Developments in Applied Phycology 6

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The Physiology of Microalgae

Springer

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Developments in Applied Phycology
ISBN 978-3-319-24943-8 ISBN 978-3-319-24945-2 (eBook)
DOI 10.1007/978-3-319-24945-2

Library of Congress Control Number: 2015960938

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

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 CO_2 fixed (and O_2 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. postgraduate 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 — Michael A. Borowitzka
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Dundee, UK — John A. Raven
June 2015

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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-ethy1-1,4-dihydro-7-methy1-4-oxo-1,8-naphtyridine-3-carboxylic acid)
nuc-DNA	nuclear DNA
pt-DNA	chloroplast (plastid) DNA
Rb	retinoblastoma protein

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 (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.

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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M.A. Borowitzka et al. (eds.), *The Physiology of Microalgae*, Developments in Applied Phycology 6, DOI 10.1007/978-3-319-24945-2_1

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 C_1 cell cycle. This terminology is based on the fact that the cells can generally divide into 2^n, where n is an integer. For binary fission, $n=1$, thus this cell cycle can be designated as C_1. The more general cell cycle pattern, C_n, or multiple fission, is described in detail in the next section. The classical cell cycle (C_1) 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

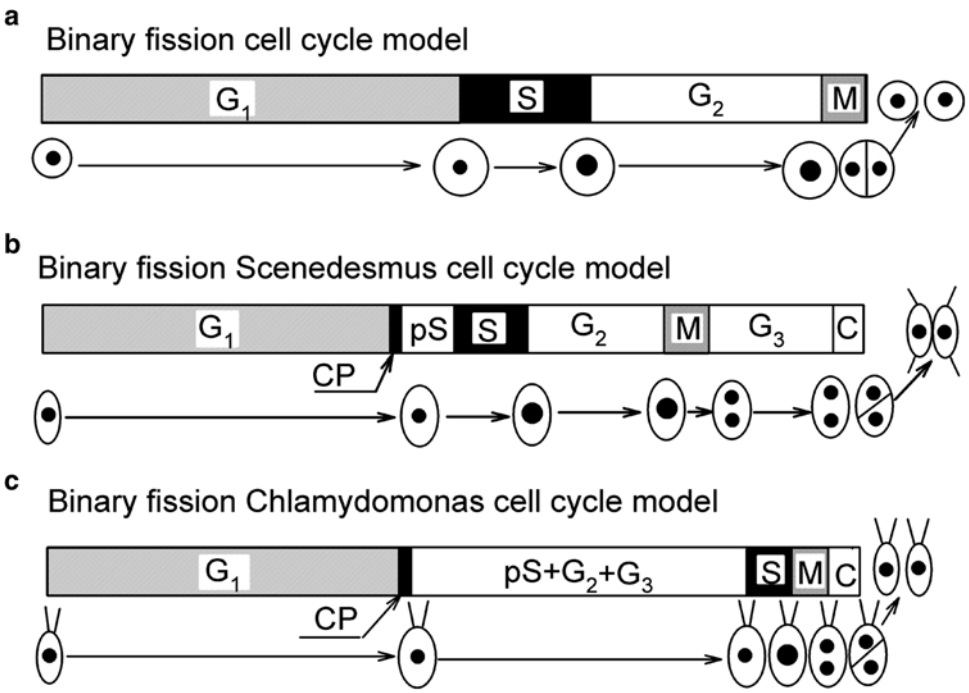

Fig. 1 Diagrams showing different types of cell cycle phases, including the classical cell cycle model and those found in *Desmodesmus* (*formerly Scenedesmus*) *quadricauda* and *Chlamydomonas*, which divide into two daughter cells. (**a**) Classical type cell cycle after Howard and Pelc (1953), (**b**) 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 C_1 type of cell cycle (number of daughter cells is 2^1). 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, stating 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 C_1 cell cycle type was introduced, where the mother cell divides into two daughter cells; many

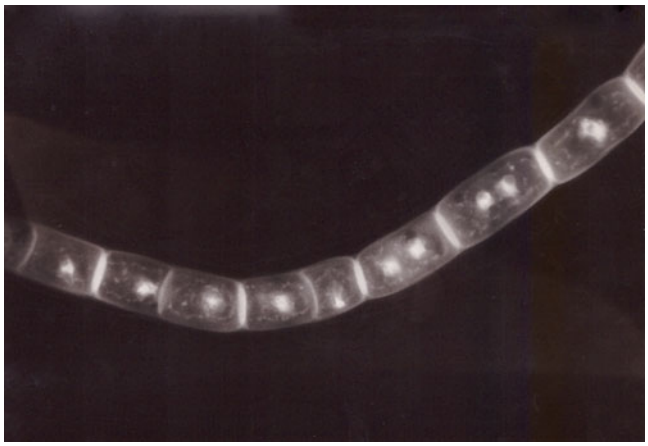

Fig. 2 Fluorescence photomicrographs of the green filamentous alga *Microspora* sp. (Ulotrichales) stained with DAPI. Different phases of the cell cycle and nuclear division can be seen in individual cells of the filament. Nucleoids are localized in chloroplasts along the cell wall (After Zachleder and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

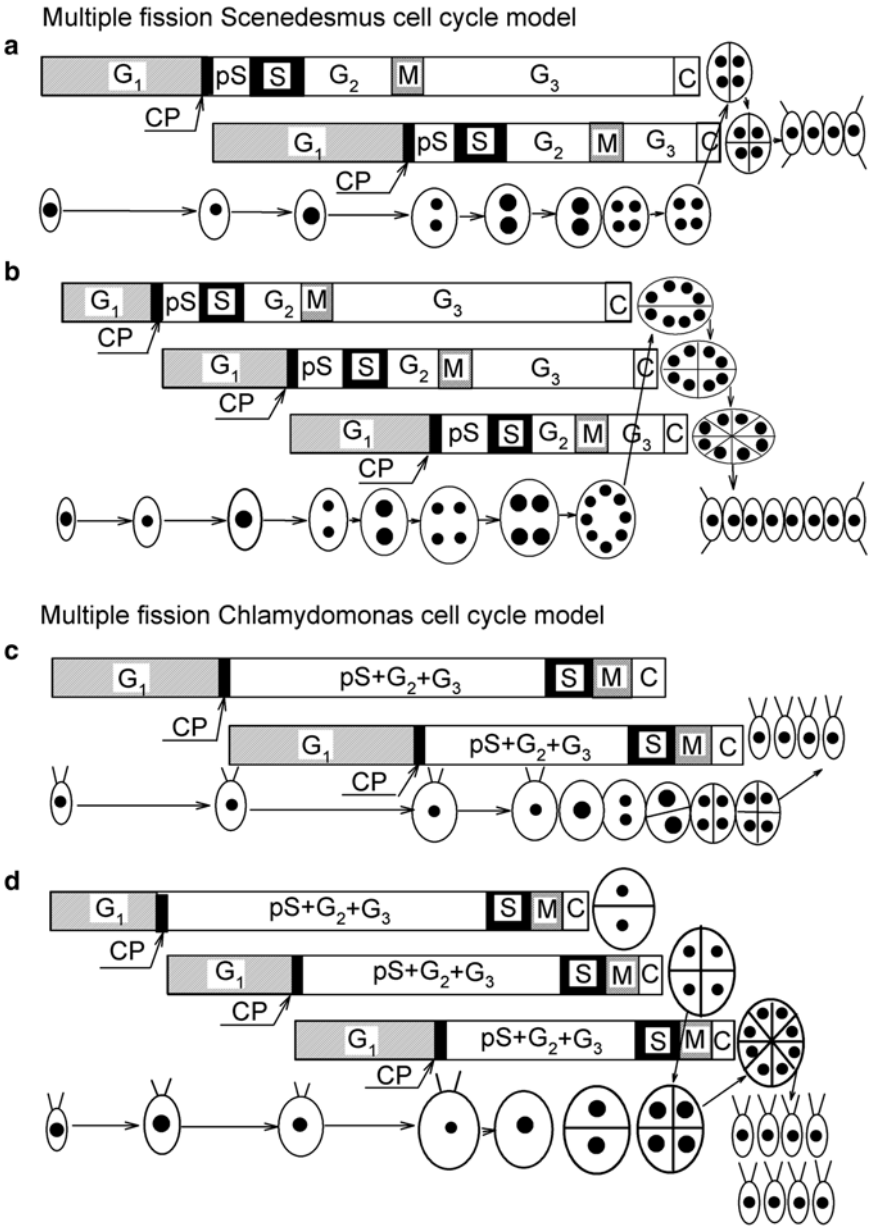

Fig. 3 Diagrams showing different types of cell cycle phases found in *Desmodesmus* (*Scenedesmus*) and *Chlamydomonas* dividing by multiple fission (cell cycle type C_n). (**a**, **b**) Scenedesmus-type cell cycle after Šetlík and Zachleder (1984), and (**c**, **d**) Chlamydomonas-type cell cycle after 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

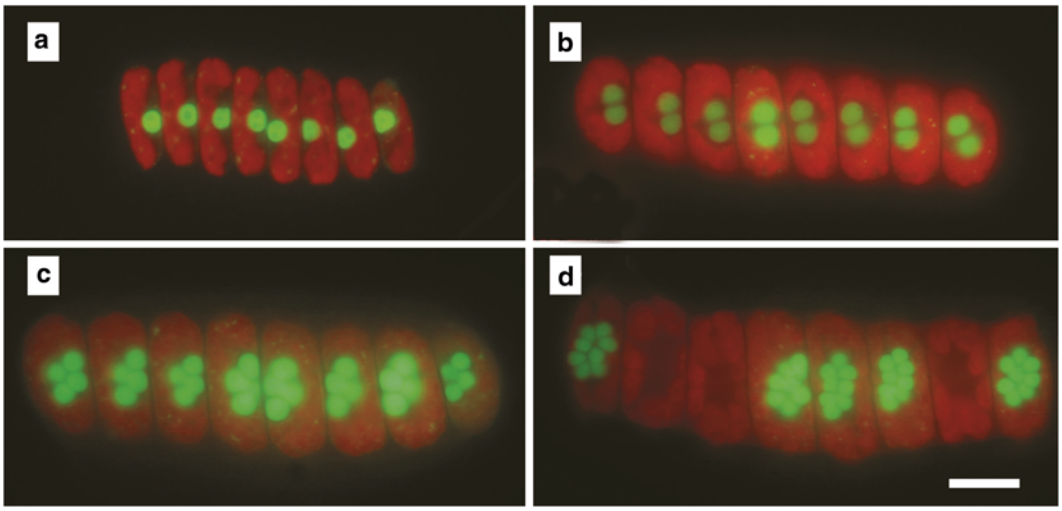

Fig. 4 Fluorescence photomicrographs of eight-celled coenobia of *Desmodesmus* (*Scenedesmus*) *quadricauda* during the cell cycle, stained with 0.3 % SYBR *green* I dye. Nuclei are visible as *yellow-green spots*. Chloroplasts are visible as a *red color*, which is autofluorescence of chlorophyll. (**a**) 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 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

[1]Wherever 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.

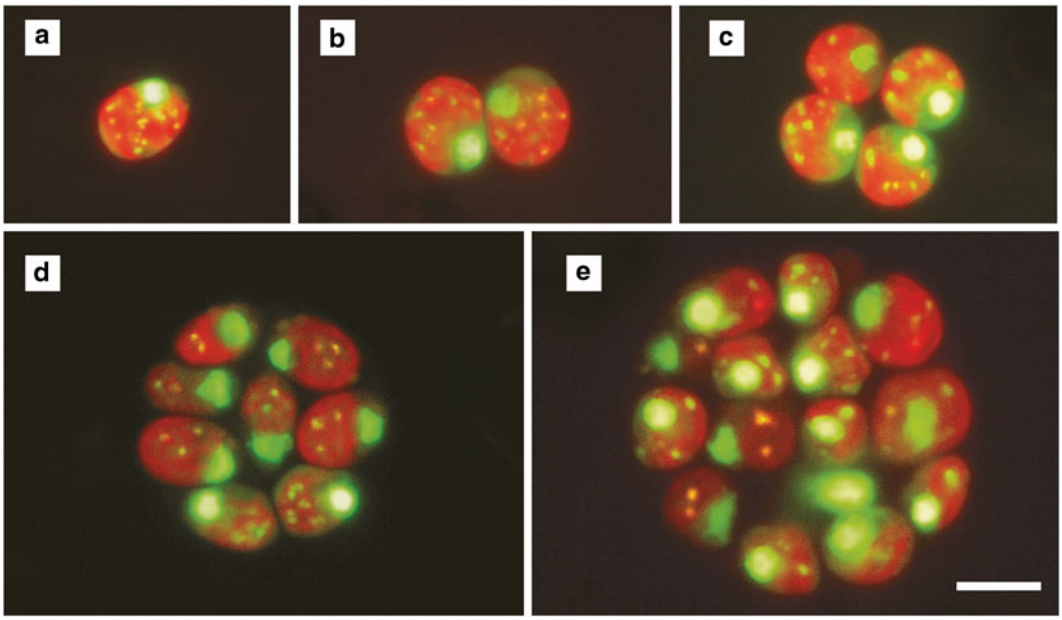

Fig. 5 Fluorescence photomicrographs of *Chlamydomonas reinhardtii* showing multiple division of protoplasts during the cell cycle. Stained with 0.3 % SYBR *green* I dye. (**a**) Uninuclear daughter cell. The nucleus is visible as a *yellow-green spot* and chloroplast nucleoids as tiny *yellow-green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

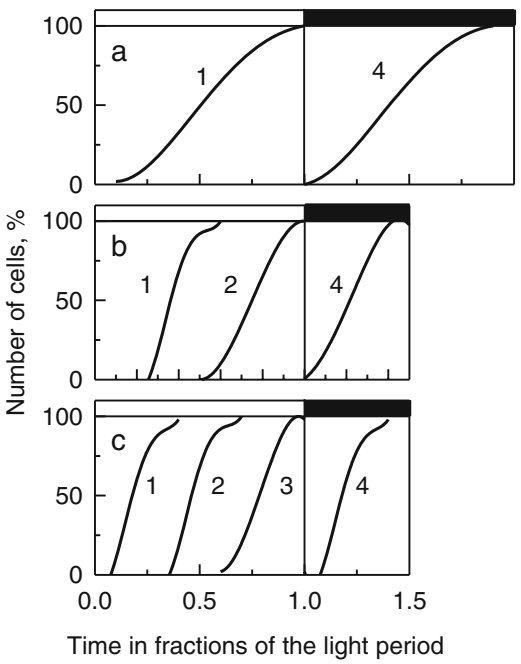

Fig. 6 Schematic drawing of commitment diagrams for three algal populations growing at low (**a**), medium (**b**), and high (**c**) growth rates. Detailed explanation in the text. Curves **1**, **2**, **3**: percentage of cells in the population committed to division into two, four and eight daughter 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 Zachleder1983)

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

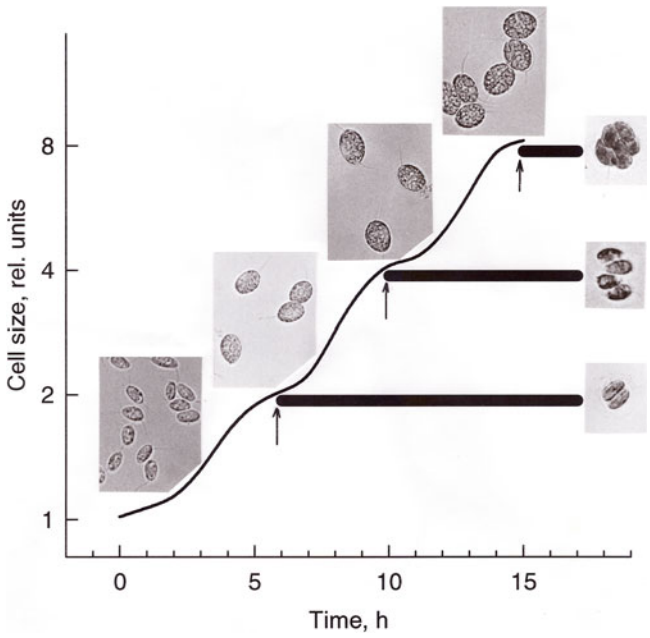

Fig. 7 Schematic illustration of the determination of commitment points to cellular division in synchronous populations of *Scenedesmus armatus*. The idealized *curve* represents the growth of cells in continuous light during the cell cycle; at the times marked by *arrows*, the subcultures were put into 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 = 4–6, 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 consists of 2 (*Didymochloris*), 4 (*Pascherina*), 8 or 16 (*Ulva*, *Spondylomorum*) 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 = 1–7), and the genus *Sorastrum* with 8128 daughter cells (n = 3–7). 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).

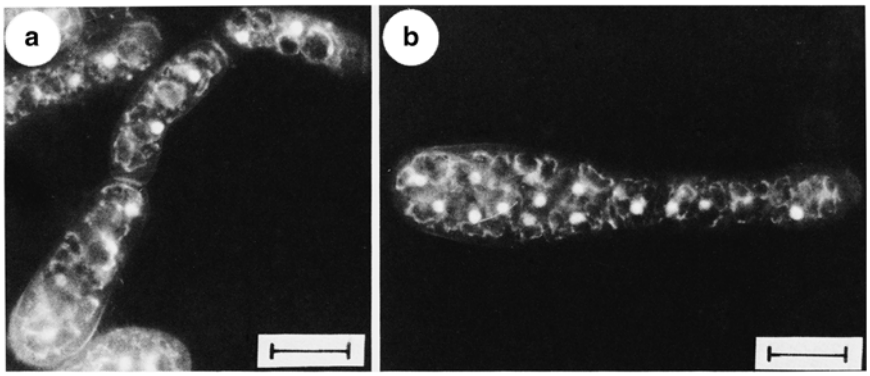

Fig. 8 Fluorescence photomicrographs of the yellow-green alga *Bumilleriopsis filiformis* (Mischococcales) stained with DAPI at different developmental stages of the cell cycle. (**a**) Binuclear and tetranuclear cells. (**b**) Multinuclear cells. Spherical arrangement of nucleoids in the individual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

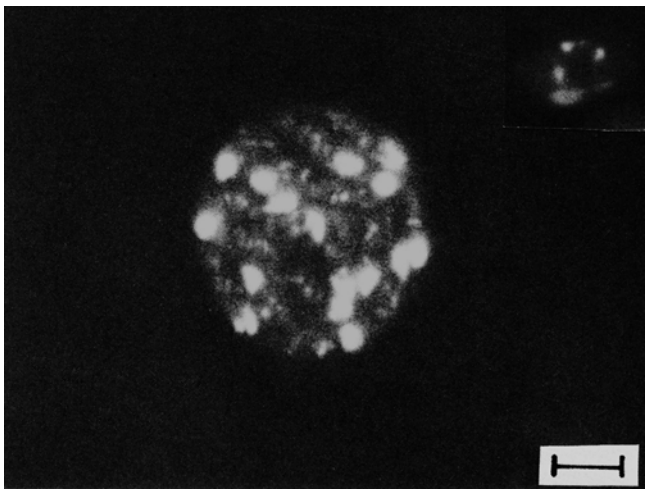

Fig. 9 Fluorescence photomicrographs of the green alga *Nautococcus piriformis* (Tetrasporales) stained with DAPI. A mature mother cell with 16 nuclei and numerous nucleoids. A young cell with one nucleus and four nucleoids is inserted in the top right-hand corner. *Scale bar* = 10 µm (After Zachleder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

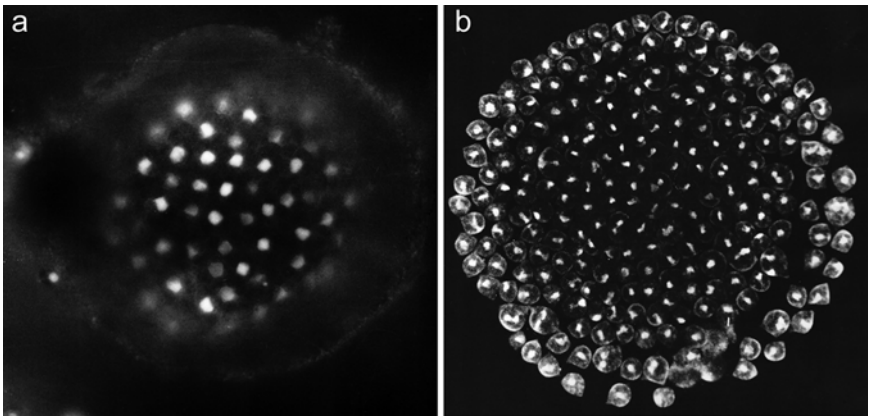

Fig. 10 Fluorescence photomicrographs of the chlorococcal alga *Kentrosphaera* sp. stained with DAPI. (**a**) A giant mother cell with an enormous number of nuclei (showing only those seen in one focal plane). (**b**) Freshly released daughter cells from one mother cell

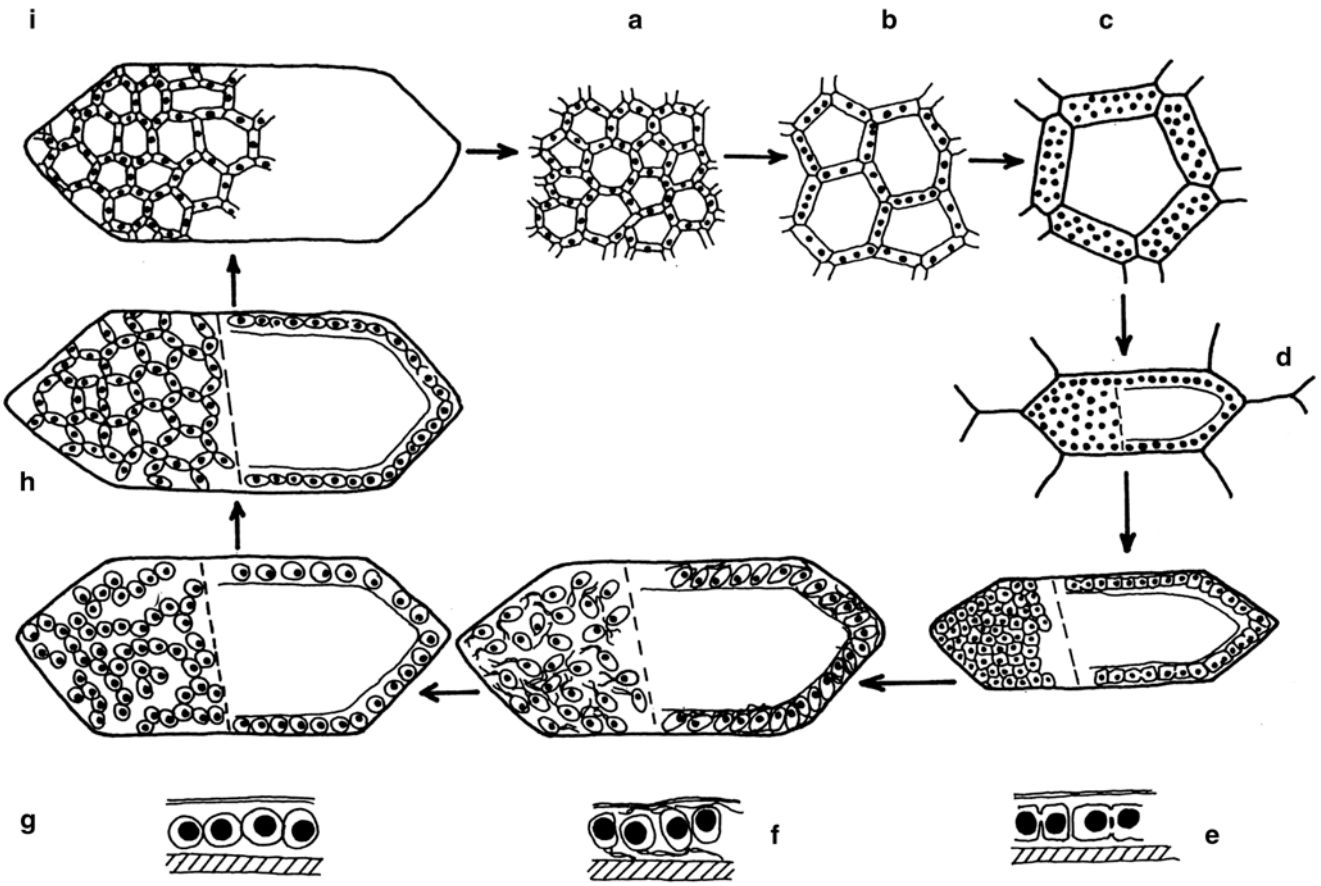

Fig. 11 *Hydrodictyon reticulatus* scheme of the cell cycle. (**a**) Uninuclear daughter cell released from mother cell wall, (**b**–**d**) multiple nuclear division in growing cells, (**e**) division into uninuclear protoplasts, (**f**) formation of biflagellar cell-wall-less zoospores, (**g**) conversion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

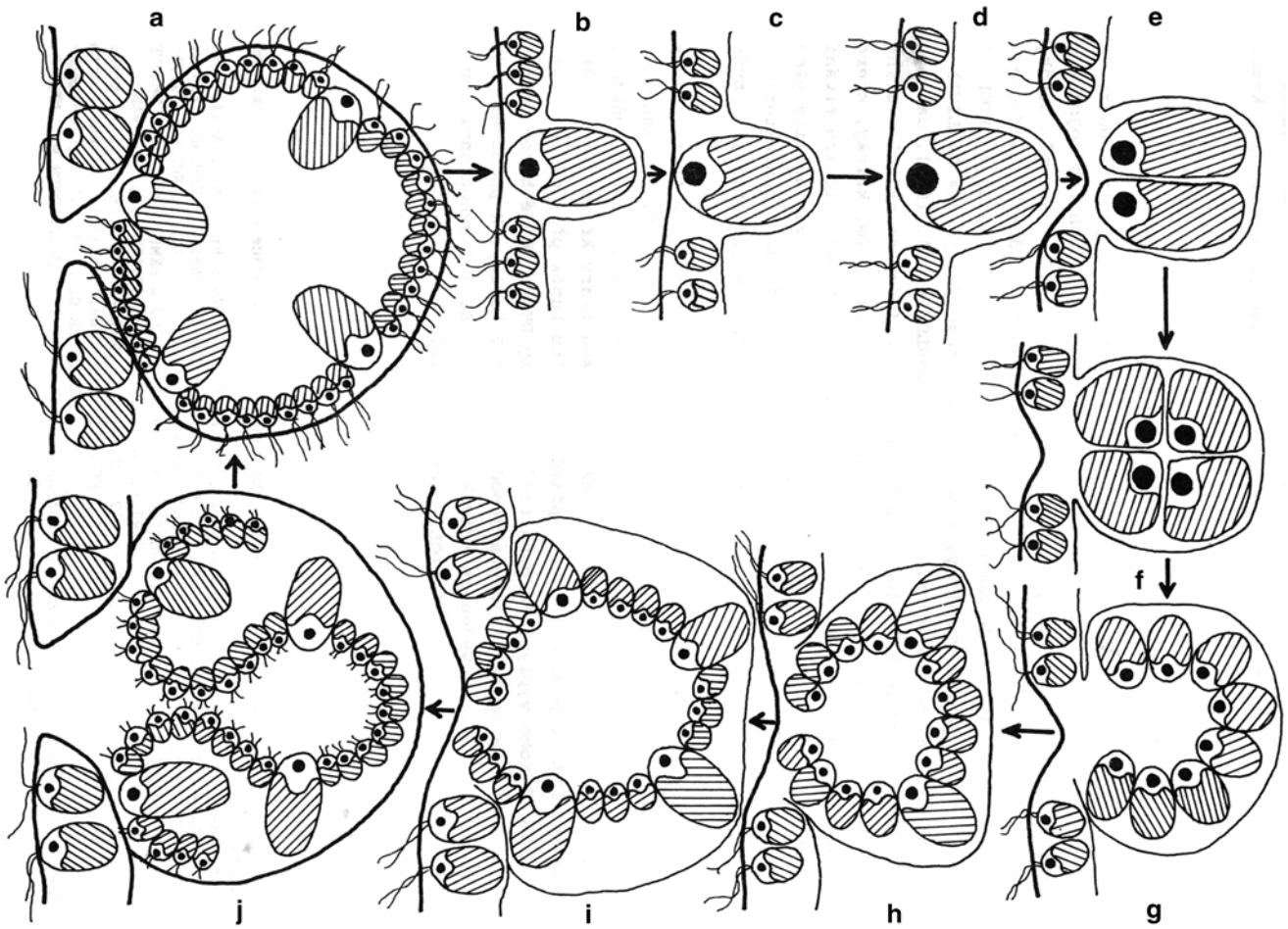

Fig. 12 *Volvox* scheme of the cell cycle. A diagram of the development of a new colony of gonidium for the genus *Volvox*. The young colony, which is released from the wall of the mother cell (**a**) nonflagellated gonidium, considerably larger than the other cells (**a**–**d**) and begins to divide (**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 Knutsen 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.

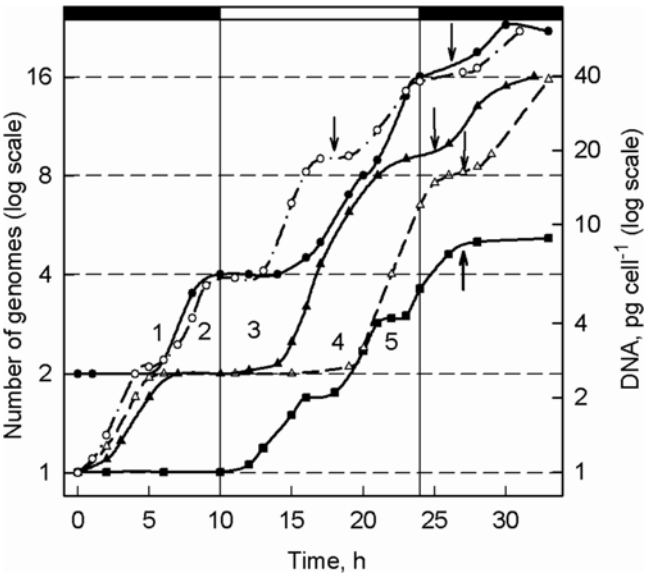

Fig. 13 The stepwise course of DNA replication under conditions of different growth rates and light-dark regimes in the cell cycle of *Desmodesmus* (*Scenedesmus*) *quadricauda*. Positions of the midpoints of cell divisions are indicated by *arrows* and the light-dark periods (for curves 2–5) are indicated 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 cell^{-1} 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 cell^{-1} 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 cell^{-1} 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 cell^{-1} h^{-1}. Curve *5*: The culture grown under alternating light-dark 14:10). Growth rate = 20 pg of protein cell^{-1} 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 Knutsen 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

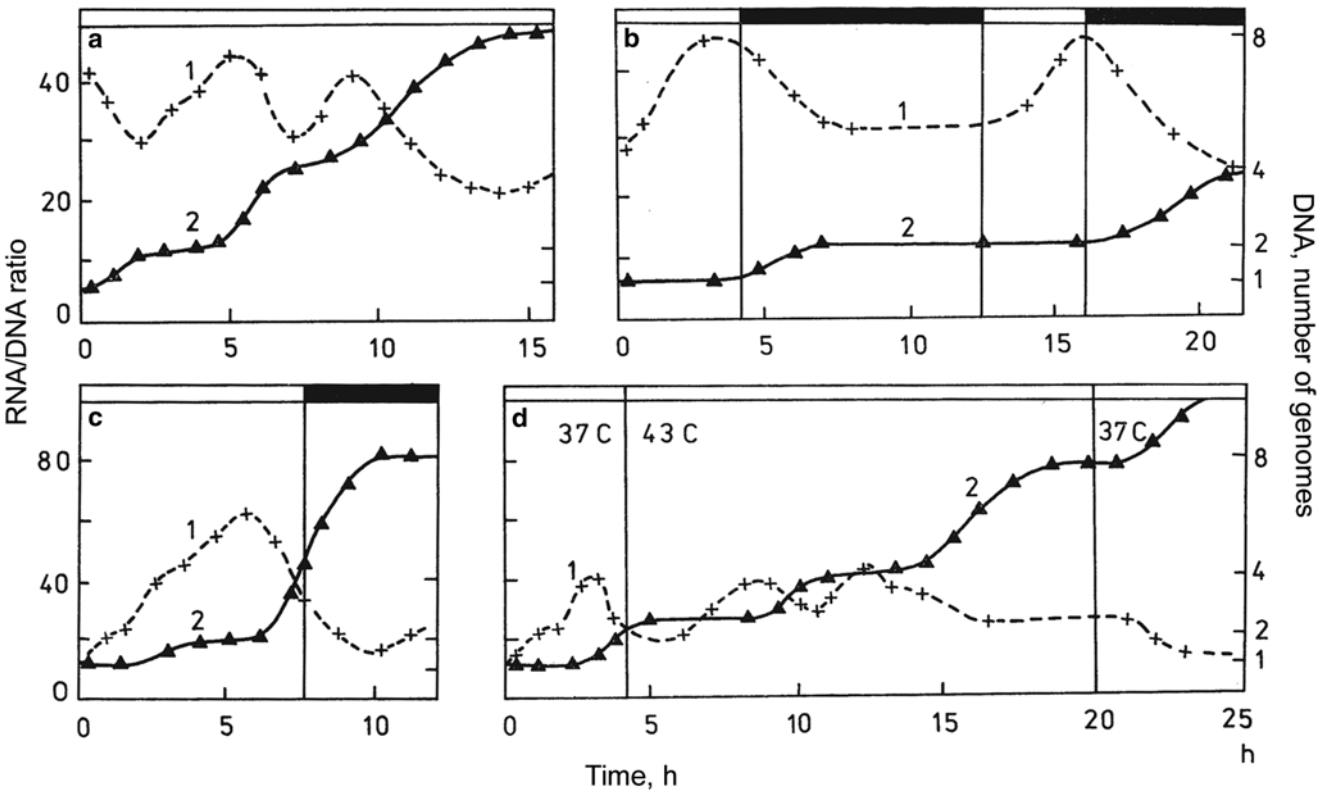

Fig. 14 Changes in RNA to DNA ratio in synchronized populations of *Desmodesmus* (*Scenedesmus*) *quadricauda*. (**a**) Continuous light (**b**) Inserted dark interval separated two growth steps. (**c**) Culture growing under alternating light and dark periods. (**d**) Inserted interval of supra-optimal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

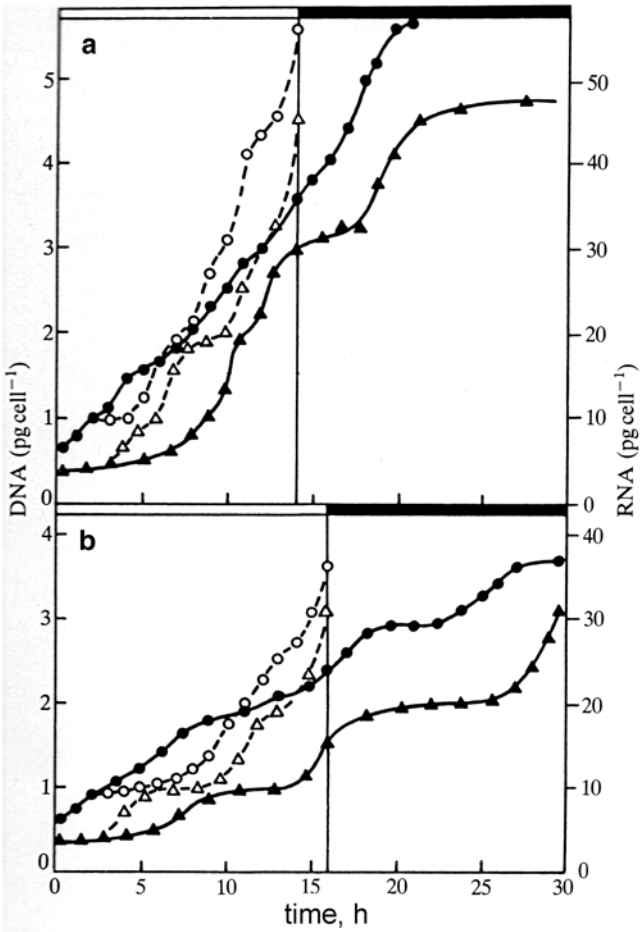

Fig. 15 Time course of RNA and DNA synthesis and their committed values in synchronous cultures of *Desmodesmus* (*Scenedesmus*) *quadricauda* grown under optimal growth conditions (**a**) and under conditions of slowed growth (**b**). Light and dark periods are indicated by *white* and *black strips* 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)

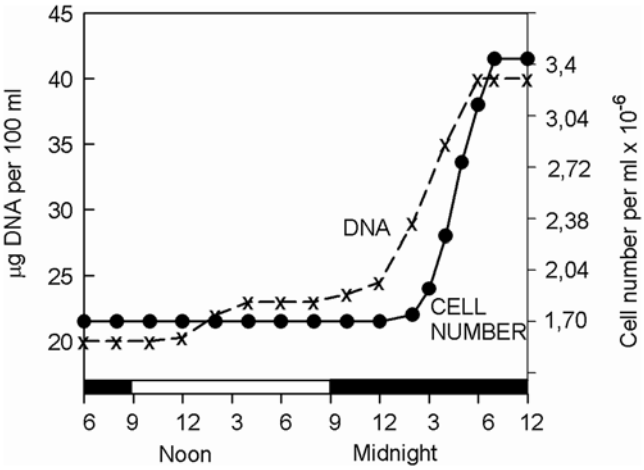

Fig. 16 Time course of DNA synthesis and cell number in a synchronized culture of *Chlamydomonas reinhardtii*. Two daughter cells were released at the end of the cell cycle under given growth conditions (After Chiang and Sueoka 1967b)

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 committed 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

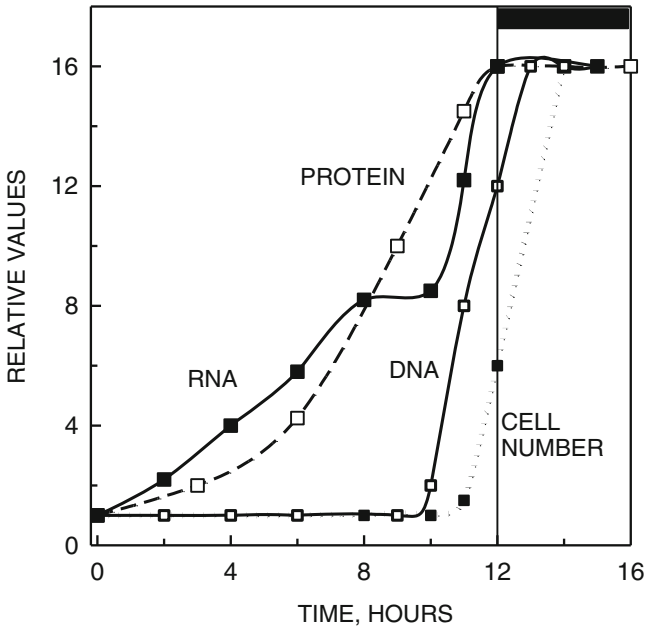

Fig. 17 Time course of increases in various parameters as indicated in the graph in a synchronized culture of *Chlamydomonas reinhardtii* (Modified after Lien and Knutsen 1979)

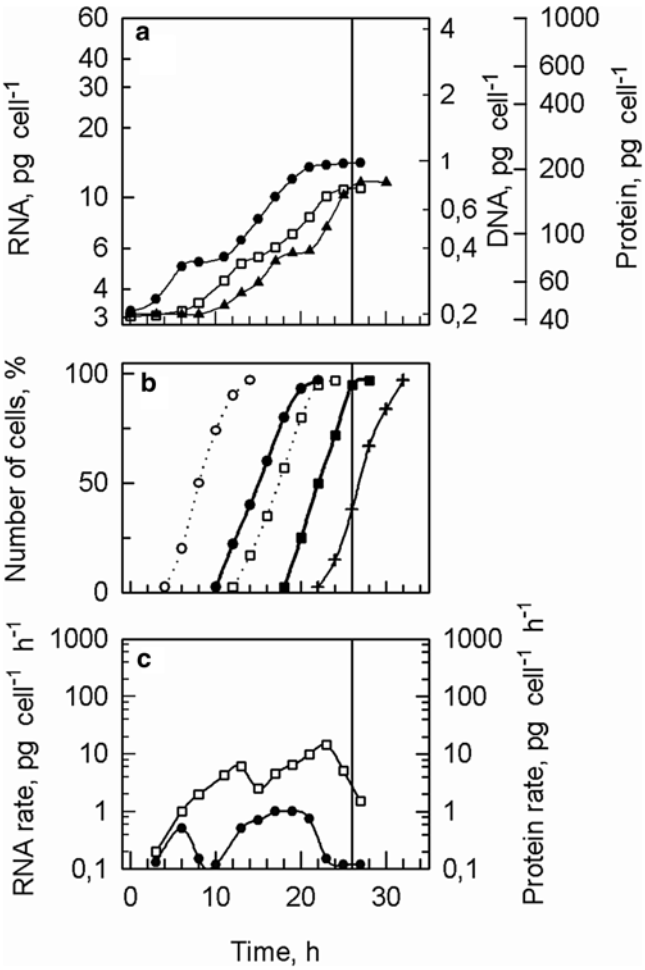

Fig. 18 Time course of growth and reproductive processes in synchronous populations of *Desmodesmus* (*Scenedesmus*) *quadricauda* grown at low irradiance. Mean irradiance 45 W m^{-2}, continuous light, temperature 30 °C. (**a**) Variation in RNA (●), protein (□), and DNA (▲) amounts per cell. The first 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)

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 (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

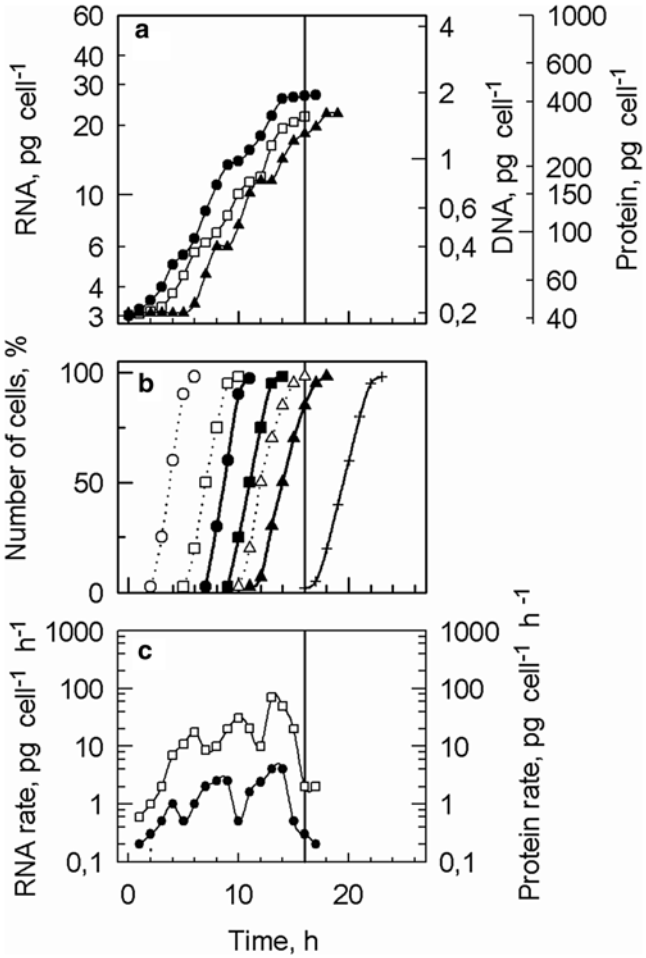

Fig. 19 Time course of growth and reproductive processes in synchronous populations of *Desmodesmus* (*Scenedesmus*) *quadricauda* grown at high irradiance. Mean irradiance 85 W m^{-1}, continuous light, temperature 30 °C. (**a**) Variation in RNA (●), protein (□), and DNA (▲.) amounts per cell (log 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; Robbens 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

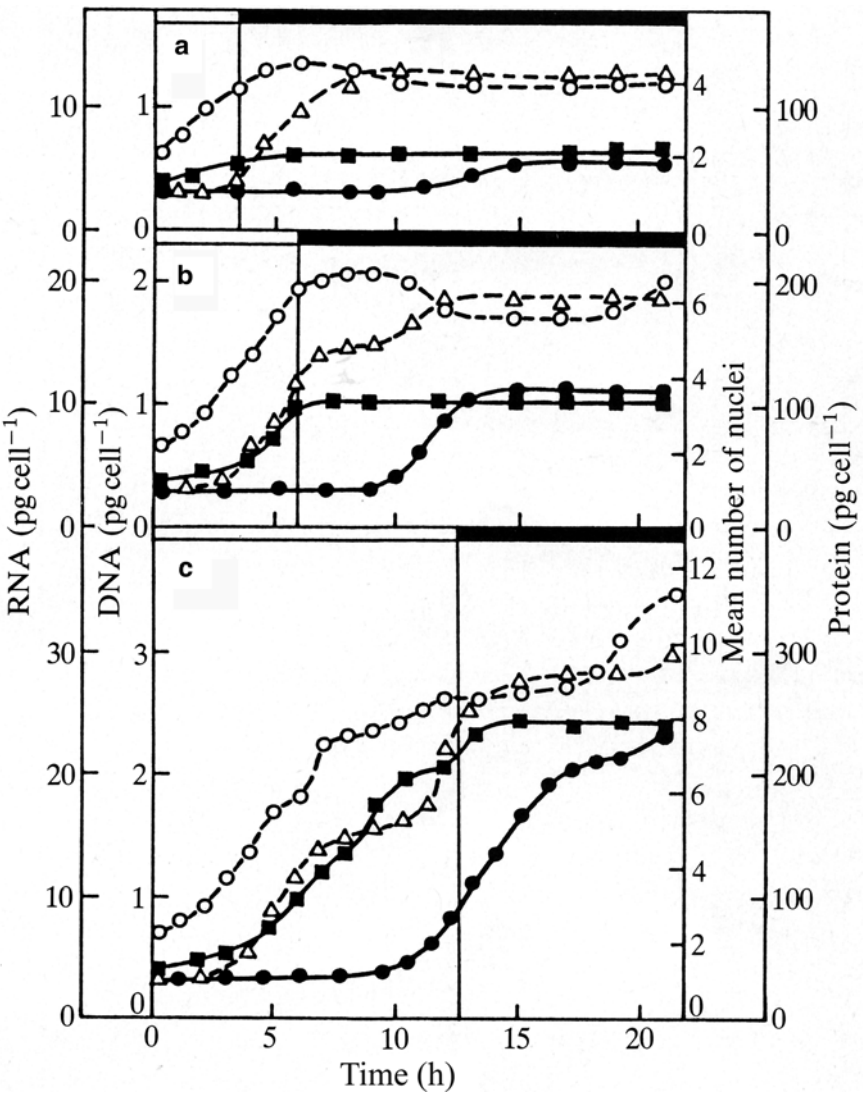

Fig. 20 Effect of darkening after different light intervals on the time course of RNA, DNA and protein synthesis and of nuclear divisions in synchronous cultures of *Desmodesmus* (*Scenedesmus*) *quadricauda* grown under optimal growth conditions. I = 95 W m^{-2}, D = 0.10 h^{-1}. The cultures were put 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

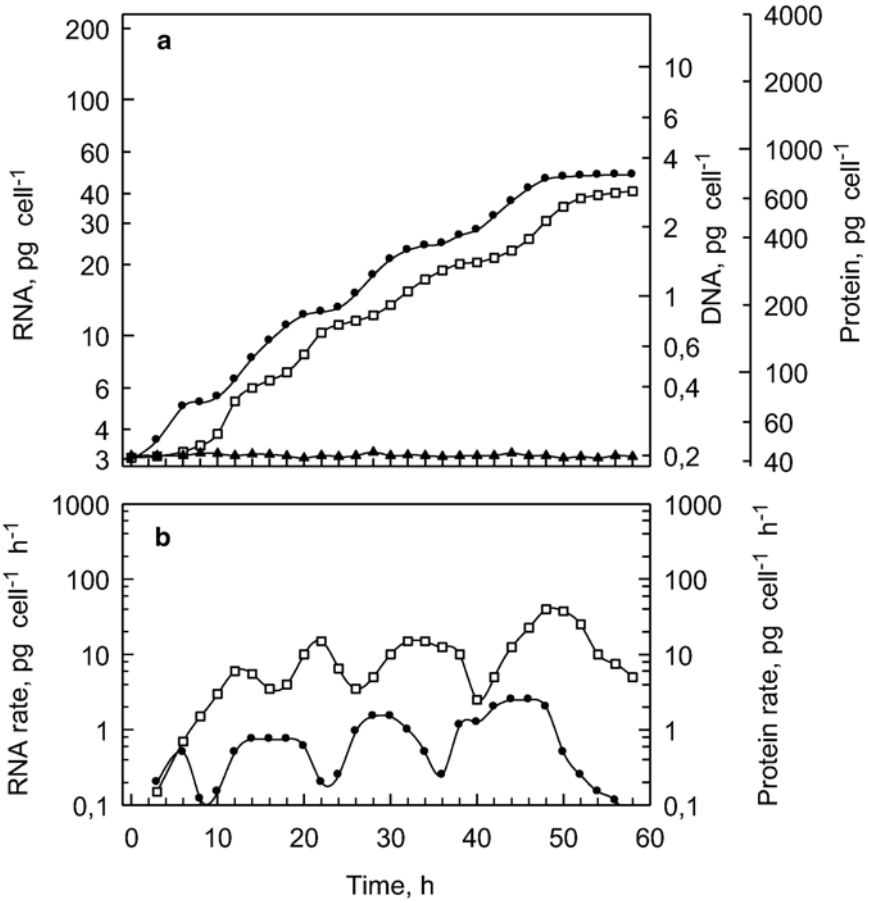

Fig. 21 The course of growth processes (RNA and protein accumulation) in synchronous populations of *Desmodesmus* (*Scenedesmus*) *quadricauda* grown at high irradiance in the presence of FdUrd. Mean irradiance 85 Wm^{-2}, continuous light, temperature 30 °C; (**a**) Variation in RNA (●), protein (□), 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 *cdc25* mutation in *Schizosaccharomyces pombe* - which may do so due to the presence of the N-terminal domain.

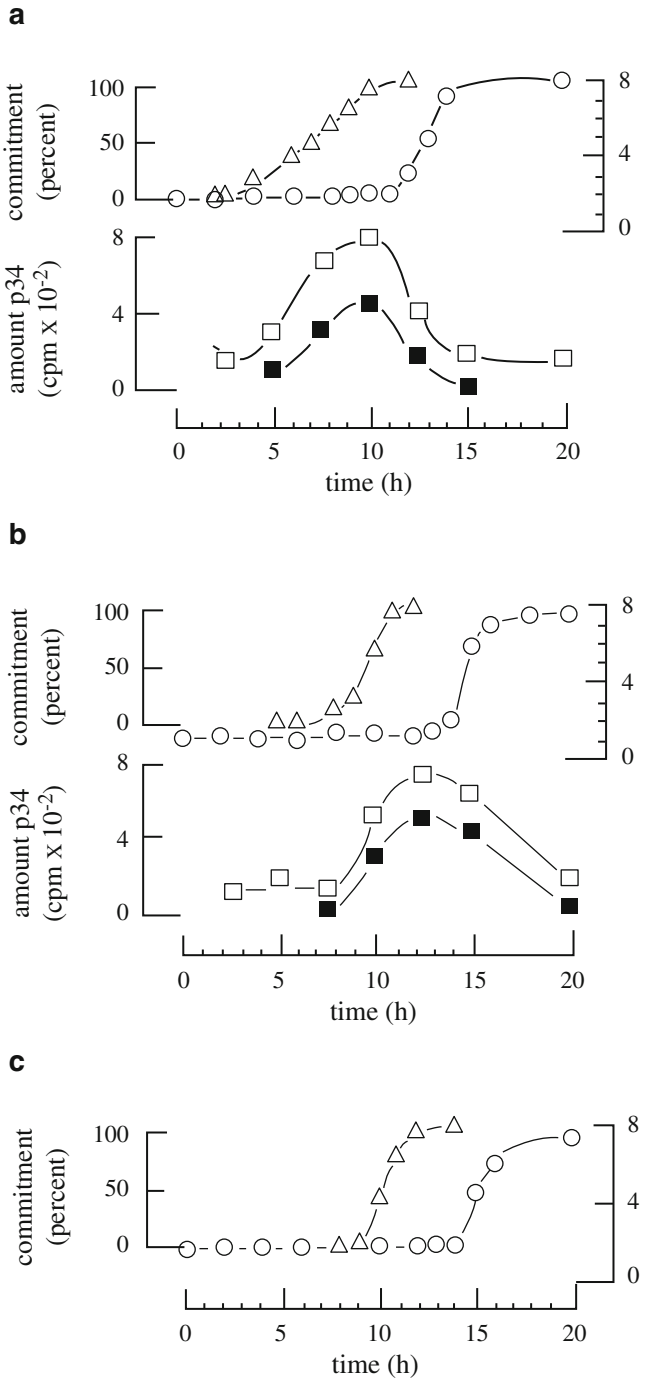

Fig. 22 Division timing and changes in the amount and phosphorylation of p34. (**a**) Very early division was caused by a reduction in phosphate to 100 µM. (**b**) Early division was caused by fast growth due to illumination at 200 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR). (**c**) Later division was caused by slower growth due to a lower light intensity of 100 µmol photons m^{-2} 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 (○) increased 6 h later. Total p34 (□) 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 (■) that are quantified in (**a**, **b**) (After John et al. 1989)

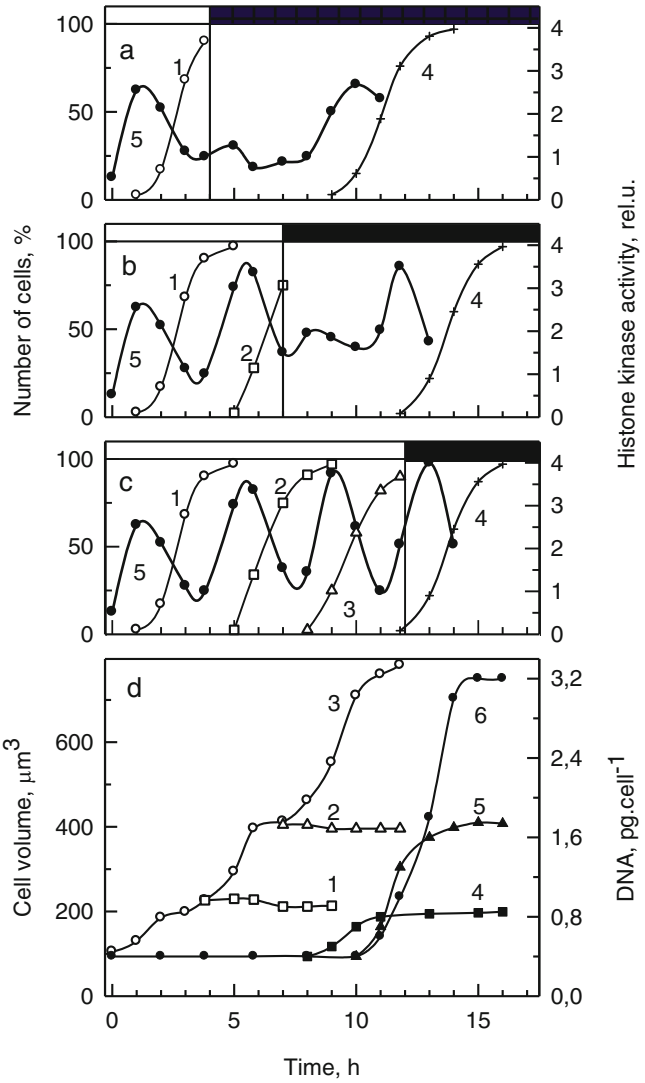

Fig. 23 Time course of commitment points to individual sequences of reproductive events, termination of these events, cell volume enlargement, and histone Hl kinase activity in synchronized populations of *Chlamydomonas reinhardtii*. The cultures were grown at a high irradiance and placed in the dark 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 Hl 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 *mat34* size mutation (Fang et al. 2006). *DP1* 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

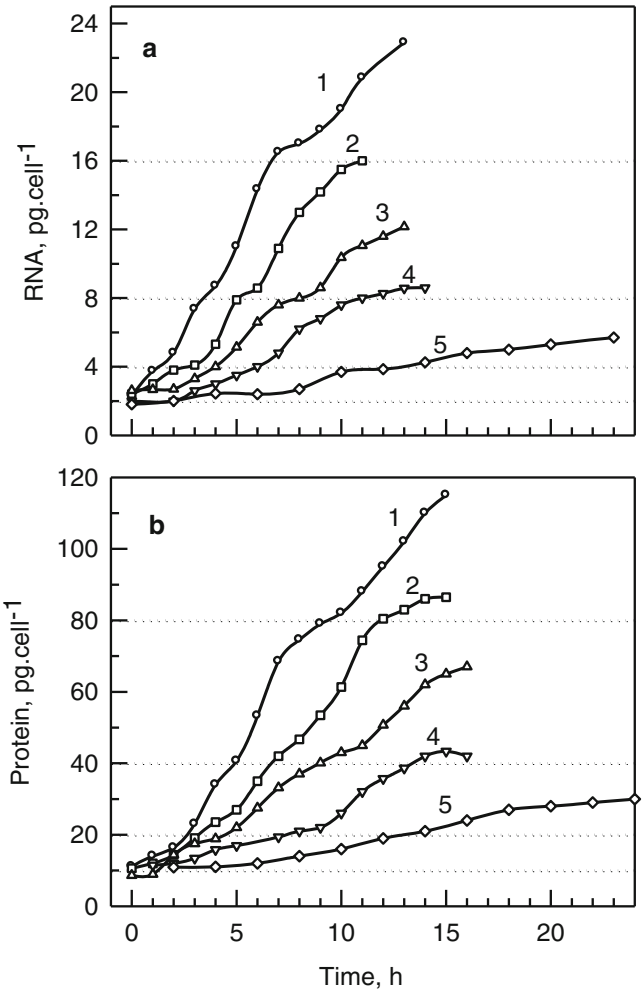

Fig. 24 Synchronous populations of *Scenedesmus armatus* grown at various mean irradiances showing (**a**) variation in RNA and (**b**) protein content per cell. Batch cultures grew for several cell cycles under alternating light (L) and dark (D) periods. In the analyzed cell cycles, the dark period 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)

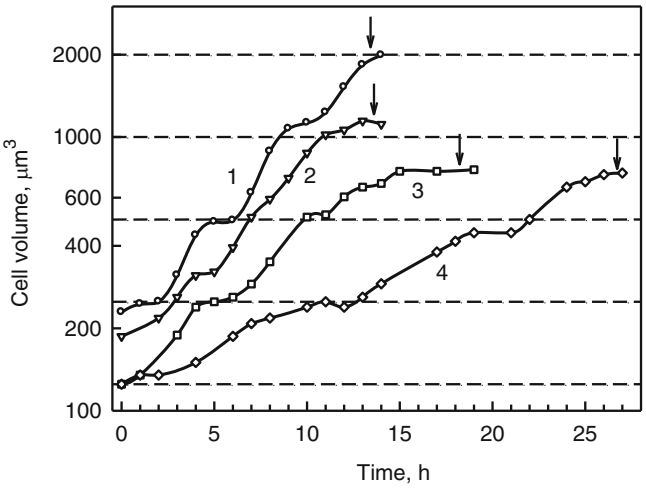

Fig. 25 Changes in mean cell volume during the cell cycle in synchronized populations of *Scenedesmus armatus* grown at different (mean) light irradiances. Curve *1*: 105 W m^{-2}; curve *2*: 85 W m^{-2}; curve *3*: 50 W m^{-2}; curve 4: 20 W m^{-2}, 15:10 h LD at 30 °C; *arrows* – the time when division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

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). 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

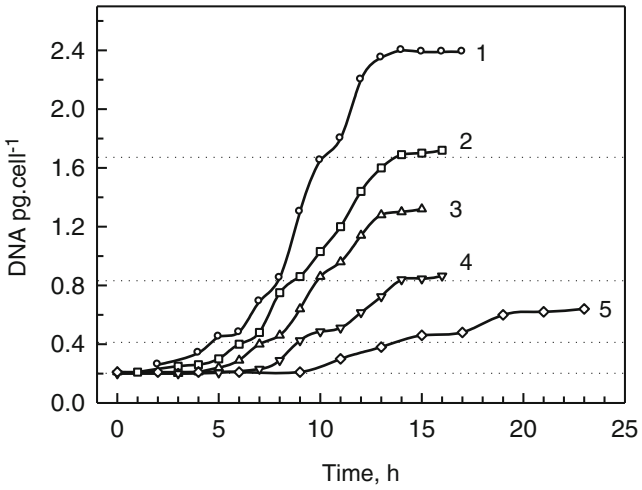

Fig. 26 Variations in DNA per cell in a synchronous population of *Scenedesmus armatus* grown at various mean irradiances. Batch cultures grew for several cell cycles under alternating light (L) and dark (D) period. In analyzed cell cycle the dark period has been omitted. Curve *1*: 100 W m^{-2}, 15:10 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)

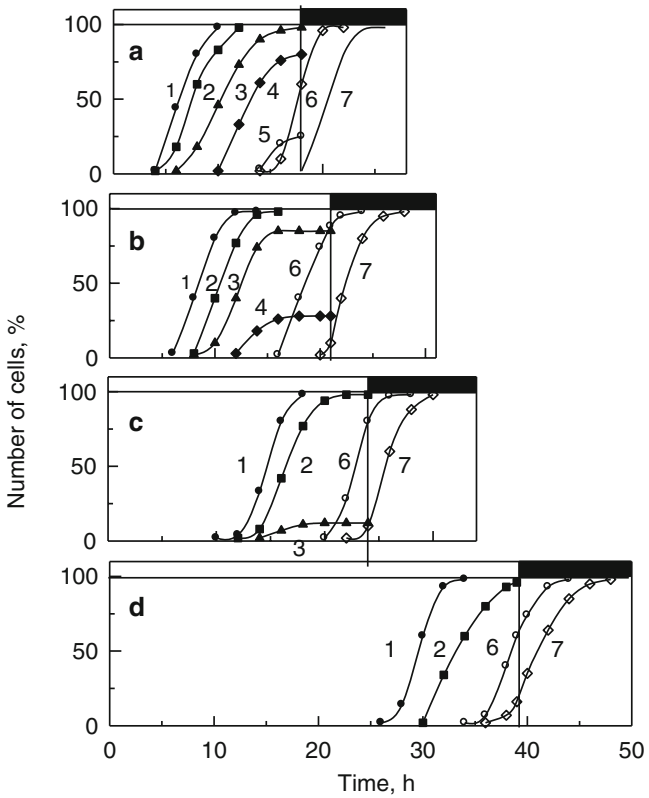

Fig. 27 Time courses of commitment points to nuclear and cellular divisions and termination of these processes in synchronous populations of *Chlamydomonas eugametos* grown at various light irradiances. (**a**) 70 W m^{-2}; (**b**) 35 W m^{-2}; (**c**) 15 W m^{-2}; (**d**) 7.5 W m^{-2}. Curves *1*, *2*, *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)

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.

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-

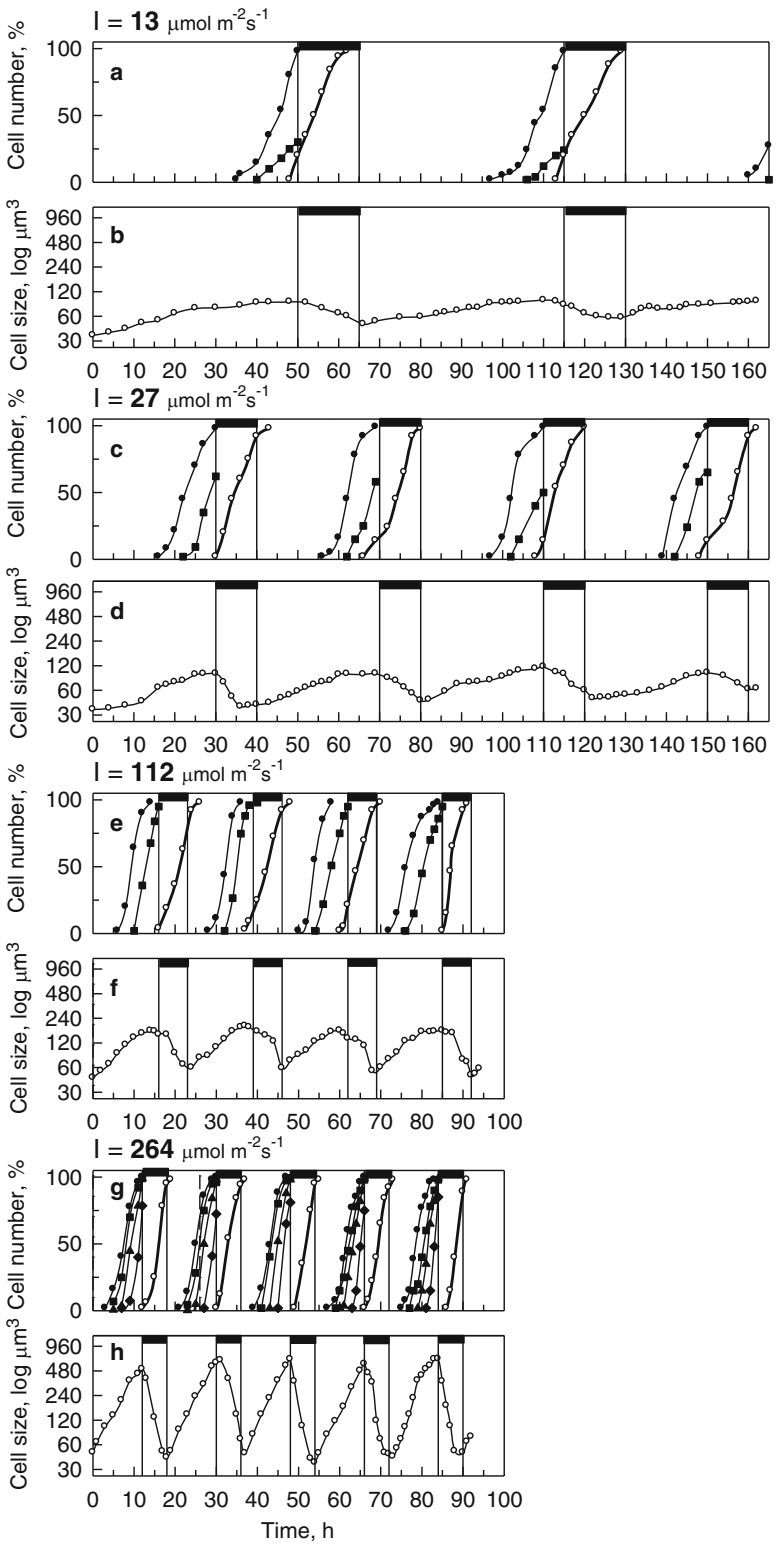

Fig. 28 Time courses of individual commitment points to cell division and daughter cell release (**a**, **c**, **e**, **g**) and changes in a mean cell volume (**b**, **d**, **f**, **h**) in synchronized populations of the alga *Chlamydomonas reinhardtii* grown at different mean light intensities (**I**) 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