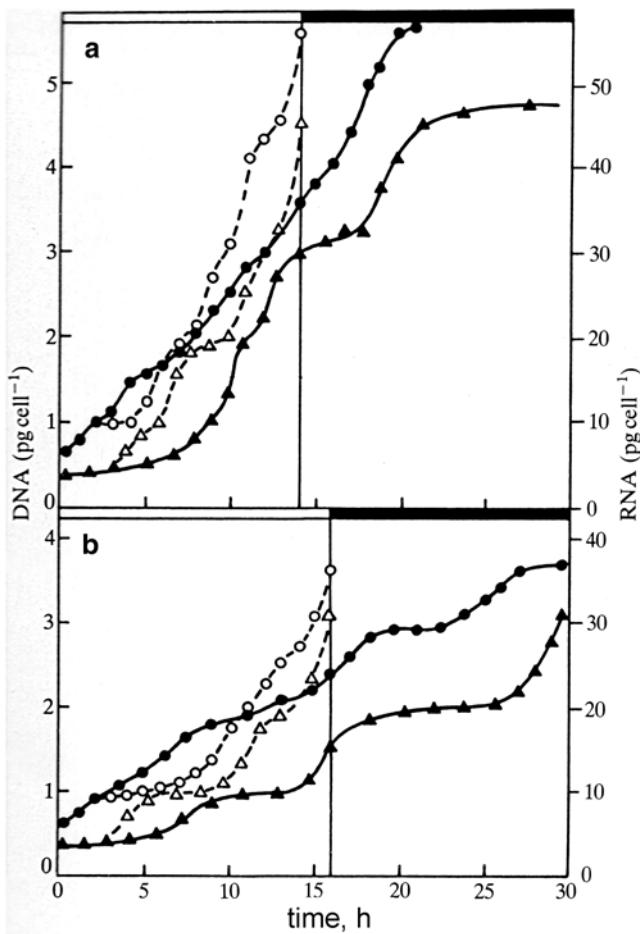


Fig. 15 Time course of RNA and DNA synthesis and their committed values in synchronous cultures of *Desmodesmus (Scenedesmus) quadricauda* grown under optimal growth conditions (a) and under conditions of slowed growth (b). Light and dark periods are indicated by white and black strips at the top of each panel and are separated by a vertical line. (●) RNA; (▲) DNA; (○) committed RNA; (Δ) committed DNA (After Zachleder and Šetlík 1988)

However, a critical question remains. “Do running DNA replication-division sequences control growth processes?” To answer this, the DNA replication-division has to be blocked and the effect of this treatment on growth needs to be assessed. In *Desmodesmus (Scenedesmus) quadricauda*, when 5-fluorodeoxyuridine was added to daughter cells, DNA replication and all subsequent reproductive events, such as nuclear and cellular division, were inhibited. On the other hand, both RNA and protein synthesis continued at a slower rate than in untreated cultures, but attained a 16-fold increase in their initial content, while DNA content was kept at its initial value (Fig. 21) (Zachleder 1994). Clearly, growth processes are a prerequisite for attaining a commitment point and initiating a DNA replication-division sequence, but completion of these initiated processes is growth-independent. Moreover, there is no impact on growth even if the commit-

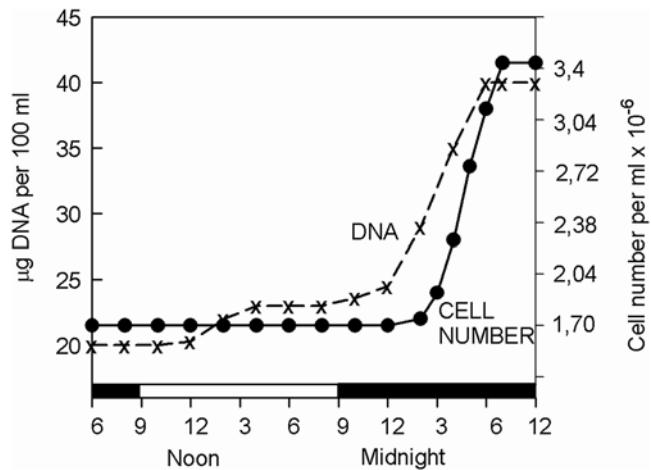


Fig. 16 Time course of DNA synthesis and cell number in a synchronized culture of *Chlamydomonas reinhardtii*. Two daughter cells were released at the end of the cell cycle under given growth conditions (After Chiang and Sueoka 1967b)

ted DNA replication-division sequence cannot be completed.

From the point of cell cycle regulation, it is very interesting that not only synthesis itself but also stepwise oscillations in the rate of synthesis of both macromolecules were preserved, even in the absence of reproductive processes (Fig. 21). This indicates that all processes required for the commitment point were probably consecutively performed, in spite of the fact that committed processes like DNA replication and nuclear and cell division themselves were blocked. This also implies that growth and cell cycle processes are regulated by distinct mechanisms. The effect of growth on cell cycle progression is probably coincidental in providing sufficient reserves for completion of a DNA replication-division sequence, but having no direct interaction. The molecular mechanisms underlying cell cycle progression are discussed in Chap. 5.

5 Molecular Mechanisms Regulating Cell Cycle Progression

The understanding of molecular mechanisms governing cell cycle regulation comes from two genetic screens performed in budding yeast (Culotti and Hartwell 1971; Hartwell 1971; Hartwell et al. 1970, 1973, 1974) and in fission yeast (Beach et al. 1982; Fantes and Nurse 1977; Nasmyth and Nurse 1981; Nurse 1975; Nurse et al. 1983; Nurse and Fantes 1977; Nurse and Thuriaux 1977; Nurse et al. 1976; Thuriaux et al. 1978) that identified master regulators of the cell cycle, denoted as CDC28 and cdc2, respectively. The two genes differed in the parts of cell cycle that they regulated and at

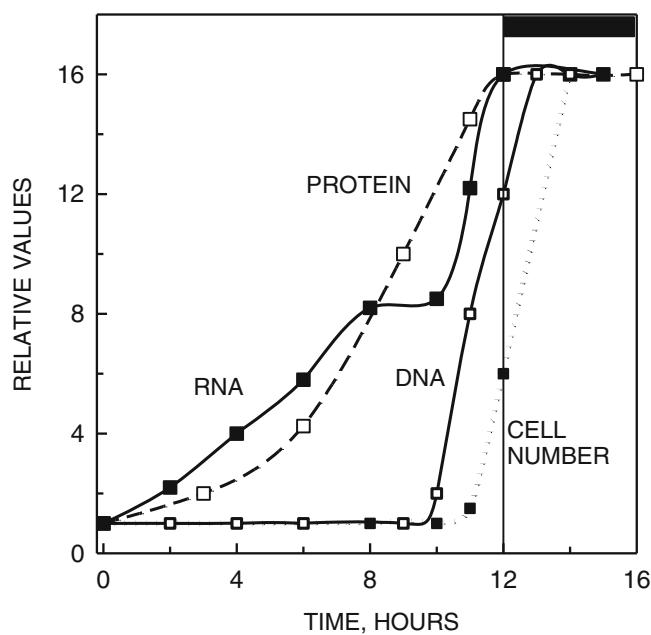


Fig. 17 Time course of increases in various parameters as indicated in the graph in a synchronized culture of *Chlamydomonas reinhardtii* (Modified after Lien and Knutsen 1979)

first sight seemed unrelated. However, in less than two decades it became clear that not only were the two genes homologous but that homologs of the gene, encoding protein kinases (Hindley and Phear 1984; Moreno et al. 1989; Reed et al. 1985; Simanis and Nurse 1986), are encoded in the human genome and have similar functions (Langan et al. 1989; Lee and Nurse 1987). Further experiments have proven the strikingly high conservation of cell cycle regulators among eukaryotes. The core cell cycle machinery includes homologs of CDC28/cdc2, denoted as cyclin-dependent kinases (CDKs). Both yeasts require only one CDK (CDC28 or cdc2) to drive the cell cycle (Mendenhall and Hodge 1998; Moser and Russell 2000). Other eukaryotes usually require more than a single gene. In humans and other mammals, there are several CDK homologs: three of them (CDK1/cdc2, CDK2 and CDK3) are considered genuine CDC28/cdc2 homologs since they possess the same canonical PSTAIRE motif in their cyclin-binding domains; another homolog/s, CDK4/6, encodes a P(I/L)ST(V/I)RE variant of the conserved motif (Lee and Yang 2003; Meyerson et al. 1992; Pines 1996; Reed 1997). Higher plants encode two classes of cell cycle regulating CDKs, A- and B-type. CDKAs possessing a PSTAIRE motif represent the genuine CDC28/cdc2 orthologs (Ferreira et al. 1991; Hirt et al. 1991) (Mironov et al. 1999) while CDKBs are a plant-specific family of CDKs with a unique expression pattern (Dewitte and Murray 2003; Dewitte et al. 2003; Mironov et al. 1999). The first green algal homologs of CDC28/cdc2 were identified in *Chlamydomonas reinhardtii* by antibody cross-reactivity

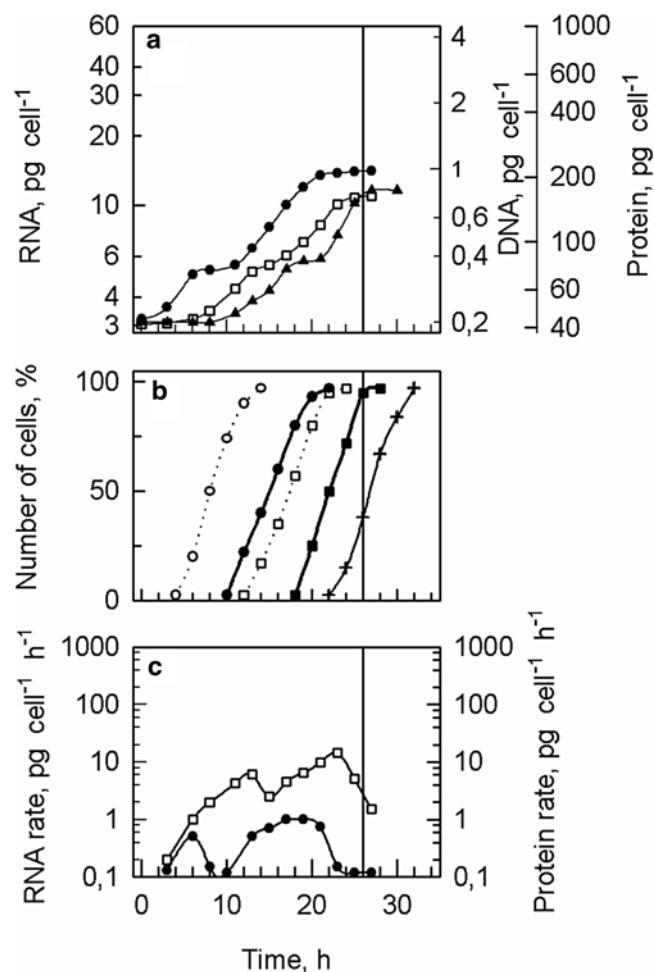


Fig. 18 Time course of growth and reproductive processes in synchronous populations of *Desmodesmus (Scenedesmus) quadricauda* grown at low irradiance. Mean irradiance 45 W m^{-2} , continuous light, temperature 30°C . (a) Variation in RNA (●), protein (□), and DNA (▲) amounts per cell. The first and second cell cycles are separated by a vertical dotted line. (b) Course of commitment to nuclear and cellular division and termination of these processes. Dotted curves: percentage of cells that attained commitment for the first (○) and second (□) nuclear divisions. Solid curves: percentage of cells in which the first (●) and second (■) nuclear divisions were terminated and percentage of cells that released their daughter cells (+). (c) Oscillations in the rates of accumulation of RNA (●) and protein (□) (After Zachleder 1995)

(John et al. 1989). Protein abundance of putative CDC28/cdc2 increased as the cells entered the commitment point and slower migrating phosphorylated forms of the protein appeared as they entered mitosis, indicating involvement of this protein in cell cycle regulation (Fig. 22). Kinase activity of CDKs is assessed by the extent of phosphorylation of histone H1, which is considered a CDK-specific substrate. In *Chlamydomonas reinhardtii*, the peak of kinase activity correlates with the attainment of commitment points and with nuclear divisions (Fig. 23), confirming the existence of putative CDK and suggesting its involvement in cell cycle regulation (Zachleder et al. 1997). A more detailed analysis of

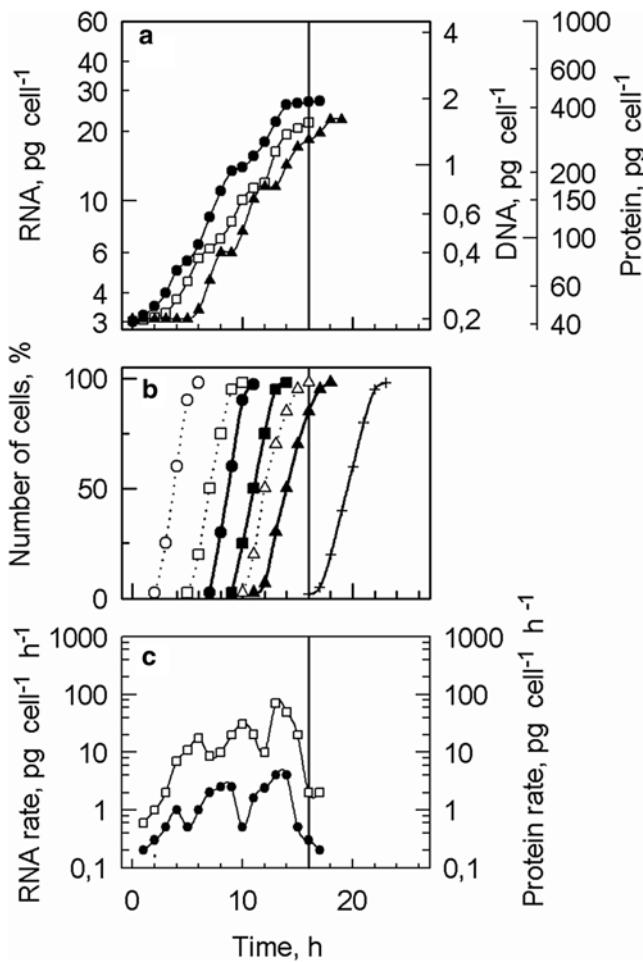


Fig. 19 Time course of growth and reproductive processes in synchronous populations of *Desmodesmus (Scenedesmus) quadricauda* grown at high irradiance. Mean irradiance 85 W m^{-2} , continuous light, temperature 30°C . (a) Variation in RNA (●), protein (□), and DNA (▲) amounts per cell (log scale). (b) Course of commitment points to nuclear and cellular division and termination of these processes. *Dotted curves*: percentage of cells that attained commitment point for the first (○), second (□), and third (Δ) nuclear divisions. *Solid curves*: percentage of cells in which the first (●), second (■), and third (▲) nuclear divisions were complete and percentage of cells that released eight daughter cells (+). (c) Oscillations in the rates of accumulation of RNA (●) and protein (□) (After Zachleder 1995)

putative CDKs in *Scenedesmus quadricauda* revealed that the two types of CDK complexes could be separated, one with activity related to growth and attainment of commitment point, and the second one with activity related exclusively to nuclear division (Bišová et al. 2000; Tulin and Cross 2014).

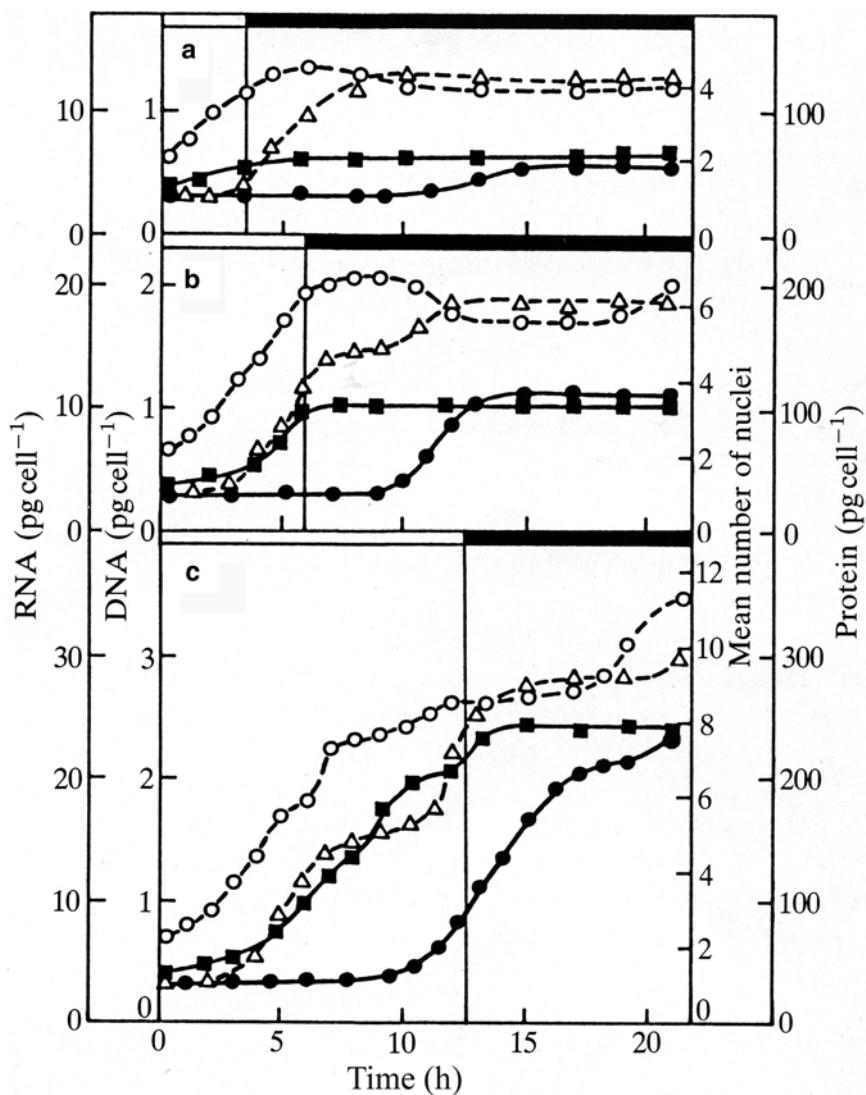
After complete sequencing of the *C. reinhardtii* genome (Merchant et al. 2007), comprehensive analysis identified homologs of all major CDKs (and cyclins, see below) present in higher plants, including plant-specific B-type CDK as well as some *C. reinhardtii*-specific CDKs with so far unknown functions (Bišová et al. 2000). The existence of

multiple CDKs, apparently involved in cell cycle regulation, and the existence of plant-specific CDKB indicate that organization of *C. reinhardtii* cell cycle genes is more plant-like and metazoan-like than are yeast (Bišová et al. 2000).

Similarly completion of genome sequencing of the green alga *O. tauri* (Derelle et al. 2006), the red alga *Cyanidioschyzon merolae* (Matsuzaki et al. 2004), diatoms *Thalassiosira pseudonana* (Armbrust et al. 2004) and *Phaeodactylum tricornutum* (Bowler et al. 2008) showed plant-like, genome-encoded cell cycle genes (<http://merolae.biol.s.u-tokyo.ac.jp/>) (Huysman et al. 2010; Robbins et al. 2005). Specific information on algal cell cycle regulators is scarce and often unravels algal-specific functions. One of the best characterized CDKs are those of *O. tauri*, where B-type CDK seems to be the main regulator of the cell cycle, in contrast to higher plants where A-type CDK is the main player (Corellou et al. 2005). OtCDKB can also be phosphorylated on tyrosine. This is in contrast to OtCDKA that is not phosphorylated although it also contains the conserved tyrosine residue; another striking difference when compared to higher plants, where the situation is quite the opposite. Isolation of temperature sensitive cell division cycle mutants in *CDKA1* and *CDKB1* genes in *C. reinhardtii* brought an understanding of their functional differences. Of the two, CDKA seems to be the key enzyme regulating cell cycle progression since it is crucial for initiation of DNA replication and cytokinesis and presumably also commitment point. On the contrary, CDKB is only required to complete the processes initiated by CDKA activity, for spindle formation, nuclear division and subsequent rounds of DNA replication (Tulin and Cross 2014). This is in line with the hypothesis of CDKB being the key regulator of mitosis in higher plants (De Veylder et al. 2011).

As the name implies, CDKs depend on and interact with another subunit, cyclin (Sherr et al. 1994). Cyclins were first discovered as proteins that were periodically degraded at each division in sea urchin eggs (Evans et al. 1983) and later, were proved to be key components of the M-promoting factor and partners of CDKs (Hunt 1989; Meijer et al. 1989; Minshull 1989; Minshull et al. 1989a, b). The three main cyclin classes comprise proteins transcribed during G1 (D-type), S (A-type) and M (B-type) phases; while the latter two are orthologous in animals and plants, the D-type cyclins are not conserved between the two kingdoms although they share the same transcriptional pattern (for review, see Abrahams et al. 2001; Mironov et al. 1999; Murray 2004; Renaudin et al. 1996). The CDKs are expressed constitutively, with the sole exception of plant-specific B-type CDK (Boudolf et al. 2004; Corellou et al. 2005; Fobert et al. 1996; Lee et al. 2003; Magyar et al. 1997; Menges et al. 2002; Porceddu et al. 2001; Segers et al. 1996; Sorrell et al. 2001) and the activity of any particular CDK-cyclin complex is, to a large extent, determined by cyclin availability. Cyclin

Fig. 20 Effect of darkening after different light intervals on the time course of RNA, DNA and protein synthesis and of nuclear divisions in synchronous cultures of *Desmodesmus (Scenedesmus) quadricauda* grown under optimal growth conditions. $I=95 \text{ W m}^{-2}$, $D=0.10 \text{ h}^{-1}$. The cultures were put into dark after attaining the first (**a**), second (**b**) and third (**c**) commitment points to divide into two, four and eight nuclei. Light and dark periods are indicated by white and black strips at the top of each panel and are separated by a vertical line. (○) RNA; (Δ) DNA; (■) protein; (●) nuclei (After Zachleder and Šetlík 1988)

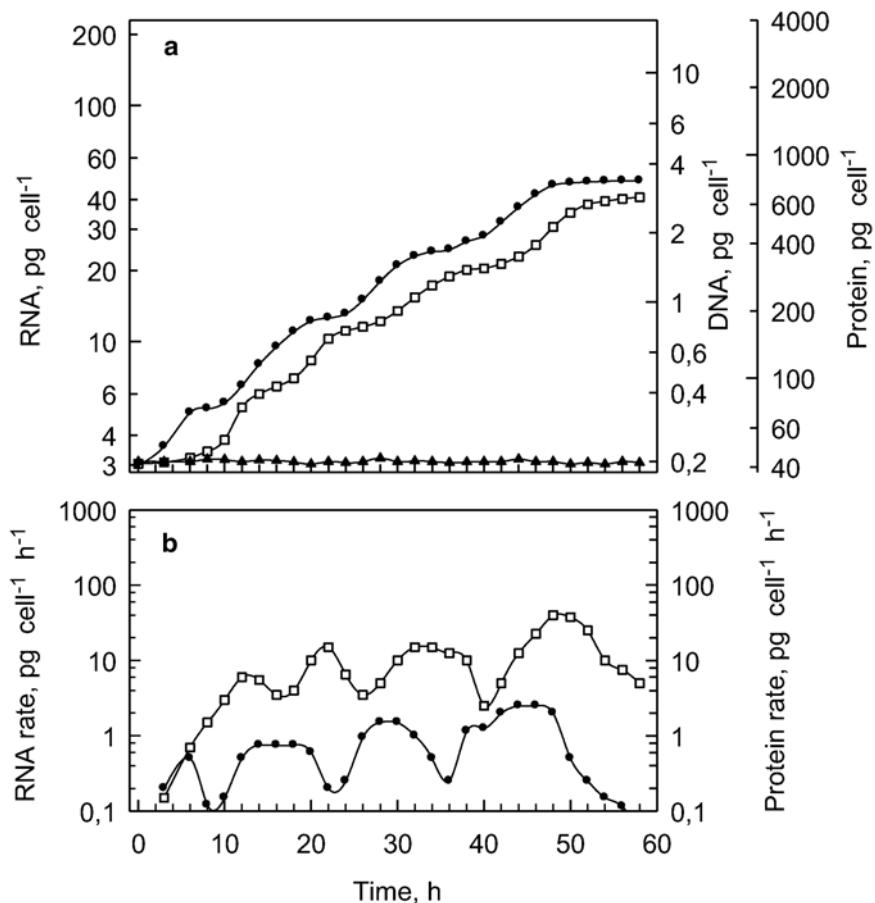


abundance is regulated both by phase specific transcription and degradation. Transcriptional patterns of different algal cell cycle genes mimic the transcriptional patterns of their higher plant counterparts (Bisova et al. 2005; Corellou et al. 2005; Farinas et al. 2006; Huysman et al. 2010; Shrestha et al. 2012), on top of which they are also differentially expressed during the light/dark cycle (Bisova et al. 2005; Huysman et al. 2010, 2013; Moulager et al. 2007, 2010). A diatom specific cyclin, dsCYC2, a partner of CDKA, is induced in a rate-dependent manner, by blue light. Interestingly, it seems to be responsible for regulation of the rate of cell division, specifically in light/dark cycles and not in continuous light (Huysman et al. 2013). This suggests that, at least in diatoms, light has not only a trophic but also a signaling role in cell cycle regulation. In *O. tauri*, *OtCYCA* was transcribed ubiquitously during the cell cycle (Corellou et al. 2005; Farinas et al. 2006) but its translation was light and cAMP-dependent. *OtCycA* interacts with retinoblastoma protein (Rb) during S phase and thus regulates S phase

entry (Moulager et al. 2007, 2010). Both G1 cyclin (cyclin A) and Rb are known sizers in other organisms. G1 cyclin Cln3 has been accepted as a sizer in budding yeast (Rupeš 2002) and Rb protein was genetically identified as a sizer in *C. reinhardtii* (for details see below) (Umen and Goodenough 2001). The proven interaction of these two proteins, and most importantly their involvement in growth-dependent S phase entry, combines the two suspected sizers into a pathway (as was hypothesized) and underlines the usefulness of *O. tauri* as a model system.

The degradation machinery consists of two families of E3 ubiquitin ligases, the Skp/cullin/F-box-containing complex and the anaphase-promoting complex/cyclosome (for review, see Teixeira and Reed 2013). Cyclin degradation, as well as degradation of other cell cycle related proteins, is crucial for one-way progression through the cell cycle. The two E3 ubiquitin ligases are thus considered key components of the core cell cycle machinery (Inagaki and Umeda 2011) and are conserved in algae (Huysman et al. 2014). Indeed, isolation

Fig. 21 The course of growth processes (RNA and protein accumulation) in synchronous populations of *Desmodesmus (Scenedesmus) quadricauda* grown at high irradiance in the presence of FdUrd. Mean irradiance 85 W m^{-2} , continuous light, temperature 30°C ; (a) Variation in RNA (●), protein (□), and DNA (▲) amounts per cell (log scale); (b) Oscillations in the rates of accumulation of RNA (●) and protein (□) (After Zachleder 1995)



of temperature sensitive mutants proved that the directed one way progression through the cell cycle, activated by anaphase-promoting factor is conserved in *C. reinhardtii* (Tulin and Cross 2014). In the red alga *Cyanidioschyzon merolae*, chloroplast DNA and nuclear DNA replications are, in contrast to the situation in *D. quadricauda*, tightly linked. The interaction is mediated by Mg protoporphyrin IX (Mg-protoIX), a molecule used by chloroplasts to signal the nucleus to modulate nuclear gene expression (retrograde signaling) (Kanesaki et al. 2009; Kobayashi et al. 2009). The signaling by Mg-protoIX activates CDKA and promotes nuclear DNA (nuc-DNA) replication. The activation of the CDKA complex is mediated through stabilization of the CDKA cyclin partner; Mg-protoIX inhibits ubiquitin E3 ligase specific for the cyclin partner and thus stabilizes the cyclin and consequently the CDK/cyclin complex (Kobayashi et al. 2011). Such a complex interaction between chloroplast and nucleo-cytosolic compartments suggests that the chloroplast and nucleus evolved to coordinate their cycles in a distinct mechanism.

Fine tuning of CDK/cyclin complex activity is ensured by protein interaction with so called CDK inhibitors and by phosphorylation of CDKs (Morgan 1995). The phosphorylation of CDK causes both its activation and inhibition

based on the kinase involved in the phosphorylation and its target site. Phosphorylation within the T-loop of the CDKs, by CDK activating kinases, is crucial for CDK/cyclin complex activation (Ducommun et al. 1991; Gould et al. 1991). In contrast, phosphorylation within ATP binding sites of CDK, executed by Wee1 kinase (Gould and Nurse 1989; Jin et al. 1996), partially inactivates already active CDK/cyclin complexes. This phosphorylation ensures the inactivation of CDKs until the G2/M transition, when they are abruptly dephosphorylated by Cdc25 phosphatases, leading to the activation of CDK-cyclin complexes, triggering mitosis (Kumagai and Dunphy 1991; Russell and Nurse 1986, 1987).

Wee1 kinases are widely conserved in both algae and plants. However, the existence of Cdc25 phosphatase homologs in the plant kingdom is a matter for discussion (Boudolf et al. 2006). The putative Cdc25 homologs in algae and higher plants are highly divergent and lack a conserved N-terminal domain (Bisova et al. 2005; Landrieu et al. 2004a, b). The only *bona fide* Cdc25 phosphatase in the plant kingdom is that of *O. tauri* (Khadaroo et al. 2004) - so far the only plant Cdc25 homolog able to complement a *cde25* mutation in *Schizosaccharomyces pombe* - which may do so due to the presence of the N-terminal domain.

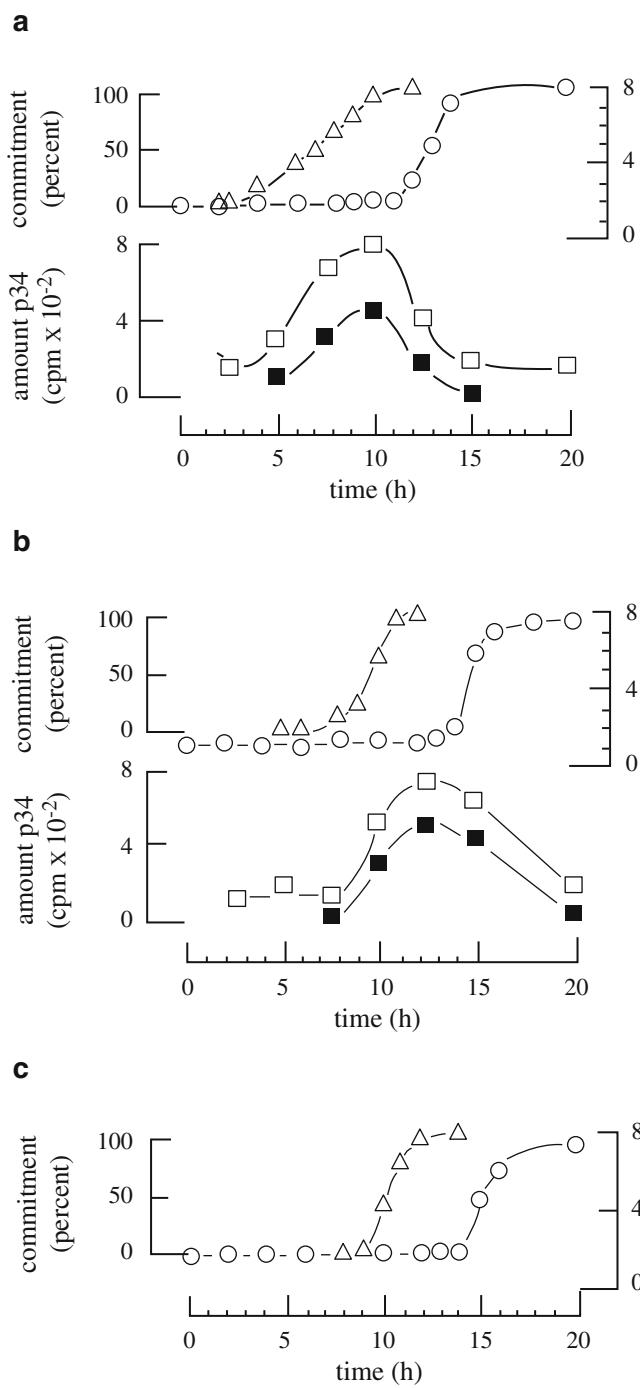


Fig. 22 Division timing and changes in the amount and phosphorylation of p34. (a) Very early division was caused by a reduction in phosphate to 100 μM . (b) Early division was caused by fast growth due to illumination at 200 μmol photons $\text{m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR). (c) Later division was caused by slower growth due to a lower light intensity of 100 μmol photons $\text{m}^{-2} \text{s}^{-1}$ PAR. During the period illustrated, cells were in continuous light. Commitment point to division (Δ) was in progress by 5 h, 8 h, and 10 h in cultures **a**, **b**, and **c**, respectively, and cell number (\circ) increased 6 h later. Total p34 (\square) increased concomitantly with commitment point to division, and 2.5 h later a high incidence of mitosis correlated with the appearance of slower migrating phosphorylated forms of p34 (\blacksquare) that are quantified in (a, b) (After John et al. 1989)

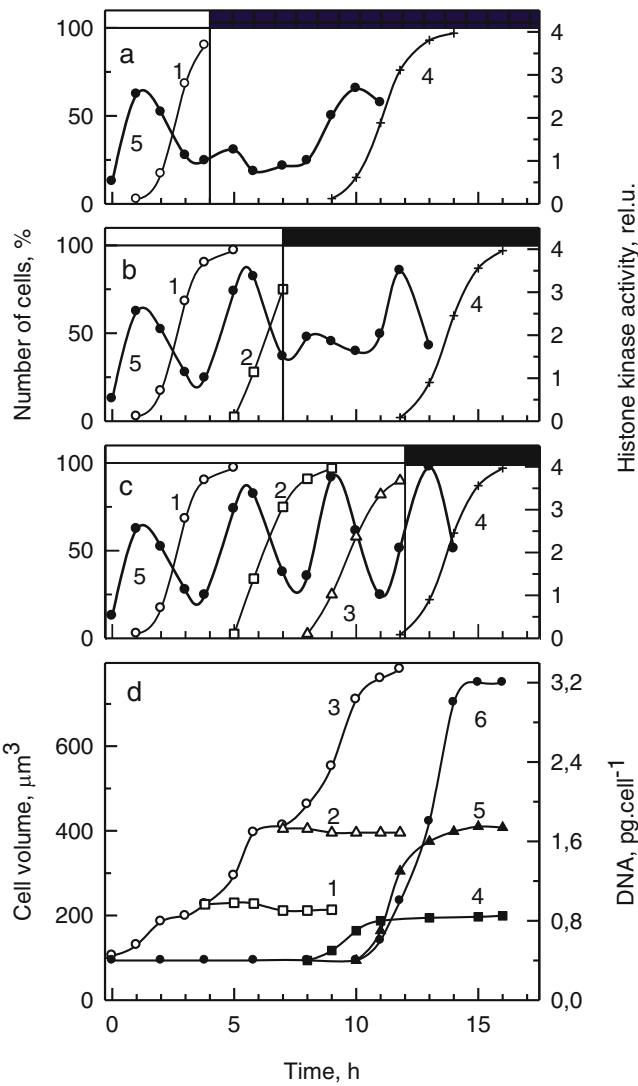


Fig. 23 Time course of commitment points to individual sequences of reproductive events, termination of these events, cell volume enlargement, and histone H1 kinase activity in synchronized populations of *Chlamydomonas reinhardtii*. The cultures were grown at a high irradiance and placed in the dark after the first (**a**), the second (**b**), and the third (**c**) commitment point to trigger a sequence of reproductive events. Panels **a–c**: Curves 1–3: the percentage of cells that reached the commitment points for the first, second, and third sequence of reproductive processes, respectively. Curve 4: the percentage of the cells that released daughter cells. Curve 5: the activity of histone H1 kinase. Panel **d**: Curves 1–3: the mean cell volume in subpopulations that were put in the dark after 4, 7, and 12 h, respectively. Curves 4–6: the concentration of DNA per cell in subpopulations that were put in the dark after 4, 7, and 12 h, respectively. Light and dark periods are marked by the lines above panels **a–c** and separated by vertical lines (After Zachleder et al. 1997)

CDK inhibitors (CKIs) represent the most diversified group of all core cell cycle regulators. While the function of such proteins has been conserved, the sequence conservation is very limited among fission and budding yeasts, animals and higher plants (Inagaki and Umeda 2011; Mironov et al.

1999; Wang et al. 1997). Higher plant CKIs are represented by two families of genes, Kip-related proteins (KRPs) with limited homology to animal CKIs, and plant-specific SIAMESE (SIM) and related proteins; each of the protein families seems to have distinct functions in cell cycle regulation. Interestingly, no homologs of CKIs have, so far, been identified in *C. reinhardtii* nor in other algal species (Bisova et al. 2005; Huysman et al. 2010; Robbens et al. 2005). However, proteins with functions attributed to CKIs are most probably present in algal cells but not identified due to sequence divergence.

CDK/cyclin activity is remembered and perpetuated by phosphorylation of its substrates and their subsequent actions. Transcription of many genes required for cell cycle progression in G1/S transition and DNA replication is controlled by binding of a heterodimer of transcription factor E2F and its dimerization partner (DP) (Inzé and De Veylder 2006; van den Heuvel and Dyson 2008). The activity of the E2F-DP dimer is controlled by interaction with the negative regulator, retinoblastoma protein, Rb. During G1 phase, Rb is hyperphosphorylated by the CDK/cyclin complex, leading to a release of active E2F/DP dimer, transcriptional activation and S phase entry (Shen 2002). The Rb/E2F pathway thus represents the best characterized substrate for the CDK/cyclin complex. Genes comprising the Rb/E2F pathway are the best characterized cell cycle genes in *C. reinhardtii*. In mutant *C. reinhardtii* containing a deletion of the Rb homolog, encoded by the *MAT3* gene, commitment point is attained at a smaller critical cell size, and when dividing, they divide excessively, giving rise to tiny daughter cells (Umen and Goodenough 2001). Thus, the Rb homolog encodes a sizer involved in the regulation of cell cycle progression in response to attainment of critical cell size. A genetic screen to isolate suppressors of *mat3-4* mutation uncovered other members of Rb/E2F pathway, E2F and DP, which were both able to suppress the *mat3-4* size mutation (Fang et al. 2006). *DPI* mutants have larger daughter cells than wild type, while *e2f* shows a similar daughter cell size. This implies the canonical Rb/E2F pathway in *C. reinhardtii* regulates cell cycle entry in response to attainment of critical cell size. It should be noted that *C. reinhardtii* represents a unique model to study the relationship between growth and cell cycle regulation, due to its multiple fission cell cycle. Cells dividing by multiple fission are, in general, less prone to change daughter cell size in response to changes in growth rates (Rading et al. 2011), and this is quite common for yeasts. Cultures of *C. reinhardtii*, and other algae dividing by multiple fission, will, after prolonged dark incubation under different growth rates and stable temperature, produce daughter cells of very uniform cell sizes ranging from a cell size just below the commitment point, to a cell size roughly half of that. Although several components of the sizing pathway were unraveled in *C. reinhardtii*, the most interesting

question remains: “What is the signal that “turns on” the sizing control”? Since *C. reinhardtii* represents an excellent genetic system, the answer to this question will most probably come from another mutant screen. Recently, a hint on the processes preceding “the sizing control” came from an unexpected organism, the red alga *C. merolae*. There E2F phosphorylation status is linked by as so-far unknown mechanism to circadian rhythm and represents a pre-requisite for the sizing control mediated by Rb phosphorylation (Miyagishima et al. 2014).

6 The Role of Light and Temperature

The cell cycle of algae starts with a period in which cells increase in size (pre-commitment period) until they reach a critical cell size and a key point of the cell cycle, commitment point, is attained. From this point, the cells are committed to divide and processes of DNA replication-division sequence are triggered. The following period (post-commitment period), during which daughter cells will be eventually formed, can be traversed without an external energy supply, and without further growth of the cells. However, if sufficient energy is supplied during this period, the cells, dividing by a C_n type of cell cycle, are able to attain another commitment point/s, leading to a higher number of daughter cells.

It is also characteristic of algae with the C_n cell cycle type that after each commitment point, growth processes that continue overlap concomitantly with running processes of triggered DNA replication-division sequences, as schematically illustrated in Fig. 3 (Sect. 2). Here we will describe the effects of light intensity and temperature, the major effectors of growth rate, on individual parts of the algal cell cycle.

6.1 Light Intensity

Most studies on the effect of light intensity were carried out on synchronized cultures of *Chlorella*, *Desmodesmus*, *Scenedesmus* and *Chlamydomonas*, as early as the 1960s (Lorenzen 1957; Nelle et al. 1975; Pirson and Lorenzen 1966; Pirson et al. 1963; Šetlík et al. 1972; Tamiya 1966; Tamiya et al. 1953; Wanka 1959, 1962, 1967; Wanka and Aelen 1973). Both the growth processes represented by an increase in RNA, protein and cell volume, and the reproductive processes, including DNA replication and nuclear division, are performed in several steps, each of which is approximately a doubling of the preceding one. With increasing light intensity, the duration of the steps in RNA and protein synthesis leading to doubling of their content per cell shortens and their number increases (Fig. 24); the same is true for an increase in cell volume (Fig. 25). In algae with a

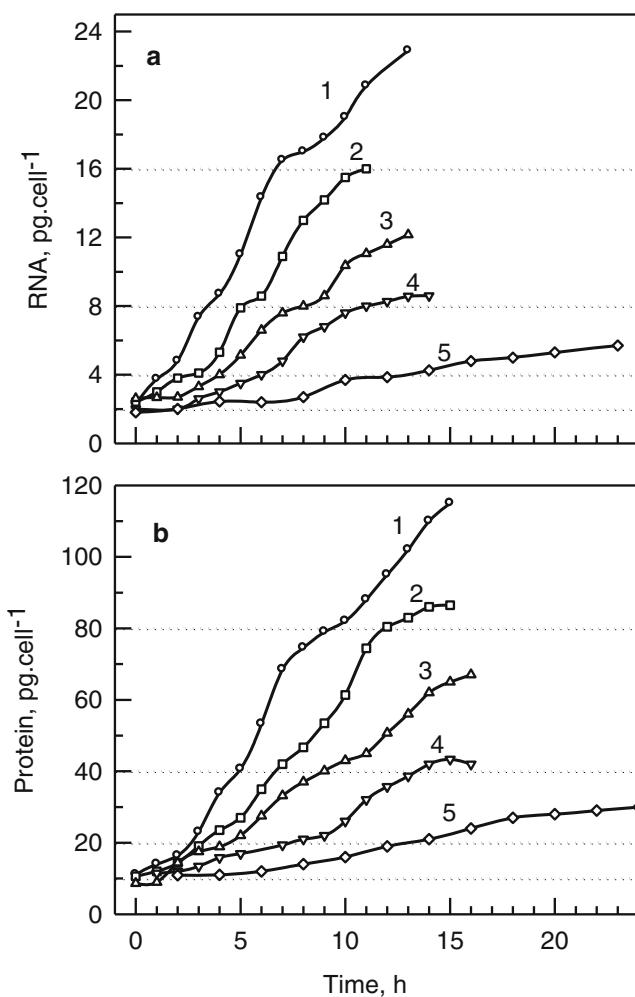


Fig. 24 Synchronous populations of *Scenedesmus armatus* grown at various mean irradiances showing (a) variation in RNA and (b) protein content per cell. Batch cultures grew for several cell cycles under alternating light (L) and dark (D) periods. In the analyzed cell cycles, the dark period has been omitted. Curve 1: 100 W m^{-2} , 15:10 h LD; curve 2: 72 W m^{-2} , 14:10 h LD; curve 3: 44 W m^{-2} , 13:10 h LD; curve 4: 17 W m^{-2} , 15:10 h LD; curve 5: 10 W m^{-2} , 20:10 h LD. Horizontal dotted lines indicate doublings of initial value (After Tukaj et al. 1996)

Scenedesmus-type cell cycle, individual doublings of DNA, e.g. individual DNA replication-division sequences, are also separated in time in Fig. 26.

The timing of individual commitment points and cellular divisions is dependent on light and temperature in synchronized cultures of *Chlamydomonas eugametos* (Zachleder and van den Ende 1992). The time interval required for attainment of the first commitment point shortened markedly (from 28 to 6 h, with increasing light irradiance from 7.5 to 70 W m^{-2} , respectively) (Fig. 27). Shortening of all consecutive pre-commitment periods with increasing light intensity caused the number of commitment points attained to increase from two to four (in 25 % of population even to five). The mother cells divided into 16 or 32 daughter cells (Fig. 27a).

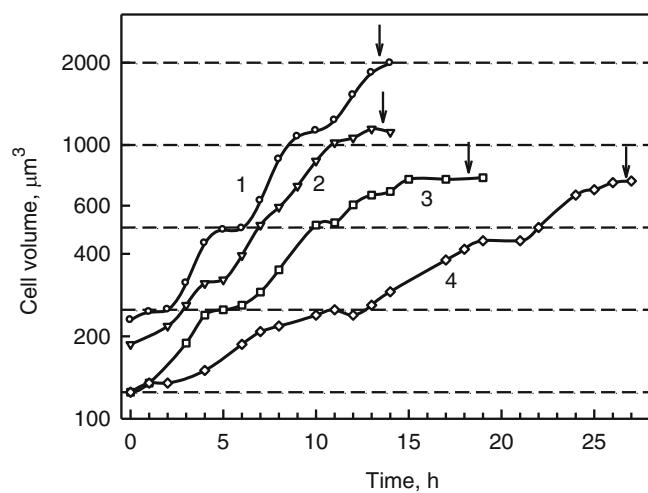


Fig. 25 Changes in mean cell volume during the cell cycle in synchronized populations of *Scenedesmus armatus* grown at different (mean) light irradiances. Curve 1: 105 W m^{-2} ; curve 2: 85 W m^{-2} ; curve 3: 50 W m^{-2} ; curve 4: 20 W m^{-2} , 15:10 h LD at 30°C ; arrows – the time when division of cell started; dashed line – doubling values for cell size levels (After Vítová and Zachleder 2005)

This was confirmed by detailed studies in distantly related *Chlamydomonas reinhardtii* (Vítová et al. 2011b). Increased growth rates (see course of cell volume in Fig. 28a–g), led to shortening of the pre-commitment periods and an increase in their number from 1 to 4. At the end of the cell cycle, cell volume was proportional to the number of daughter cells; these increased from 2 at the lowest light intensity (Fig. 28a), to 16 at the highest light intensity (Fig. 28g). The growth rates were solely dependent on mean light intensities and were not affected by dark period. When grown in continuous light, the length of the cell cycle shortened with increasing light intensity (increasing growth rate), from about 73 h at the lowest growth rate (Fig. 29a) to 15 h at the highest growth rate (Fig. 29c). Furthermore, the same dependency on mean light intensity for setting the growth rate and the length of the cell cycle was seen if the daughter cells from an asynchronous culture were separated by sedimentation or gentle centrifugation (Vítová et al. 2011b) (Fig. 30). This supports the view that rules for regulation of cell cycle length are the same in asynchronous cultures as in cultures synchronized by light/dark regime.

General rules for regulating the lengths of pre- and post-commitment phases of the algal cell cycle are the following:

1. The length of the pre-commitment period depends on irradiance, suggesting that a finite amount of photosynthetic work must be completed before the cell becomes committed. This supports the early idea that the main (if not the only) factor determining the timing of commitment point is growth rate, which is set by the rate of photosynthesis (Spudich and Sager 1980). Until reaching the “threshold

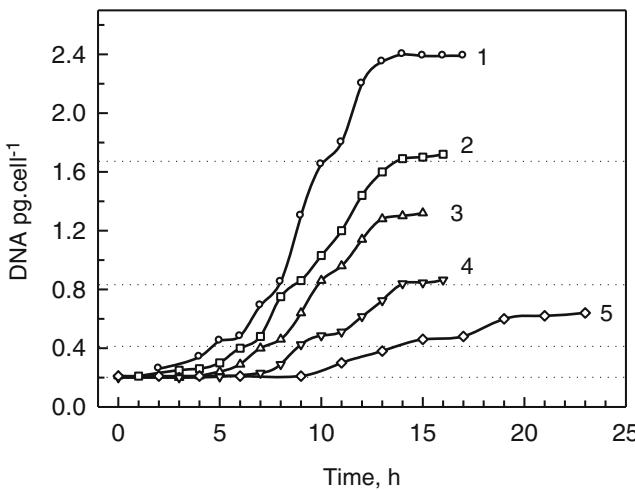


Fig. 26 Variations in DNA per cell in a synchronous population of *Scenedesmus armatus* grown at various mean irradiances. Batch cultures grew for several cell cycles under alternating light (L) and dark (D) period. In analyzed cell cycle the dark period has been omitted. Curve 1: 100 W m^{-2} , 15:10 h LD; curve 2: 72 W m^{-2} , 14:10 h LD; curve 3: 44 W m^{-2} , 13:10 h LD; curve 4: 17 W m^{-2} , 15:10 h LD; curve 5: 10 W m^{-2} , 20:10 h LD. Horizontal dotted lines indicate doublings of initial value (After Tukaj et al. 1996)

photosynthetic work" required for attaining commitment point, the pre-commitment period can be interrupted by dark periods affecting its length within wide limits. As illustrated in Fig. 31 for *Desmodesmus* (*Scenedesmus*) *quadricauda* (Šetlík and Zachleder 1983) and in Fig. 32 for *Chlamydomonas reinhardtii* (Vítová et al. 2011b), if the synchronized population is darkened in the pre-commitment period for a certain interval of time, the only result is the postponement of commitment points and all post-commitment events for an equal interval of time. On the other hand, the pre-commitment period in a population growing at an irradiance well below saturation may be markedly shortened by inserting a comparatively short (2 h) interval of saturating irradiance (Figs. 33c, d). This treatment has no effect on the course of post-commitment events.

2. The time between commitment point and daughter cell release at a given temperature remains approximately constant at different irradiances (Figs. 27, 28, and 29). In sharp contrast to growth, the processes in the DNA replication-division sequence (post-commitment period) are independent of the simultaneous supply of external energy to the cell. The timing of the first commitment point determines, in principle, the timing of daughter cell release. This depends entirely on the growth rate (and hence on irradiance) and whether, in the period after the first commitment point, another commitment point will be attained before the cells divide. If this occurs, a new DNA replication-division sequence is initialized and a higher division number will be reached within the time between the first commitment point and daughter cell release.

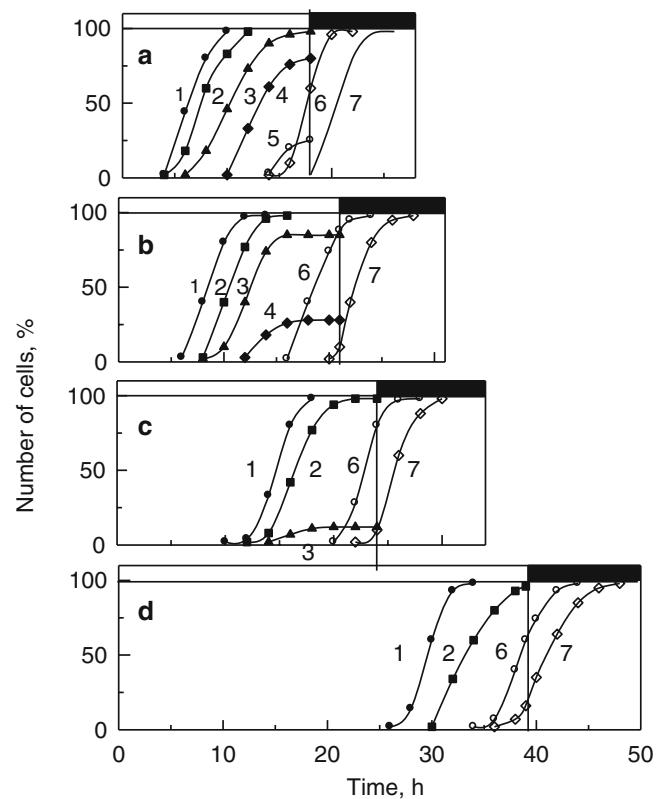
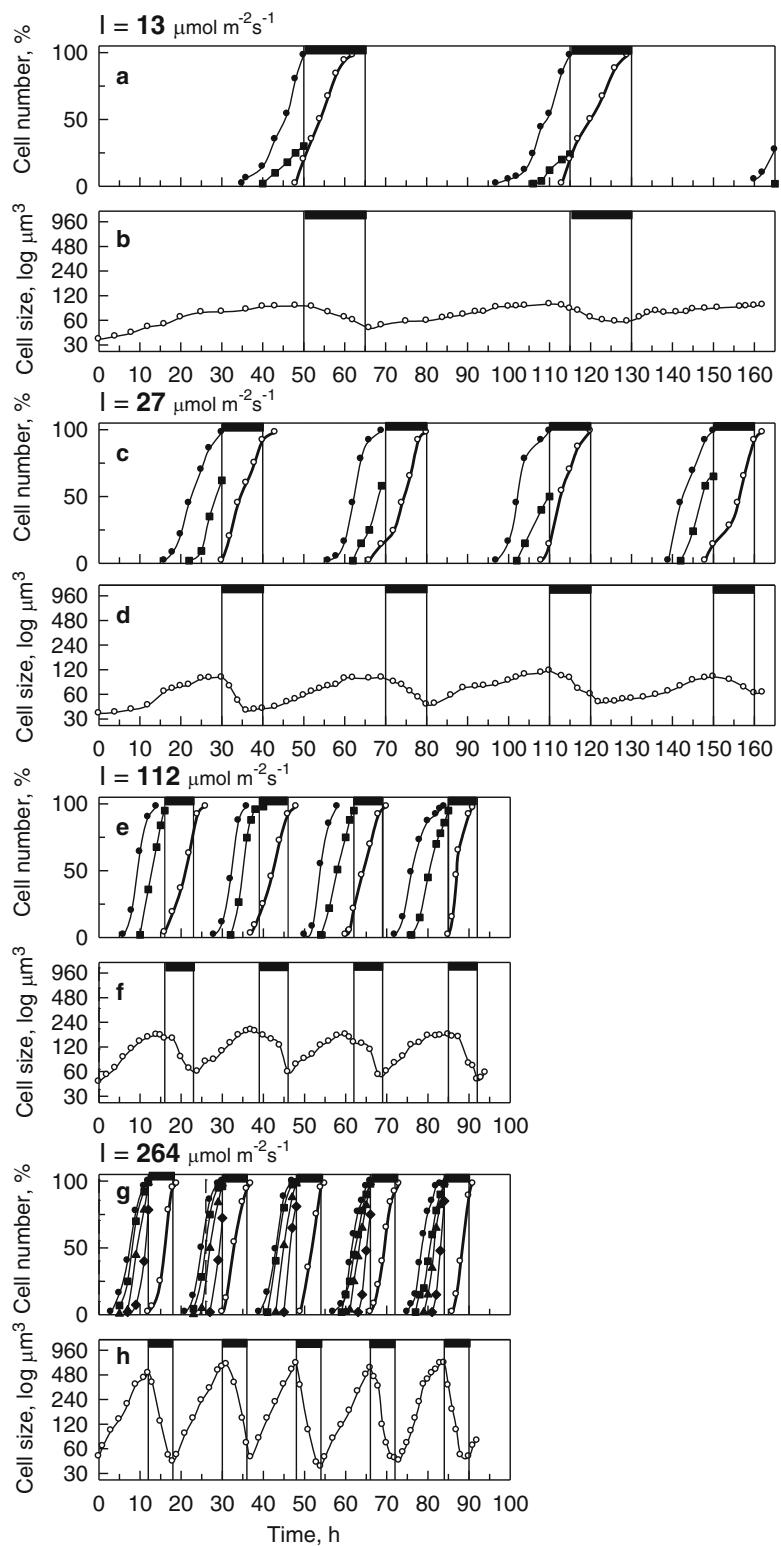


Fig. 27 Time courses of commitment points to nuclear and cellular divisions and termination of these processes in synchronous populations of *Chlamydomonas eugametos* grown at various light irradiances. (a) 70 W m^{-2} ; (b) 35 W m^{-2} ; (c) 15 W m^{-2} ; (d) 7.5 W m^{-2} . Curves 1, 2, 3, 4, 5: percentage of the cells that attained commitment points for the first, second, third, fourth and fifth nuclear divisions, respectively, curve 6: percentage of the cells in which the first protoplast fission occurred, curve 7: percentage of the cells that released their daughter cells. Light and dark periods are indicated by white and black strips above panels and separated by vertical lines (After Zachleder and van den Ende 1992)

6.2 Temperature

Most biological reactions vary with temperature such that with every 10°C increase in temperature, the reaction rate approximately doubles; this is expressed as a temperature coefficient (Q_{10}) of about 2. It could be therefore assumed that the same rule will apply for processes involved in the regulation of cell cycle events. This was verified practically more than half a century ago by (Morimura 1959), who provided the first information on the effect of different temperatures on synchronized cultures of *Chlorella ellipsoidea* (Fig. 34). The basic rule has been repeatedly verified in other species of algae: A decrease in temperature decreases the algal cell growth rate and consequently, the cell cycle is prolonged in a manner inversely proportional to the temperature. The question remaining is how temperature affects individual phases of the cell cycle. Particularly in cell cycle type C_n, where, at high growth rates, a complex overlapping of sev-

Fig. 28 Time courses of individual commitment points to cell division and daughter cell release (**a, c, e, g**) and changes in a mean cell volume (**b, d, f, h**) in synchronized populations of the alga *Chlamydomonas reinhardtii* grown at different mean light intensities (I) under **alternating light and dark periods**. Full symbols: percentage of the cells, which attained the commitment point for the first (circles), second (squares), third (triangles) and fourth (diamonds) protoplast fission, respectively; open symbols: percentage of the cells, which released their daughter cells. Dark periods are marked by *black stripes* and separated by *vertical solid lines* (After Vítová et al. 2011b)



eral sequences of growth (pre-commitment phases) and DNA replication-divisions (post-commitment phases) occurs, as shown in preceding chapters). The comparison between the effect of light and temperature can be seen in Figs. 35 and 36. As discussed above, with increasing light

intensity, the cell cycle shortens due to shortening of pre-commitment periods. Post-commitment periods are independent of light intensity (Fig. 35). Due to this distinct effect of light, only variations in pre-commitment phases determines the final length of the cell cycle, and thus the

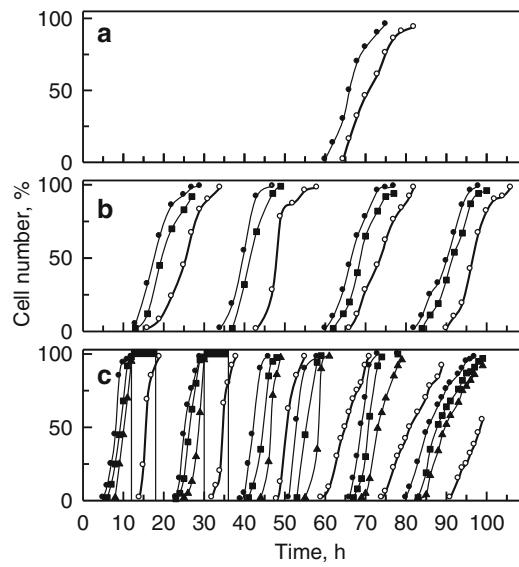


Fig. 29 Time courses of individual commitment points to cell division and daughter cell release in synchronized populations of *Chlamydomonas reinhardtii* continuously illuminated and grown at different mean light. Full symbols: percentage of the cells, which attained the commitment point for the first (circles), second (squares) and third (triangles) protoplast fission, respectively; open symbols: percentage of the cells, which released their daughter cells (After Vítová et al. 2011b)

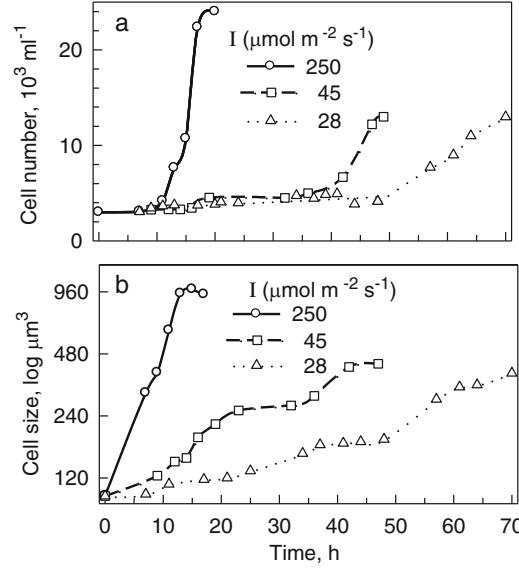
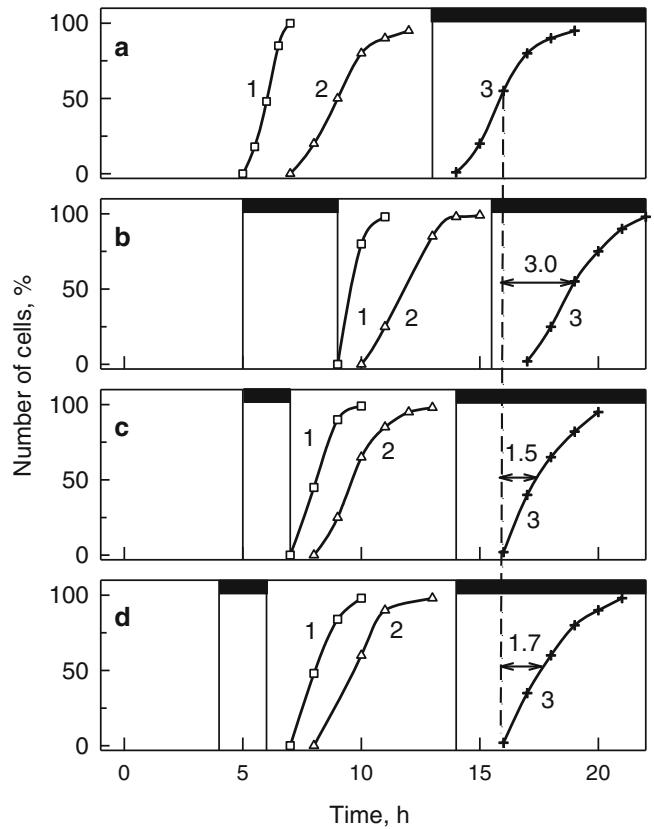


Fig. 30 Time courses of daughter cell release (a) and variation of the cell size (b) in populations of *Chlamydomonas reinhardtii* synchronized by size-selection and grown continuously illuminated at different mean light intensities (I) (After Vítová et al. 2011b)

relative position in the cell cycle varies markedly with light intensity (see insert in Fig. 35). This is in sharp contrast to the effect of temperature.

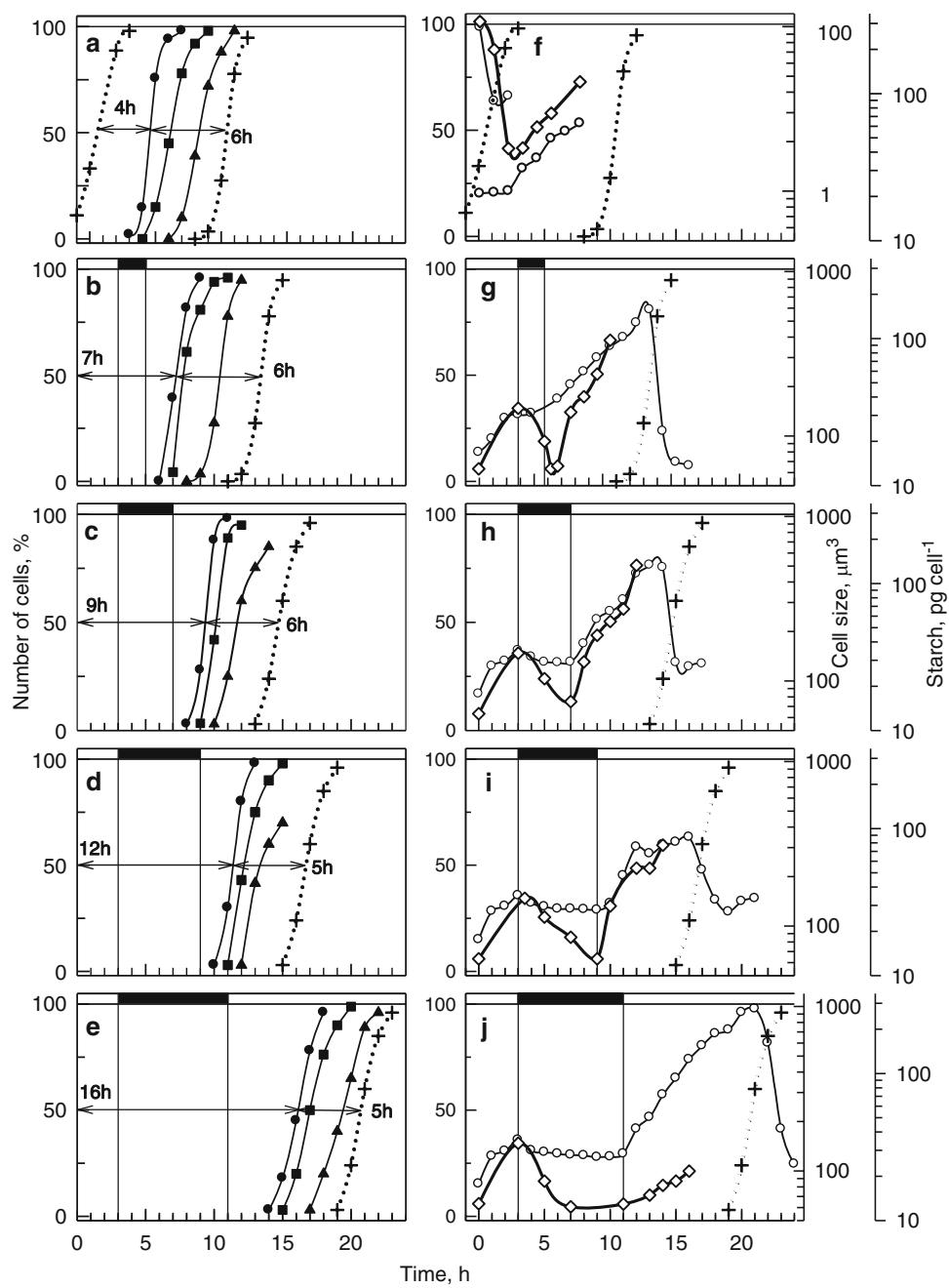
Changes in temperature, similarly to light intensity, affect growth rate and the cell cycle shortens with increasing temperature (Fig. 36). However, temperature affects all processes

Fig. 31 Influence of interrupting illumination in the precommitment period on the timing of commitment points and of cell division in a synchronous culture of *Desmodesmus (Scenedesmus) quadricauda*. Batch cultures started from a common inoculum were grown at 30°C , incident irradiance 80 W m^{-2} . The mid-point of cell division in a control culture (panel a) is indicated by the vertical dashed line. The delay of cell division caused by interrupting the light period for 4 h (panel b) and 2 h (panels c, d) are marked by horizontal arrows with figures indicating the delay in hours. Duration of post-commitment period, the distance between curve 1 and 3 remains approximately constant. White and black strips above the panels indicate the intervals of light and dark (After Šetlík and Zachleder 1983)

in the cell, not only growth, as was the case for light intensity. When the temperature coefficients (Q_{10}) for the duration of the cell cycle, pre- and post-commitment periods were determined, the values were close to 2, indicating a doubling in the rate of metabolism with a temperature increase of 10°C (Vítová et al. 2011a). This is well above the values expected for temperature-compensated processes and suggests metabolic dependency of both pre- and post-commitment periods solely on temperature. Consequently, the lengths of both pre- and post-commitment phases are affected by temperature in the same way, and the relative position in the cell cycle does not change substantially with temperature (see insert in Fig. 36d).

Under constant temperature, the length of the post-commitment period is rather constant and is independent of growth rate. Although this is clearly the case, there are conditions under which the length of the post-commitment period

Fig. 32 Time courses of individual commitment points to cell division and the course of daughter cell release (**a–e**) and changes in mean cell volume and starch level (**f–j**) in synchronized populations of *Chlamydomonas reinhardtii* grown at constant light irradiance ($I = 264 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and different illumination regimes. **a–e** Solid lines, full symbols: percentage of the cells, which attained the commitment points for the first (circles), second (squares) and third (triangles) protoplast fission, respectively; dotted line, crosses: percentage of the cells that released their daughter cells. The pre- and post-commitment phases are marked by horizontal arrowed lines connecting midpoints of the phases, numerals indicate their duration in hour. **(f–j)** Solid thick lines, diamonds: starch amount; thin solid lines, circles: cell size; dotted lines, crosses: percentage of cells that released their daughter cells. Dark periods are marked by black stripes in panels and separated by vertical solid lines (After Vítová et al. 2011b)



will be prolonged, even at stable temperatures, such as in cultures of *Chlamydomonas eugametos* grown at very high light intensities (Zachleder and van den Ende 1992). This is probably caused by the insertion of additional DNA replication-division sequence/s after additional commitment points were attained. This would result in prolongation of the period from the first commitment point to the final division due to more processes being required to be completed. The prolongation is longer, the higher number of commitment points attained, and can be seen particularly well in *Chlamydomonas*, dividing into 32 daughters with 5 commitment points attained

within one cell cycle (Zachleder and van den Ende 1992) (Fig. 37).

The cell cycle, comprising pre- and post-commitment periods, is quite complex and is affected by a combination of light and temperature. At low light intensities, light availability limited growth, even at higher temperatures. Consequently, the length of the pre-commitment period did not change at various temperatures, giving the impression that the length of the cell cycle was temperature-independent. On the other hand, at low temperatures (below 20 °C), growth processes were so slow that even low light intensities

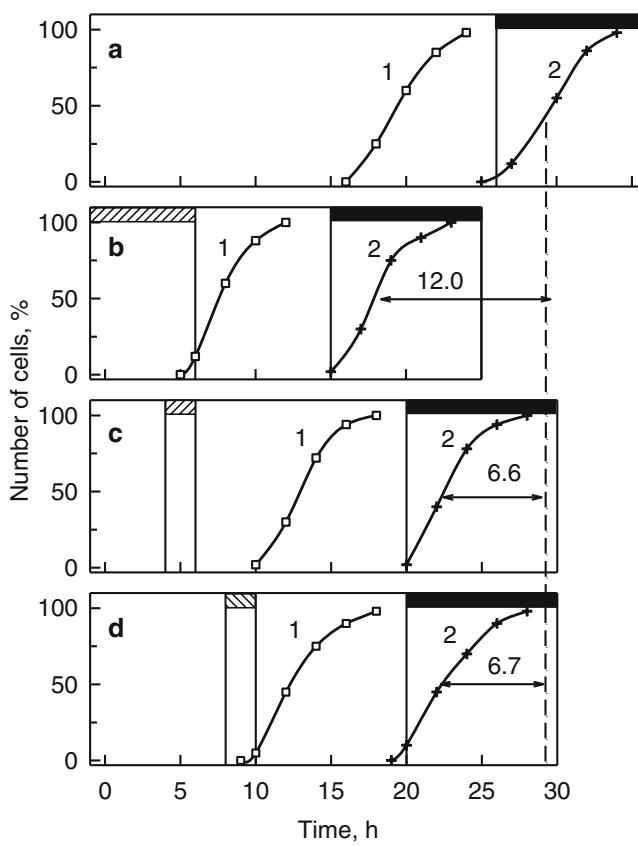


Fig. 33 Influence of a short time increase in irradiance during the pre-commitment period on the timing of commitment points and cell division in a synchronous culture of *Desmodesmus* (*Scenedesmus*) *quadricauda*. Batch cultures started from a common inoculum and were grown at 30 °C and incident irradiance 45 W m⁻² (panel a). The shortening of the cell cycle caused by increasing irradiance to 80 W m⁻² for 6 h (panel b) and 2 h (panels c, d). Dashed, white and black sectors of the strip above the panels indicate the periods of high irradiance, of low irradiance and of darkness, respectively (After Šetlík and Zachleder 1983)

were sufficient to saturate their photosynthetic demands. So the length of the cell cycle appeared to be light-insensitive. This constant duration of the cell cycle under a certain range of growth conditions found by some authors in *Chlamydomonas reinhardtii* (Donnan and John 1983) and *Chlorella* (Lorenzen 1980; Lorenzen and Albrodt 1981) led them to postulate the existence of a timer (pacemaker, Zeitgeber), or circadian rhythms (Goto and Johnson 1995; Lorenzen and Schleif 1966), which are triggered at the beginning of the cell cycle, causing division of cells after a constant time period, regardless of the growth rate. The finding in *Desmodesmus* (*Scenedesmus*) *quadricauda*, as well as those published for *Scenedesmus armatus* (Tukaj et al. 1996), *Chlamydomonas eugametos* (Zachleder and van den Ende 1992) and *Chlamydomonas reinhardtii* (Spudich and Sager 1980), and recent ones in the same species (Vítová et al. 2011a, b), clearly refute any role of any type of timer,

endogenous oscillator or circadian rhythm in determining the length of their cell cycle, as well as cell cycle phases. Rather, the cell cycle duration is set by the combination of durations of pre- and post-commitment periods that are differentially sensitive to light and temperature.

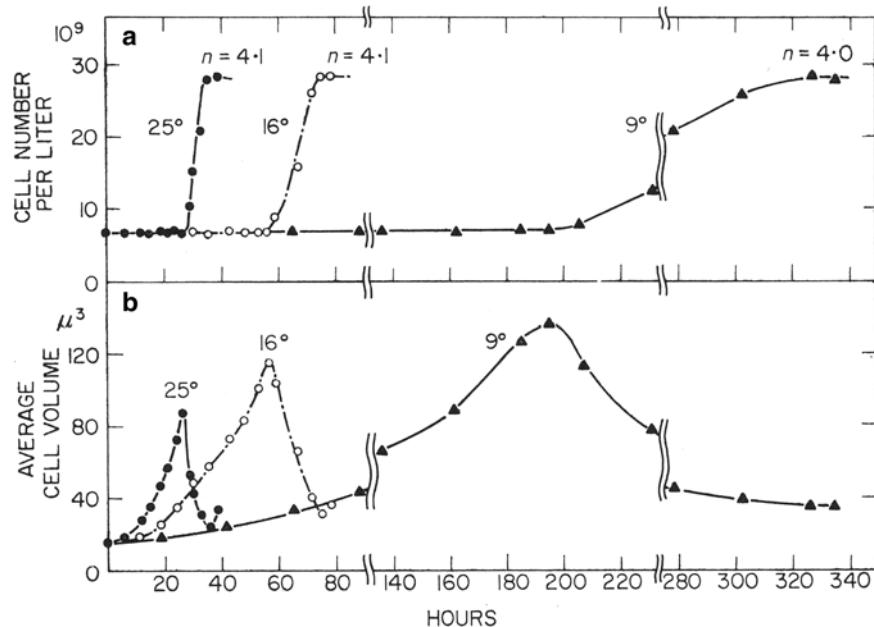
As a crucial conclusion from the effect of light and temperature on the cell cycle, it should be kept in mind that the cell cycle is not a “clock” since its length is, to great extent, varied by external conditions. This was succinctly summarized by (Tyson and Novak 2008): “Under constant favorable conditions, cells progress through the DNA replication – division cycle with clocklike regularity, but the cell ‘cycle’ is not a ‘clock’.”

Even more, the cell cycle is in fact not a “cycle”; it only appears to be one. This was lucidly put by Cooper: “The word ‘cycle’ implies that at cell division something is initiated, the cell cycle. Actually, nothing starts at cell division but it is merely the end of a sequence of events which start with the accumulation of some initiation potential, and which was followed in succession by the initiation of DNA synthesis, the preparations for cell division following termination of DNA synthesis, and the final cell division. The final cell division is the end of the process and the beginning of nothing” (Cooper 1979, 1984). This might not be so clear when studying a classical cell cycle, binary fission, but it is in striking agreement with cell cycle regulation in the C_n cell cycle type. In this, everything overlaps with everything else: growth after the last commitment overlaps with preparation for DNA replication and/or DNA replication of the just committed DNA replication-division sequence, and with preparation for and/or the running of nuclear division of the previously started DNA replication-division sequence. Clearly, all the processes are happening at the same time and the cell is neither in G1 nor G2 phases; in fact it is, at the same time, in G1, G2, S and M phases simultaneously. From this point of view, the generally accepted semantic distinction of individual cell cycle phases is bringing more confusion than help and might be limiting our understanding of cell cycle organization and regulation.

7 Chloroplast Cycle

As discussed above, light condition (duration of light interval and light intensity) is one of the most important factors affecting growth of phototrophic algae. The chloroplast, the photosynthesizing organelle, is mostly considered only as a supplier of energy and material, however it also has its own chloroplast division cycle that it is affected by light (Zachleder et al. 2004). Chloroplasts can occupy about 50 % of the total cell volume, chloroplast RNA is about 30 % of total RNA (Chiang and Sueoka 1967a) and about 3–15 % of cellular DNA (Zachleder et al. 1989, 1995). Thus, a great

Fig. 34 Effect of temperature on the cell cycle of *Chlorella ellipsoidea* (under the condition of light saturation 10 kilolux). Temperatures 25, 16 and 9 °C. (a) Number of cells at indicated temperatures. Number of daughter cells per one mother cell expressed as division number n above the curves; (b) Changes in average cell volume at indicated temperatures (After Morimura 1959)



deal of the chloroplast photosynthetic capacity, as well as cellular synthetic machinery, are utilized for growth and reproduction of the organelle itself, implying that coordination of the chloroplast and the nucleo-cytosolic compartment in growth and reproduction is essential for survival of autotrophic algae.

7.1 Regulatory Relationships between Chloroplast and Nucleo-cytosolic Compartments

Chloroplasts, as photosynthetic organelles, clearly support the metabolism of the entire cell grown under autotrophic conditions via production of energy and assimilation of carbon. On the other hand, since the majority of chloroplast proteins are nuclear encoded, the high growth rate of the chloroplast requires corresponding activity in the cytoplasm. Indeed, the assembly of functional chloroplast ribosomes depends on a supply of proteins made by nuclear-encoded DNA-dependent RNA polymerase. Many essential chloroplast structures consist of supramolecular complexes involving both proteins synthesized in the chloroplast and cytoplasm (Ohad 1975; Parthier 1982). The “selfish” chloroplast, therefore, has to keep the cytoplasmic protein-synthesizing machinery running at a rate to satisfy its demands. This interdependence creates a system of feedback controls that coordinate and even couple growth and development of chloroplast and nucleo-cytosolic compartments. This is, reflected by strict proportionality in the increase in chloroplast and cytoplasmic ribosomes during the course of the *Chlamydomonas* cell cycle (Wilson and

Chiang 1977). The timing and extent of reproductive processes in chloroplast and nucleo-cytosolic compartments are also dictated by the supply of energy and organic compounds produced by photosynthesis, which in turn, is strictly regulated by light conditions (irradiance and the illumination regime) (Šetlík et al. 1972; Zachleder and Šetlík 1982). The content of pt-DNA, and the number of nucleoids in which pt-DNA is located, vary markedly during the chloroplast cycle and are strictly regulated by external conditions. The number of nucleoids increases during the cell cycle, reaching a maximum at the time of chloroplast division. The rate of increase, as well as the final number of nucleoids, is strictly controlled by light intensity (Figs. 47, 48 and 49). The higher the light intensity, the more nucleoids are formed, and the rate of nucleoid divisions is strictly light-dependent (Figs. 48 and 49). This reflects the trophic effect that light intensity has on growth and consequently, on chloroplast (plastid) DNA (pt-DNA) replication and nucleoid division. Daughter cells, divided in the dark, have a lower number of nucleoids, as compared to the cell from the same culture but divided in light. This is similar to the overlapping nuc-DNA replication-division sequences in the nucleo-cytosolic compartment, as well as to other prokaryotes, and it clearly supports Cooper’s continuum model of the cell cycle (Cooper 1979, 1984).

Clearly, the growth of the chloroplast is a prerequisite for cellular growth, which again is a prerequisite for attaining commitment point and allowing nuc-DNA replication-division sequences. This leads to the hypothesis that the chloroplast plays a decisive role in the regulation of cell cycle processes (Chiang 1975; Šetlík and Zachleder 1983). There are two reasons to support this notion:

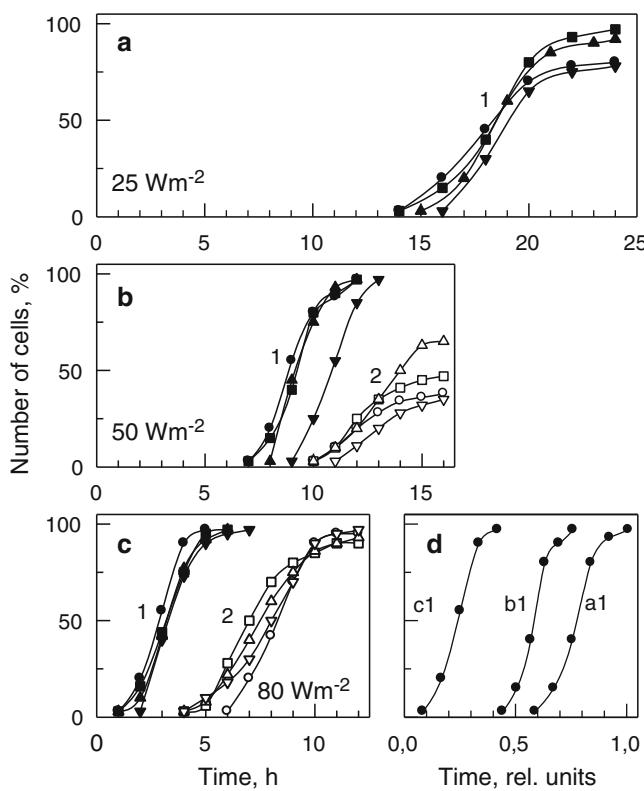


Fig. 35 The effect of light intensity on time courses of attainment of commitment points to cellular divisions in synchronous populations of *Desmodesmus* (*Scenedesmus*) *quadricauda*. Light irradiance (a) 25 W m⁻², (b) 50 W m⁻², (c) 80 W m⁻². Temperature 30 °C was used for all variants. Curves 1 (solid symbols): The percentage of cells that attained the commitment point to divide into four daughter cells (quadruplet coenobia), curves 2 (open symbols): the percentage of cells that attained the commitment point to divide into eight daughter cells (octuplet coenobia). Curves for 4 subsequent cycles (circles, squares, triangles, and diamonds respectively) are illustrated in all variants. (d) a1, b1, c1: The curves correspond to the average of curves 1 from the cultures presented in variants (a–c). The length of the cell cycle is divided into ten units irrespective of its actual duration (After Vítová and Zachleder 2005)

1. Chloroplast DNA replication precedes the corresponding reproductive events in the nucleo-cytosolic compartment.
2. The number of pt-DNA replications is equal to the number of nuc-DNA replications (and the corresponding nuclear and cell division), i.e. two processes occurring much later in the cell cycle (Dalmon 1970; Dalmon et al. 1975; Chiang 1971; Chiang and Sueoka 1967b; Iwamura et al. 1982). One experimental approach to study mutual regulatory relationships between reproductive events in the chloroplast and nucleo-cytosolic compartments was to follow the course of growth and reproductive processes within one compartment, while some of these processes were prevented in the second one. Such experiments should answer some of the questions concerning mutual relationships between chloroplast and nucleo-cytosolic

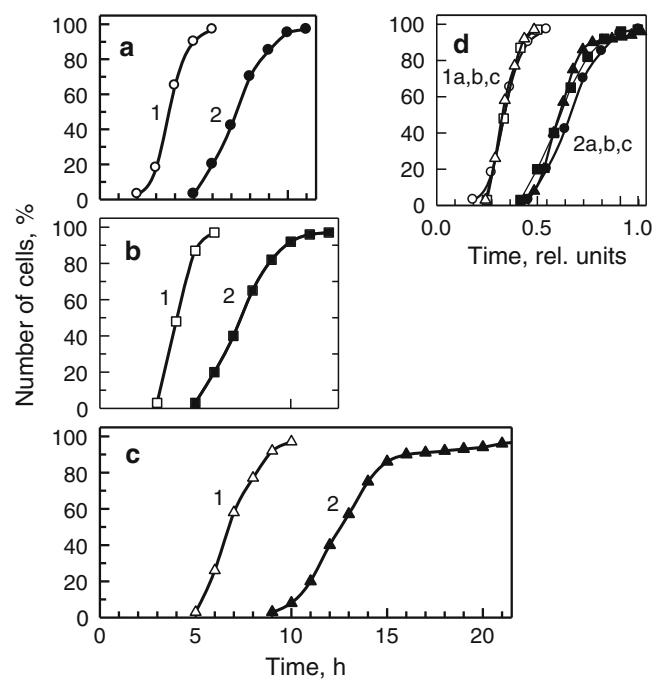


Fig. 36 The effect of temperature on time courses of attainment of commitment points to cellular divisions in synchronous populations of *Desmodesmus* (*Scenedesmus*) *quadricauda*. Temperature (a) 35 °C, (b) 30 °C, (c) 25 °C. Light irradiances 100 W m⁻² was used for all variants. Curves 1 (open symbols): The percentage of cells, which attained the commitment point to divide into four daughter cells (quadruplet coenobia) (open symbols in d), curves 2 (solid symbols): the percentage of cells, which attained the commitment point to divide into eight daughter cells (octuplet coenobia) (solid symbols in d). D: The curves are derived from corresponding curves in panels a–c. Triangles are valid for a, squares for b and circles for c. The length of the cell cycle is divided into ten units irrespective of its actual duration (After Vítová and Zachleder 2005)

compartments and can be divided into the following simple hypothesis-driven experiments:

- (1) How are growth and reproductive processes in chloroplast and nucleo-cytosolic compartments affected by blocking chloroplast DNA-replication?
- (2) How far are the reproductive processes in the nucleo-cytosolic compartment controlled by, or dependent upon, chloroplast reproductive events?
- (3) What is the course of growth and reproduction in the chloroplast under conditions of blocked DNA replication?

The outcome of the experiments, namely those with 5-fluorodeoxyuridine (FdUrd) and nalidixic acid described below, indicate that there is no regulatory mechanism directly coordinating the two cycles, and the relationship is based solely on the trophic role of the chloroplast on the nucleo-cytosolic compartment; there is a continuum of processes that depend on each other but do not require a specific regulating mechanism.

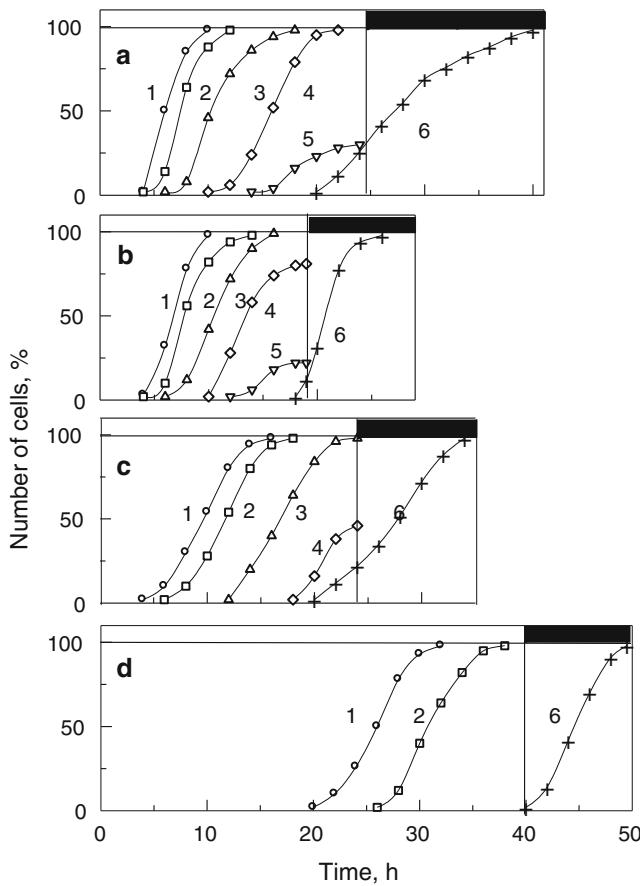


Fig. 37 Time courses of commitment points to nuclear and cellular divisions and termination of these processes in synchronous populations of *Chlamydomonas eugametos* grown at various temperatures. **(a)** 35 °C; **(b)** 30 °C; **(c)** 25 °C; **(d)** 20 °C. Curves 1, 2, 3, 4, 5, percentage of the cells that attained commitment points for the first, second, third fourth and fifth nuclear divisions, respectively. Curve 6, percentage of the cells that released their daughter cells. Light and dark periods are indicated by white and black strips above panels and separated by vertical lines (After Zachleder and van den Ende 1992)

7.2 Chloroplast DNA

For the study of pt-DNA and nuc-DNA, the crucial experiments were with algae having a *Chlamydomonas*-type cell cycle, i.e. multiple reproduction events that occurred clustered at the end of the cell cycle, immediately following each other (see Fig. 3, Chap. 3). In such cell cycles, it is relatively easy to distinguish between the course of chloroplast (pt-DNA) and nuclear (nuc-DNA) replications since they occur at different times during the cell cycle. Moreover, due to different GC contents of pt-DNA and nuc-DNA molecules in *Chlamydomonas reinhardtii*, they can be separated by fractionation and differential centrifugation (Iwamura 1962, 1966, 1970; Iwamura and Kuwashima 1969; Sueoka et al. 1967). The first experiments were carried out on slowly growing *C. reinhardtii*, with mother cells dividing only into two daughter cells (cell cycle type C₁) (see Fig. 16, Chap. 4)

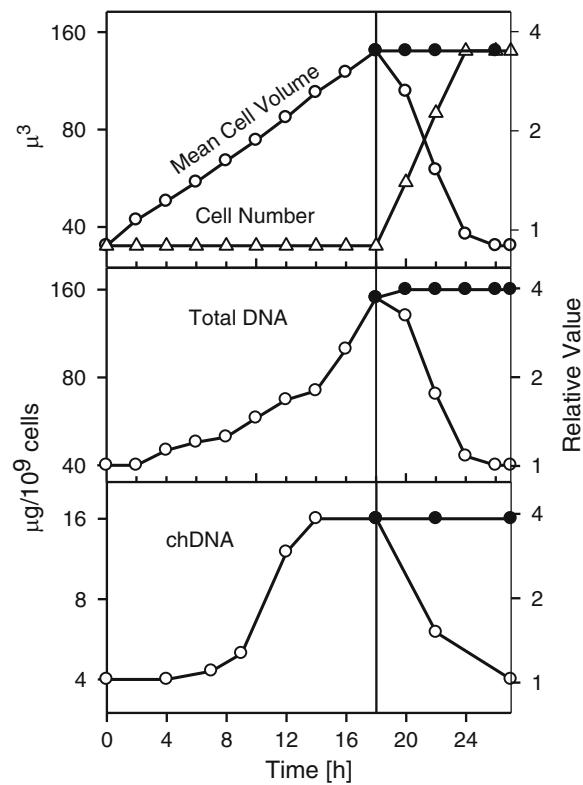


Fig. 38 Synchronous growth of the *Chlorella ellipsoidea* and synthetic patterns for total cellular and chloroplast DNAs formed during the cell cycle. Hollow and solid bars in the upper part of the figure represent the light- and dark-phases, respectively. Mean cell volume was calculated by dividing the packed cell volume (PCV) by the cell number per unit volume of culture, this latter unit being shown only as values relative to the value at T₀. The solid circles represent values estimated after assuming that no cellular division occurred after T₁₈. They express the net change in each of the variables during the dark-phase (After Iwamura et al. 1982)

(Chiang and Sueoka 1967b). A distinct chloroplast DNA replication occurred earlier in the cell cycle, preceding for several hours nuclear DNA (nuc-DNA) replication. Chiang and his collaborators (Grant et al. 1978; Chiang 1971, 1975; Kates et al. 1968) later confirmed this finding for the same species dividing into more than two daughter cells (cell cycle type C_n). Multiple rounds of pt-DNA replication occurred in the middle of the growth phase of the cell cycle, and were always equal to the number of nuc-DNA replications (and the corresponding number of nuclear and cell divisions) that occurred at the end of the cell cycle. Later, a similar course of pt-DNA was also observed in the synchronized algae, *Chlorella ellipsoidea* (Fig. 38) (Iwamura et al. 1982), where pt-DNA quadrupled during the synthetic period, corresponding to the four chloroplasts present in the mother cells prior to cell division into four daughter cells (Iwamura et al. 1982). While the replication of pt-DNA was performed early in the cell cycle in *C. reinhardtii* (Chiang and Sueoka 1967a, b; Chiang 1975), leading to an increase in nucleoid size, nucle-

Fig. 39 Fluorescent image of *Desmodesmus (Scenedesmus) quadricauda* cells stained with DAPI. Left side: the photomicrograph of two cells in quadruplet daughter coenobia. Right side: a schematic drawing of the cell structures seen in the DAPI stained cells. Note: Chloroplasts are seen as red structures due to the autofluorescence of chlorophyll but are not clearly shown in the black and white photograph. Very faint fluorescence of the rest of the cell makes the cytoplasm and the outline of cell obscure (After Zachleder and Cepák 1987a)

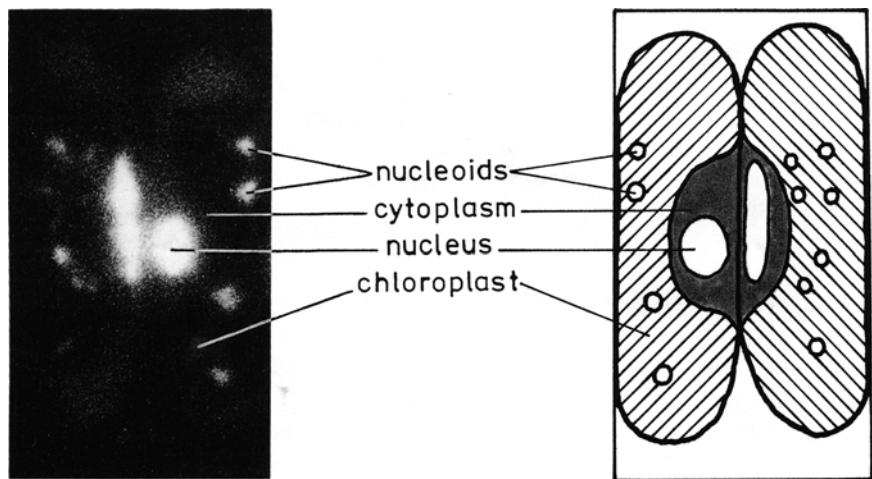


Fig. 40 Fluorescence photomicrographs of DAPI-stained cells of *Desmodesmus (Scenedesmus) quadricauda* at the division phase into four or eight cell nuclei. Chloroplast nucleoids are small, even sized; many are dumbbell shaped (see arrows) indicating intense division. Bar = 10 µm (After Zachleder et al. 1975)

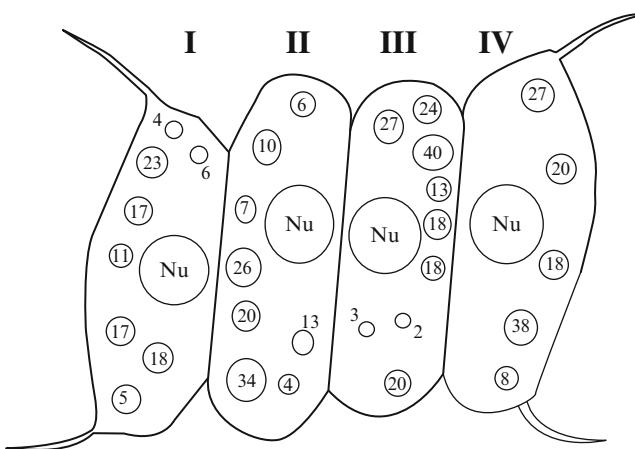
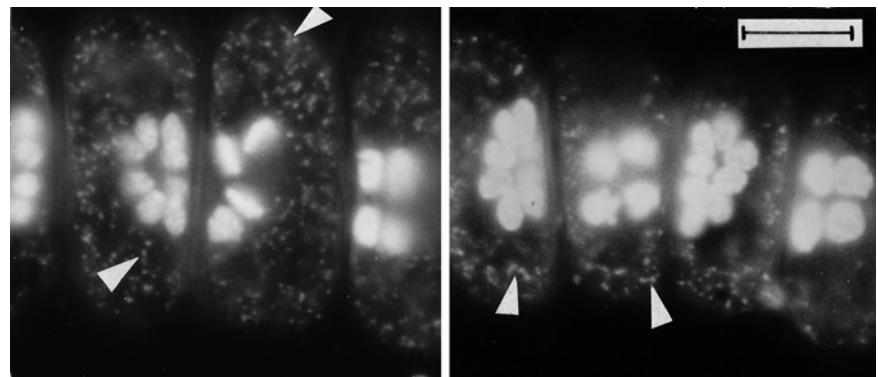


Fig. 41 Schematic drawing of the localization and size of nucleoids and cell nuclei (Nu) in cells of *Desmodesmus (Scenedesmus) quadricauda*. Numerals indicate DNA amount ($\times 10^{-16}$ g) in individual nucleoids (After Zachleder et al. 1995)

oid division was restricted to the very end of the cell cycle. This however, seems to be a specific case since in the closely related *Volvox carteri* and *Volvox aureus*, each pt-DNA replication was followed by the division of nucleoids (Coleman and Maguire 1982). The xantophycean alga *Bumilleriopsis* displays the consecutive Scenedesmus-type of cell cycle, i.e. multiple reproduction events occur consecutively during the whole cell cycle (see Fig. 3, Chap. 3). The alga has numerous chloroplasts dividing during the growth phase of the cell cycle; their division rate traces a curve similar to the rate of pt-DNA synthesis in the algae mentioned above (Hesse 1974).

Similarly as it was found in *Desmodesmus (Scenedesmus) quadricauda* (Zachleder 1995), the recent study of the course of the pt-DNA in a synchronous culture *C. reinhardtii* (Kabeya and Miyagishima 2013) proved that the chloroplast DNA is replicated independently of the timing of chloroplast division and the cell cycle. Chloroplast DNA replicates under the continuous light condition correlated with chloroplast and cell sizes increase to keep the proper DNA content per cell/chloroplast volume.

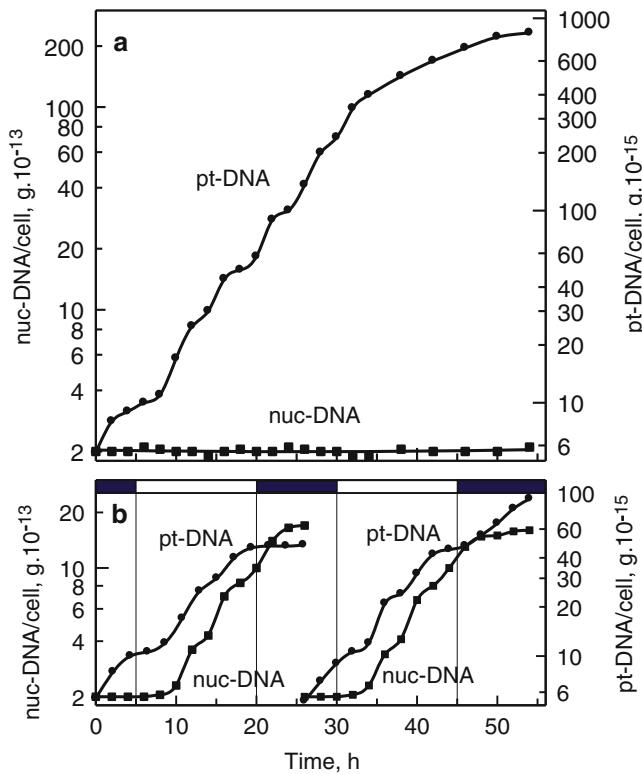


Fig. 42 Variations in chloroplast and nuclear DNA per cell in the FdUrd treated (a) and control (b) synchronized populations of the *Desmodesmus (Scenedesmus) quadricauda*. The values for pt-DNA are the mean values from about 30 cells in which total pt-DNA was calculated as the sum of pt-DNA in individual nucleoids. Values for cell-nuclear DNA were obtained by biochemical assay. The FdUrd treated culture (a) was continuously illuminated. Time intervals including sequences of dark and light periods in control culture (b) are indicated by black and white bars at the top of panel (After Zachleder et al. 1996)

Taken together, DNA replication is initiated at each doubling of chloroplast size and it is thus linked with chloroplast growth in a manner characteristic of prokaryotic cells.

Early experiments characterized the bulk of pt-DNA synthesis, however, the structure formed by pt-DNA was, at the time, unknown. This was significantly changed by the introduction of the fluorescent dye, DAPI (4,6-diamidino-2-phenylindole), which can bind with DNA (James and Jope 1978; Williamson and Fennel 1975) and thus made it possible to view such tiny structures as compactly organized DNA-protein complexes (Zachleder and Cepák 1987a, b, c), called nucleoids (Kawano et al. 1982; Kuroiwa et al. 1981; Nagashima et al. 1984) (a term will be used from now on) or chloroplastic nuclei (Kuroiwa et al. 1982, 1991; Nemoto et al. 1990, 1991) (Fig. 39).

Staining with DAPI also simplified research into pt-DNA replication in algae with a Scenedesmus-type cell cycle, where the rounds of replication of nuc-DNA overlap with the replication of pt-DNA and thus cannot be easily followed as for the Chlamydomonas-type cell cycle. *D. quadricauda*

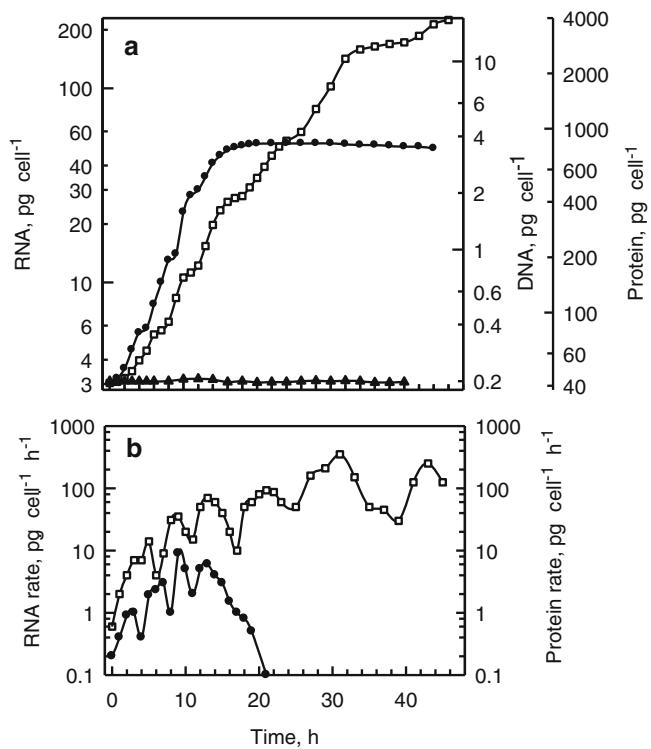
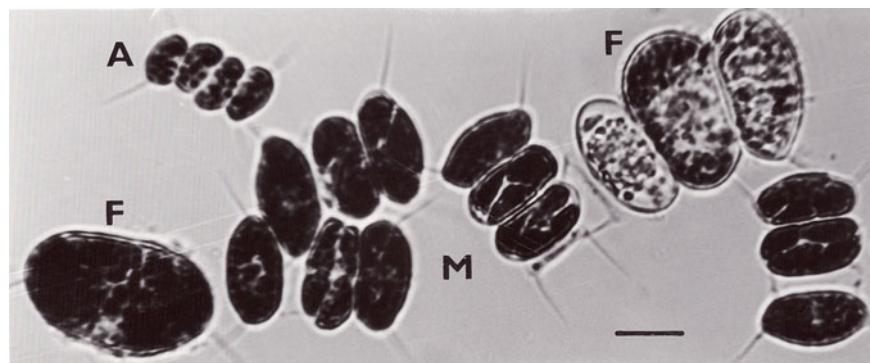


Fig. 43 The course of growth processes (RNA and protein accumulation) in synchronous populations of *Desmodesmus (Scenedesmus) quadricauda* grown at high irradiance in the presence of FdUrd. Mean irradiance 85 W m⁻², continuous light, temperature 30 °C, (a) Variation in RNA (●), protein (□), and DNA (▲) amounts per cell; (b) Oscillations in the rates of accumulation of RNA (●) and protein (□) (After Zachleder 1995)

cells possess a cup-shaped (often lobed) chloroplast; 2–16 nucleoids can usually be observed in daughter cells at the beginning of the cell cycle. During the cell cycle, the nucleoids divide intensively by binary fission, forming many tiny ones in mother cells at the end of the cell cycle (Fig. 40). In *Desmodesmus quadricauda*, dividing into eight daughter cells, three consecutive steps in the replication of chloroplast DNA occurred over the cell cycle. The first step was performed shortly after release of the daughter cells (even in the dark), the second and third steps occurred consecutively during the cell cycle. While the first round of pt-DNA replication occurred earlier than nuclear DNA, the other two steps overlapped the first two steps in nuc-DNA replications (Fig. 42b).

Once the DNA-containing structures (cellular, chloroplast and mitochondrial nuclei) are stained with DAPI, fluorescence intensity can be used to estimate DNA content during the mitotic cycle in synchronized populations of the chlorococcal algae. An example of such an estimate is presented in Fig. 41, where the size, localization and DNA content of individual pt-nuclei are schematically drawn; the DNA content per nucleoid inside a single chloroplast varied between 2 and 40 × 10⁻¹⁶ g pt-DNA.

Fig. 44 Comparison of mother cell size of *Desmodesmus (Scenedesmus) quadricauda* at the end of the control cell cycle with a cell grown for 48 h in the presence of FdUrd. The cells from control and FdUrd treated cultures were mixed together and stained with Lugol solution to visualize starch content. Mother cells in four/celled coenobia (**M**) are dividing their protoplasts and releasing four-celled daughter coenobia (**A**); FdUrd treatment produced a giant cell (**F**). Bar = 10 µm (After Zachleder 1995)



7.2.1 Inhibition of Nuclear DNA Replication

One of compounds used to uncouple chloroplastic and cytoplasmic processes in *Desmodesmus quadricauda* is 5-fluorodeoxyuridine (FdUrd), a specific inhibitor of thymidylate synthase. Replication of nuc-DNA was specifically blocked by FdUrd, leading to a blockage of nuclear division/s and cytokinesis/es. Under the same treatment regime however, growth of the chloroplast was accompanied by continuous intensive replication of pt-DNA while nuc-DNA replication was completely inhibited (Fig. 42a). Growth processes continued for at least one cell cycle (24 h) in the case of the nucleo-cytosolic compartment, and even longer than 48 h in the chloroplast compartment. The first process affected by the treatment was RNA in the nucleo-cytosolic compartment, while bulk protein synthesis continued (Fig. 43) simultaneously with chloroplast growth. Eventually, the FdUrd-treated cells grew to a giant size (Fig. 44) with only a single nucleus containing its initial content of nuclear DNA.

Due to the blocking of nuc-DNA replication, all consequent energy and carbon consuming processes, such as nuclear division/s and cytokinesis, were inhibited, leading to a massive accumulation of unspent and continuously synthesized starch grains in a growing giant chloroplast (see, Fig. 53, Chap. 8). Once the inhibitor was removed, the cell rapidly underwent all committed processes of the DNA replication-division sequence. Even in the case of *D. quadricauda*, this would lead to cell cycle progression resembling that of *Chlamydomonas* (Zachleder et al. 2002).

7.2.2 Inhibition of Chloroplast DNA Replication

As is discussed above, inhibition of nuclear DNA effectively blocked all consecutive processes of the DNA replication-division sequence, e.g. nuclear and cellular division/s. In contrast, the chloroplast cycle, i.e. replication of chloroplast (plastid) DNA (pt-DNA) and division of nucleoids, was not affected by the absence of reproductive processes in the nucleo-cytosolic compartment, leading to the production of

giant cells with a single nucleus and its initial DNA content. In spite of inhibition of the DNA replication-division sequence, growth was not substantially affected, even in the nucleo-cytosolic compartment.

Similarly to blocking nuc-DNA replication by FdUrd, due to its prokaryotic nature, pt-DNA in algae can be inhibited by prokaryotic-specific DNA inhibitors such as nalidixic acid (1-ethyl-1,4-dihydro-7-methyl-1,4-oxo-1,8-naphthyridine-3-carboxylic acid) (NAL), an inhibitor of DNA gyrase. NAL inhibits chloroplast DNA synthesis with no effect on nuc-DNA synthesis in higher plants (*Nicotiana tabacum*) (Heinhorst et al. 1985), in green flagellates (*Euglena*) (Hashimoto and Murakami 1982; Pienkos et al. 1974), green algae (*Chlamydomonas reinhardtii*) (Robreau and Le Gal 1974), and the unicellular red algae (*Cyanidioschyzon merolae*) (Itoh et al. 1997).

The application of NAL to synchronized cultures of *D. quadricauda* inhibited both pt-DNA replication (Fig. 45b) and nucleoid division (Fig. 45c) (Zachleder et al. 2004). Chloroplast growth was not defective because the chloroplast nucleoids usually contain multiple DNA molecules so that growth is not limited by DNA template, even if pt-DNA replication is blocked (Zachleder et al. 2004). Interestingly, chloroplast division was not affected and was performed in coordination with cellular division. The divided chloroplasts had reduced numbers of nucleoids and, in some cases, only one or even no nucleoid was observed in daughter cell chloroplasts. Similarly, no nucleoids were present under some conditions in the red alga *Cyanidioschyzon merolae* (Itoh et al. 1997), the green flagellate *Euglena gracilis* (Hashimoto and Murakami 1982) and part of the chloroplast population in *Acetabularia* (Dasycladales) (Woodcock and Bogorad 1970).

The rate of growth processes in the nucleocytoplasmonic compartment was about half that in the control, probably due to reduced accumulation of rRNA and ribosomes. However, all processes ran normally with the CPs reflecting the slower

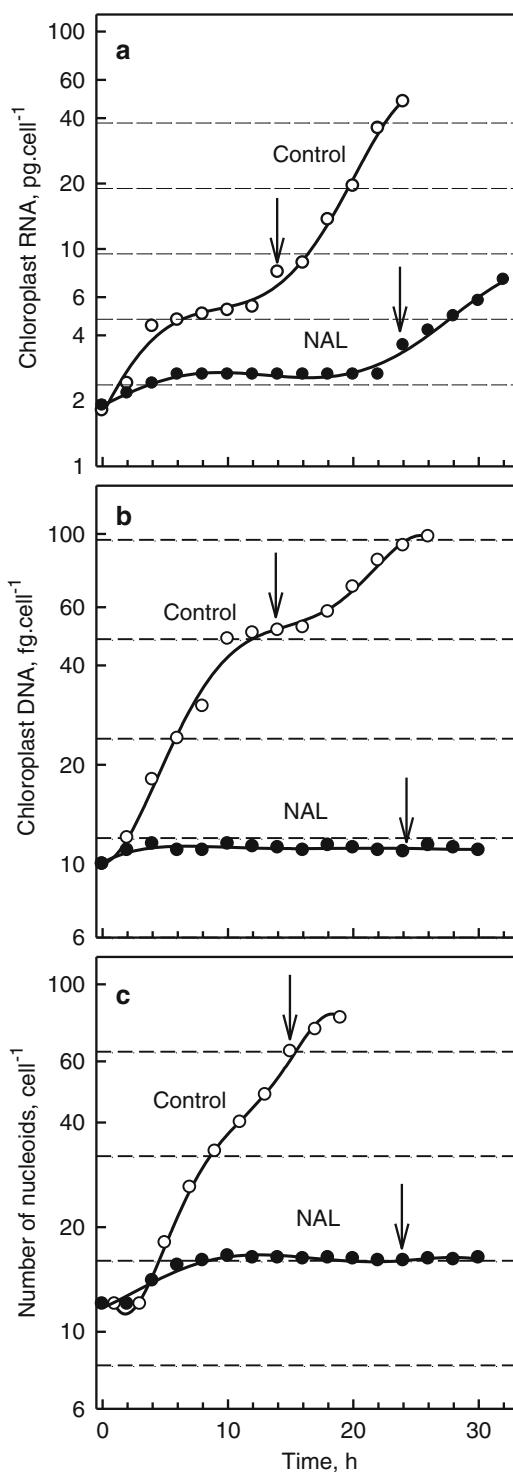


Fig. 45 Changes in chloroplast RNA (a), chloroplast DNA (b), and the number of chloroplast nucleoids (c) per initial cell during the cell cycle in synchronized populations of *Desmodesmus (Scenedesmus) quadri-cauda* grown at an irradiance of 100 W m^{-2} in NAL-free medium (Control open symbols) or in the presence of nalidixic acid (NAL solid symbols) from the beginning of the cell cycle. Horizontal dashed lines mark doubled values (After Zachleder et al. 2004)

growth rate with no affect on the nuclear DNA replication-division sequence. The control cells, as well as those grown in the presence NAL, divided into the same number of daughter cells, but in the case of NAL-treated cells, with reduced numbers of nucleoids per chloroplast (on average 2, range 1-4) (Fig. 46b). The existence of chloroplast division, even in the presence of NAL, indicates that it is, at least to some extent, independent of pt-DNA and nucleoid replication and is directed by the “master” cell division (Figs. 47, 48 and 49).

7.3 Chloroplast RNA

7.3.1 Effect of Light on RNA Synthesis

Changes in the availability of light to individual cells of *C. reinhardtii* cause fluctuations in photosynthetic capacity, levels of starch, rates of respiration, and the acquisition of enzyme activities (Donnan et al. 1985; Donnan and John 1983). Other processes affected by light availability are the accumulation of chloroplast and cytoplasmic RNA. Both cytoplasmic and chloroplast RNA accumulate exponentially when grown in light but the rate of accumulation and final content of rRNA per cell is strictly light intensity-dependent. Time courses of cytoplasmic and chloroplast RNA synthesis are more or less parallel (Fig. 50a, b).

7.3.2 Inhibition of Chloroplast-Protein and RNA Synthesis

Chloroplast protein synthesis can be blocked by a specific inhibitor, chloramphenicol, leading also to a cessation in chlorophyll (Fig. 51) and chloroplast ribosomal RNA (chl-RNA) synthesis (Fig. 52). Under these conditions, autotrophic growth of algal daughter cells is completely prevented due to the inability to use light and CO_2 as an external source of energy and carbon via photosynthesis. Thus, glucose must be used as a source of energy and carbon in order to study the relationship between chloroplast and nucleo-cytosolic compartments and the cells grow heterotrophically. Under heterotrophic conditions, chl-RNA synthesis (Fig. 52), chlorophyll synthesis and starch synthesis in the chloroplast (Fig. 51a), were not affected in the presence of glucose. In spite of the presence of glucose, chloramphenicol, however completely stopped the synthesis of chlorophyll (Fig. 51) and chl-RNA (Fig. 52) regardless if grown in the light or dark (Fig. 52, empty and full symbols, respectively). Surprisingly, in the presence of chloramphenicol, starch synthesis was not affected and continued as in heterotrophically grown control cultures (Fig. 52, G and G+CAP, dashed line). In spite of a severe limitation in chloroplast growth processes

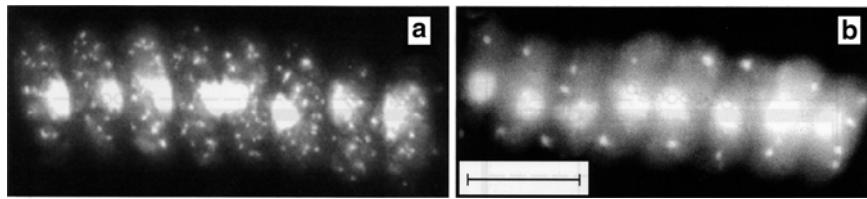


Fig. 46 Fluorescence photomicrographs of DAPI daughter coenobia *Desmodesmus (Scenedesmus) quadricauda* liberated from control (**a**) and NAL-treated (**b**) mother cells; intensive fluorescence of DAPI-

stained structures is seen as *white spots*; the largest spots represent the nuclei; nucleoids are seen as *small spots* localized in chloroplast. Bar = 10 μm (After Zachleder et al. 2004)

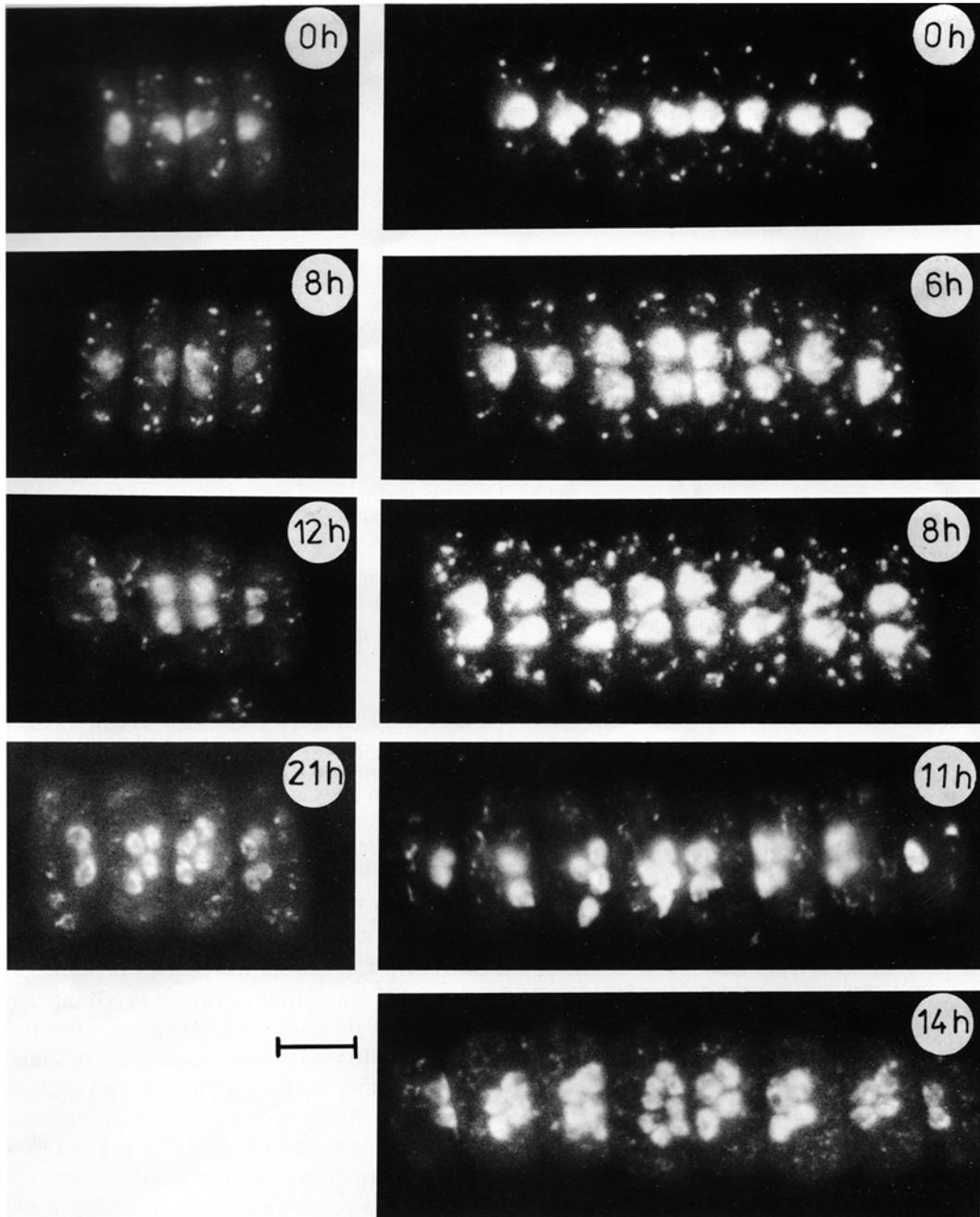


Fig. 47 Fluorescent microphotographs of typical DAPI-stained coenobia in synchronous populations of *Desmodesmus (Scenedesmus) quadricauda* grown at low and high irradiances. The age of the cells in hours, from the beginning of the cell cycle, is indicated at the top-right corner of individual microphotographs. Left column: The cells in quadruplet coenobia grown at the mean irradiance 30 W m^{-2} . Right column:

The cells in octuplet coenobia grown at the mean irradiance 100 W m^{-2} . Intensive bluish fluorescence of DAPI-stained structures can be seen as *white spots* in the black and white photographs. *Big spots* represent nuclei. Nucleoids are seen as small spots distributed seemingly at random in the chloroplasts. Bar=10 μm (After Zachleder and Cepák 1987b)

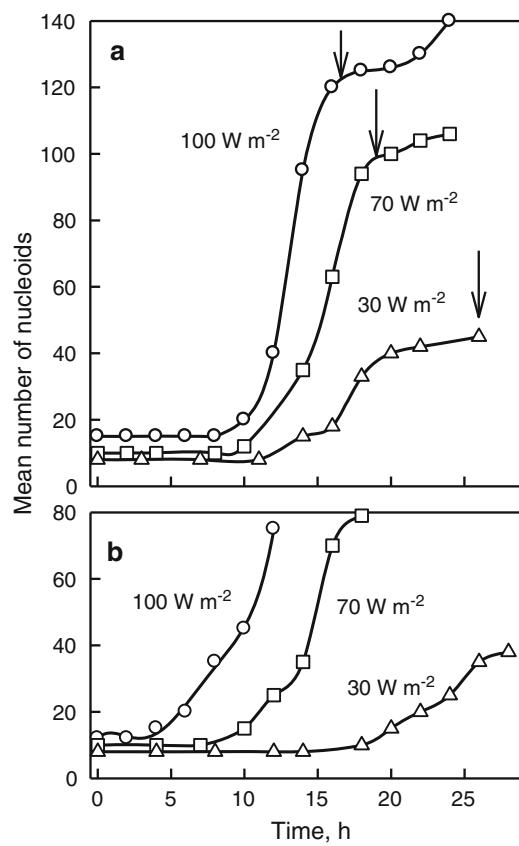


Fig. 48 Variations in the mean number of chloroplast nucleoids in synchronous populations of *Desmodesmus (Scenedesmus) quadricauda* grown under continuously illumination at high (100 W m^{-2}), moderate (70 W m^{-2}), and low (30 W m^{-2}) irradiance. Arrows indicate the moment when 50 % of the population has divided. (a) The first cell cycle: Daughter cells were released during dark period of the preceding cell cycle. The moment of the illumination is taken at the beginning of the cell cycle. (b) The second cell cycle: Daughter cells were released in light. The times when 50 % the population has released the daughter cells are taken as the beginning of the cell cycle. Only parts of the second cell cycles at three irradiances are illustrated (After Zachleder and Cepák 1987b)

due to inhibition of proteosynthesis, events in nucleo-cytosolic compartment were not substantially influenced. Growth processes in the nucleo-cytosolic compartment were not affected by the presence of chloramphenicol, as illustrated by the time course of cytoplasmic ribosomal RNA (cyt-RNA) (Fig. 52) regardless if kept in the presence of glucose in the dark or in light. The time course of the DNA replication-division sequence was the same as in the control culture (Fig. 51b). The cells were able to complete the entire cell cycle and release four small daughter cells.

Ribosomal RNA is a crucial component of growth since it is required for building ribosomes. Furthermore, a threshold

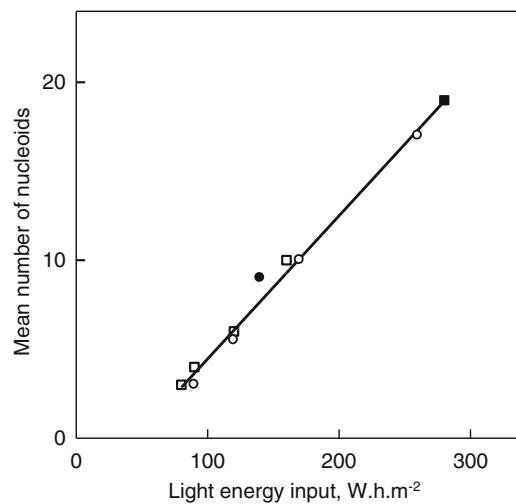


Fig. 49 Relationship between the total amount of light energy obtained by one daughter cell of *Desmodesmus (Scenedesmus) quadricauda* and the number of chloroplast nucleoids. Light energy (E) was calculated from the light intensity (I), interval of illumination (t) under which mother cells grew and number of daughter cells formed (n) according to the formula: $E = I \cdot t/n$. □ daughter cells in four-celled coenobia from mother cells grown at 25 Wm^{-2} PAR, daughter cells in four-celled coenobia (■) and in eight-celled coenobia (●) from mother cells grown at 75 Wm^{-2} PAR, ○ daughter cells in eight-celled coenobia from mother cells grown at 130 Wm^{-2} PAR (After Zachleder and Cepák 1987a)

level of nuc-RNA is a prerequisite for DNA replication (Singer and Johnston 1979; Zachleder and Šetlík 1988). DNA replication in the chloroplast often preceded replication events in the nucleus, and the number of chloroplast DNA replications seemed to be predictive of the final number of DNA replications in the nucleus. It was suggested that the chloroplast might play an important role in regulating cell cycle events in photoautotrophic organisms (Chiang 1975; Chiang and Sueoka 1967b; Šetlík and Zachleder 1984). Experiments with uncoupling DNA replication in the chloroplast and nucleus by blocking nuc-DNA replication by FdUrd suggested that there is no mechanism blocking the chloroplast cycle when the nuclear DNA replication-division sequence is blocked. On the other hand, experiments with nalidixic acid indicated that inhibition of chloroplast growth will not directly block the nuclear cycle processes. Finally, the effect of growth inhibition of the chloroplast had a mere trophic effect, suggesting a growth dependency of the nuclear cycle on the chloroplast but no supporting the existence of any exclusive coordination mechanism.

7.3.3 Role of Starch

Algal cells develop fully active chloroplasts, even after long-term cultivation (15 years) in the dark under heterotrophic

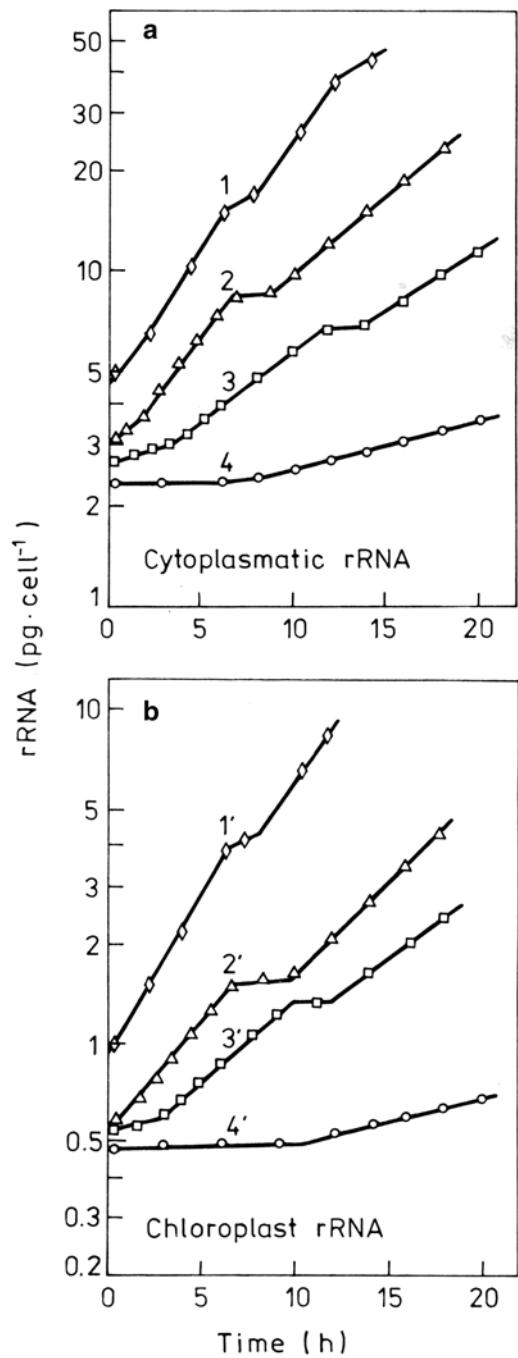


Fig. 50 Accumulation of cytoplasmic (a) and chloroplast (b) RNA during the second cell cycle in a synchronous population of *Desmodesmus (Scenedesmus) quadricauda* grown and continuously illuminated at four different mean irradiances. Curves 1: 150 W m⁻²; curves 2; 75 W m⁻²; curves 3: 40 W m⁻²; curves 4: 20 W m⁻² (After Cepák and Zachleder 1988)

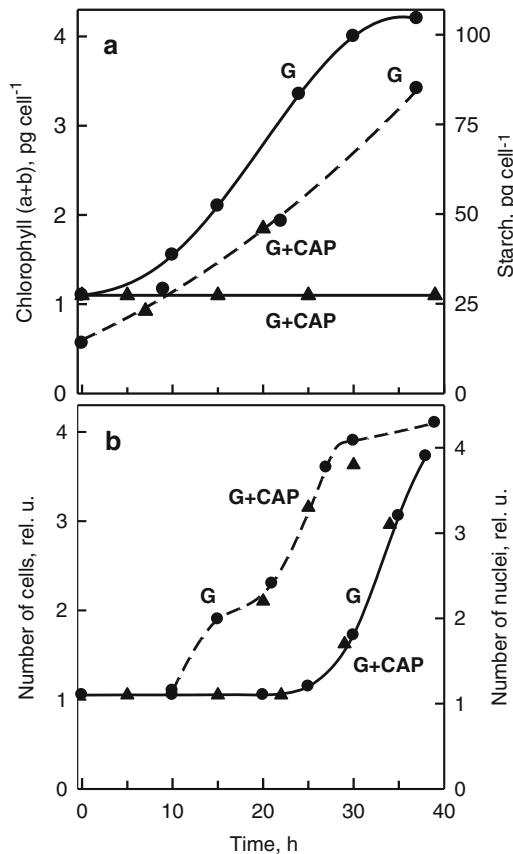


Fig. 51 Accumulation of chlorophyll (solid line) and starch (dashed line) (a) and the time course of the nuclear (dashed line) and cellular division (solid line) (b) in synchronous populations of *Desmodesmus (Scenedesmus) quadricauda* grown in dark in the presence of 1% glucose (G, ●) or in the presence of glucose and chloramphenicol (G+CAP, ▲) (After Zachleder et al. 1990)

conditions (Iwamura et al. 1982). This indicates that certain functions of the chloroplast must be preserved to enable normal progress through the cell cycle. One candidate is starch synthesis. It is not affected by light, even in the presence of chloramphenicol, if a substitute source of external energy (glucose) is provided. Accumulation of starch remains active under a wide range of restrictive conditions such as limitation by nitrogen (Ballin et al. 1988), sulphur (Šetlík et al. 1988) or phosphorus (Zachleder et al. 1988), or under benzo-pyrene inhibition (Zachleder et al. 1983), when most other synthetic processes were more or less inhibited. Chloroplastic starch reserves are used exclusively for performing the nucle-DNA replication-division sequence in *Chlorella* (Wanka

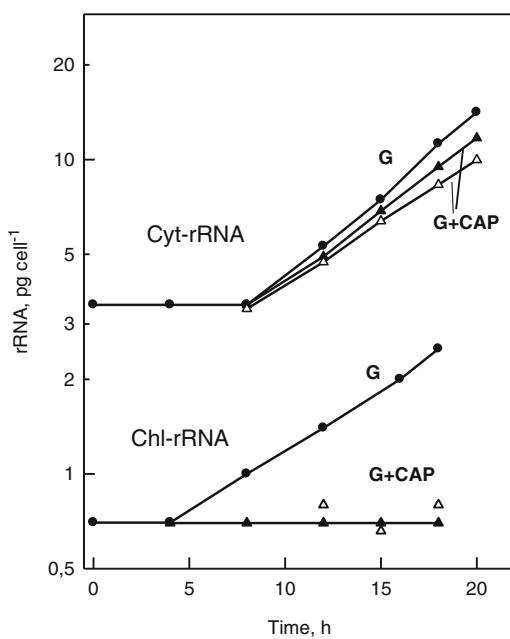


Fig. 52 Accumulation of cytoplasmic and chloroplast RNA in synchronous populations of *Desmodesmus (Scenedesmus) quadricauda* grown in presence of glucose in dark (G, ●), glucose and chloramphenicol (G+CAP) in dark (▲) or in light (Δ) (After Zachleder et al. 1990)

1968) and *Chlamydomonas* (Spudich and Sager 1980; Vítová et al. 2011b) by cells grown both in the light and dark. Furthermore, sufficient starch reserves are required for attainment of commitment points (Spudich and Sager 1980; Vítová et al. 2011b). This indicates the amount of starch or the rate of its degradation might be the process connecting/ coordinating the DNA replication-division sequences in chloroplast and nucleo-cytosolic compartments.

8 Energy Reserves

The amount of sun light, the sole source of energy for growth and division of phototrophically grown algae, varies during the day in addition to day/night alternation. While growth processes can easily adapt to variations in energy supply by changing their rate, the variation or even absence (during nights) of an external energy source it is extremely challenging for vital high energy demanding

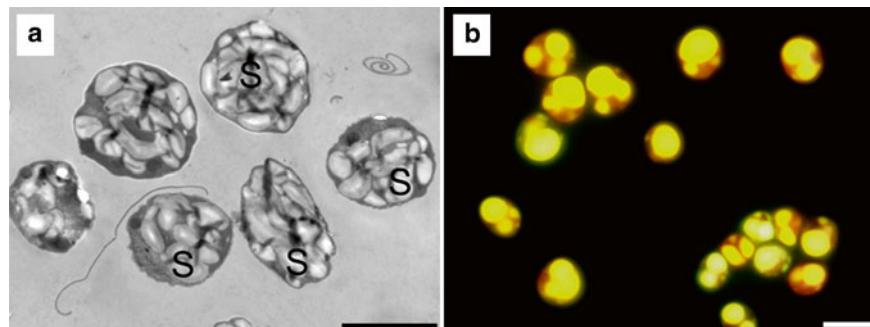
reproductive processes, DNA replication, nuclear and cellular division. Producing energy reserves for reproductive processes is therefore essential in order to compensate for differences in the rate of energy production. Algae have, in principle, two main types of energy reserves: starch (and other polyglucans) and lipids. Both components serve both as an energy source and as a supply of carbon allowing the cells to become completely independent of a direct supply of energy and carbon from photosynthetic activity.

Surprisingly, with the exception of a few early papers (Ballin et al. 1988; Duynstee and Schmidt 1967; Šetlík et al. 1988; Zachleder et al. 1988), little research has focused on this crucial process. The recent boom in energy reserve research in algae, motivated by the idea that algae could serve as a high-yielding source of energy-rich components for bioethanol production from starch or biodiesel from lipids has, however, resulted in a considerable body of information on starch and lipids in algae.

Starch is produced in most green algae as a primary store of carbon and energy. Other algae produce different types of polyglucans such as chrysotaminarin in diatoms, floridean starch and glycogen in red algae and paramylon in euglenophytes (Hildebrand et al. 2013). Lipids are the primary carbon and energy store in algal species from eustigmatophytes like *Nannochloropsis*, (Rodolfi et al. 2009) and *Trachydiscus* (Řezanka et al. 2010) apparently unable to produce glucans, but are also produced under specific conditions by other algae. Depending on the conditions, some strains of *Chlorella* or *Parachlorella* can overproduce starch (Fig. 53a) (Brányiková et al. 2011), while some strains of the same species overproduce lipids instead (Fig. 53b) (Li et al. 2013). A diatom *Odontella aurita* is industrially used to produce omega-3-fatty acids, while it also produces chrysotaminarin (Xia et al. 2014). A detailed description of this topic is out of the scope of this Chapter and would duplicate recently published reviews (Ahmad et al. 2011; Brennan and Owende 2010; Cheng and Timilsina 2011; John et al. 2011; Lam and Lee 2011; Mata et al. 2010; Rodolfi et al. 2009; Singh and Olsen 2011; Přibyl et al. 2013; Zachleder and Brányiková 2013).

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Fig. 53 Electron photomicrographs of *Chlorella vulgaris* grown in sulfur free mineral medium (a) S large starch bodies filling most of the cell volume. Scale bar = 10 μm (After Brányiková et al. 2011). (b) Overproduction of lipids during batch cultivation of *Parachlorella kessleri* in nitrogen free medium. Lipid bodies were stained using Nile Red (yellow); autofluorescence of chloroplasts is seen in red. Scale bar = 10 μm (After Li et al. 2013)



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