

**Macromolecular Syntheses and the Course of Cell Cycle Events
in the Chlorococcal Alga *Scenedesmus quadricauda*
under Nutrient Starvation:
Effect of Sulphur Starvation**

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Abstract. Daughter cells of the chlorococcal alga *Scenedesmus quadricauda* were incubated under photosynthesizing conditions in a sulphur-free medium. The course of the cell cycle under these conditions was changed in daughter cells which differed in their stage of development. In absence of sulphur, advanced daughter cells with two nuclei and 2 or 4 genomes passed a cycle identical with that of control in sulphur containing medium. Each cell yielded eight binuclear daughter cells. With less advanced daughter cells (one nucleus and 1 or 2 genomes) restriction of RNA synthesis occurred near to the end of the cell cycle and protein synthesis ceased two hours later (practically at the time of the protoplast fission). The last round of DNA replication found in the control culture was not initiated in sulphur-starved culture and uninuclear daughter cells with one genome were released.

If the daughter cells coming from the starved populations were kept further in the sulphur-free medium, macromolecular syntheses were dramatically restricted. Only photosynthesis continued to produce starch at a similar rate as in normally grown cells. Thus, a very large amount of starch accumulated. Supported by these reserves, starved cells refeed with sulphur passed an entire cell cycle in the dark and divided into eight daughter cells. In sulphur-supplied cells, both in the dark and in light, RNA, protein and DNA synthesis started without any delay in a similar way as in the control culture. Competition for sulphur reserves occurred between the growth and division processes; the former were preferred in the light and the latter in the dark.

As early as 1931 RAPKINE published his observation on what is nowadays known as the "thiol cycle" (RAPKINE 1931). He reported that the concentration of free acid-soluble SH-groups fluctuated in sea urchin eggs and reached a maximum at cell cleavage. At the present time, the oxidoreduction state of SH-groups is taken as essential for the proper functioning of various proteins (for review see PAPADOPULOS-ELEOPULOS 1982), *e. g.* the sulphur-rich tubulins, which play a decisive role in the nuclear and cellular divisions (INOUE 1981).

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A specific role for sulphur in cell reproduction processes has been proposed by HASE *et al.* (1958, 1959, 1960 a, b, c, TAMIYA 1964): they assumed that specific sulphur-containing substances control cellular division in *Chlorella*. Although several research projects became stimulated by this idea (*e.g.* KYLIN 1964, GORYUNOVA *et al.* 1970 a, b), the substances partially characterized by the above authors remained elusive. Nevertheless, HASE *et al.* (1958) emphasized that growth and reproduction processes may compete for limiting supply of sulphur.

Any factor that would selectively arrest either nuclear or cellular division while keeping other processes in the cell cycle running would be of great use for the cell cycle control. Some of the above authors suggest that sulphur compounds might play this role. This was the main reason why we performed the present analysis.

MATERIALS AND METHODS

The strain of *Scenedesmus quadricauda* and the conditions of its culture, the methods of assessment of various cell characteristics and the terminology used were described or specified in BALLIN *et al.* (1988).

In sulphur-free nutrient solution the sulphates were replaced by equimolar concentrations of chlorides. To start the starvation the population of daughter cells was washed twice and resuspended in sulphur-free nutrient solution.

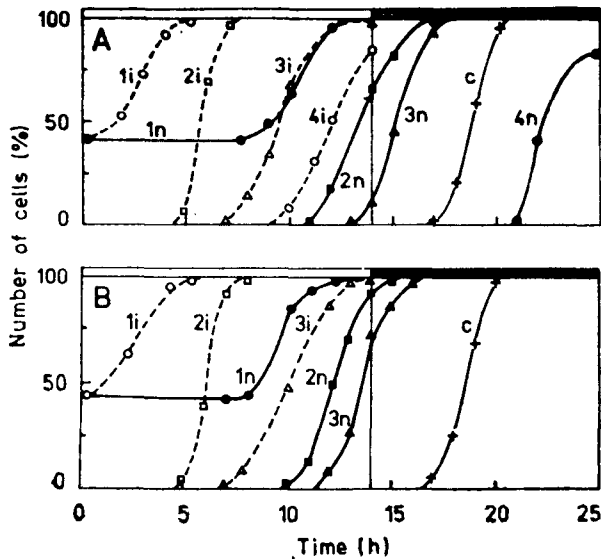


Fig. 1. Time courses of commitments to nuclear and cellular divisions and termination of these division processes during the first cell cycle in control (A) and sulphur-starved (B) synchronous populations of *Scenedesmus quadricauda*. Culture conditions: mean irradiation 70 Wm^{-2} (PhAR), dilution rate 0.1 h^{-1} , S-withdrawal at the beginning of the light period. 1i, 2i, 3i, 4i: fraction of the cells attaining commitment to the first, second, third, and fourth nuclear divisions. 1n, 2n, 3n, 4n: fraction of the cells in which the first, second, third, and fourth nuclear divisions were terminated. c: fraction of the cells which released daughter coenobia. Light and dark periods are indicated by white and black strips at the top of panels.

RESULTS

The population of daughter cells was divided into two parts. One was grown in a complete nutrient medium (control culture). The other was washed, transferred to a sulphur deficient medium (sulphur-starved culture) and maintained under similar conditions to the control culture for a period corresponding to two cell cycles.

The First Cell Cycle

Two experiments have been carried out with populations of daughter cells which differed in their stage of development.

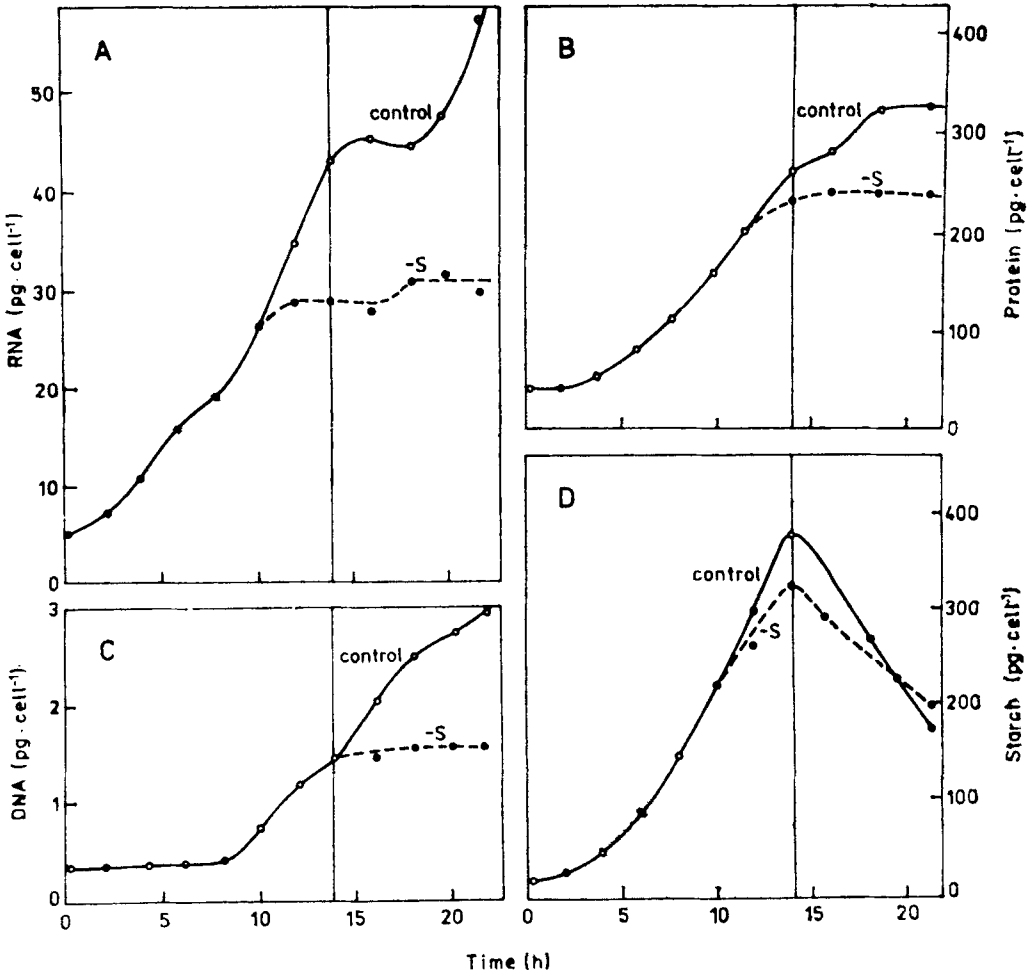


Fig. 2. Variation in RNA (A), protein (B), DNA (C) and starch (D) contents during the first cell cycle in control (open symbols) and sulphur-starved (closed symbols) synchronous populations of *Scenedesmus quadricauda*. Culture conditions: see Fig. 1. The beginning of the dark period in both cultures is indicated by a vertical line.

The first one started with a population in which about 40 % of daughter cells were binuclear (Fig. 1A). Lack of sulphur changed the pattern of macromolecular syntheses and reproductive events considerably late in the cell cycle, *i. e.*, around the tenth hour of the cycle (Figs. 1 and 2). At this time the commitment to divide into eight cells had been reached in all cells of the

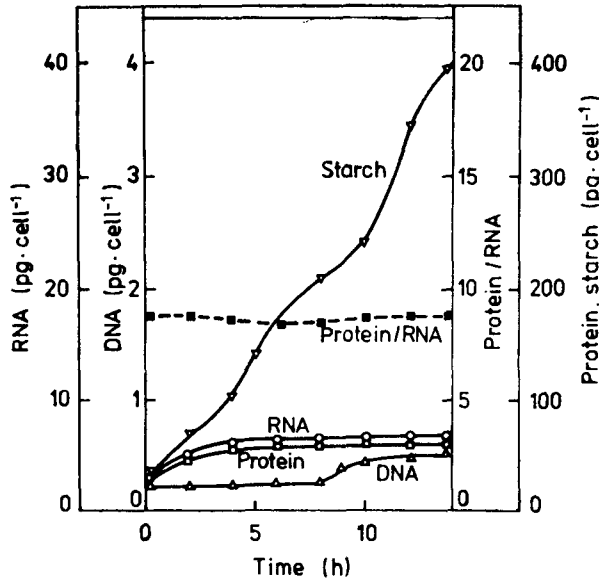


Fig. 3. Variation in RNA, protein, DNA, starch contents and in the protein to RNA ratio during the second cell cycle in a sulphur-starved synchronous population of *Scenedesmus quadricauda*. Culture conditions: see Fig. 1, only the values for the light period are presented.

population. However, the fourth commitment, which indicates that nuclei in the daughter cells will divide during the dark period, did not occur, so that all cells born from this cycle were uninuclear (Fig. 1B).

RNA synthesis was the first process arrested upon sulphur withdrawal. Two hours later, the curves for protein content per cell in the starved and control cultures started to diverge (Fig. 2). It is difficult to judge what effect the sulphur deficiency exactly had on the course of macromolecular syntheses over the next few hours. In the time when protoplast fissions occur, macromolecular syntheses were also restricted in control cycles. But the sulphur deficiency evidently affected the freshly released daughter cells while still in the dark. In such cells in the control culture, RNA, protein synthesis and, consequently, also DNA replication and nuclear division occurred (Fig. 2). All these processes were arrested in the absence of sulphur (Fig. 2).

In the second experiment with the progressed daughter cells, the course of macromolecular syntheses and the reproductive events in sulphur depleted cells were the same as in the control culture (data not shown). The first effects of the sulphur deficiency occurred at the beginning of the next cell cycle in a similar way as in the cells born from the less progressed population (see next section).

The Second Cell Cycle

When daughter cells coming from the starved population illustrated in Figs. 1 and 2 were illuminated to start the next cycle (Fig. 4), the macromolecular syntheses were dramatically restricted, although not entirely

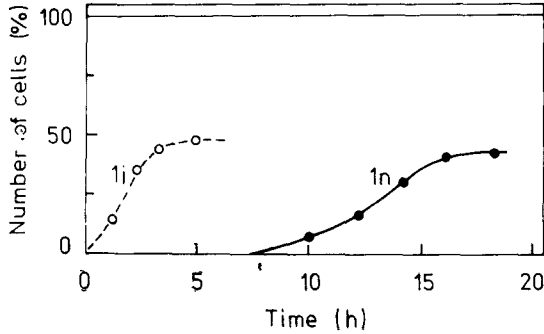


Fig. 4. Time courses of commitment to nuclear division and its termination during the second cell cycle in a sulphur-starved synchronous population of *Scenedesmus quadricauda*. For the culture conditions and labelling of curves see Fig. 1.

arrested. The daughter cells doubled their size and protein content (Fig. 3). Approximately 40% of the cells in the population divided the nuclei (Fig. 4). During the whole second light period, photosynthesis continued and starch

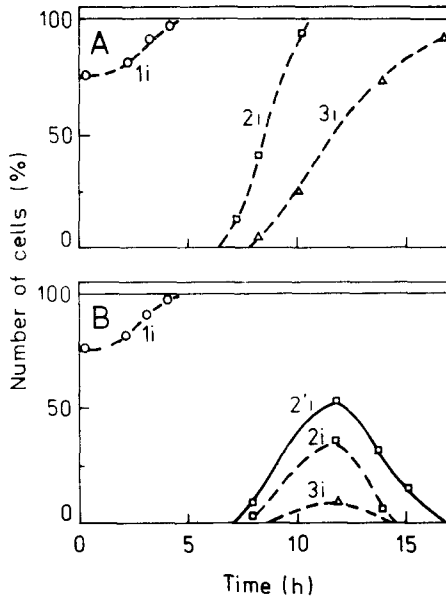


Fig. 5. Variation in the capacity of cells to divide if deprived of light in the presence (A) and absence (B) of exogenous sulphur during the second cell cycle in sulphur-starved populations of *Scenedesmus quadricauda*. Culture conditions: see Fig. 1, only the values for the light period are presented, 1i, 2'i — fraction of cells which attained the commitment to the first and the second nuclear division, respectively; 2i, 3i — fraction of cells which attained the commitment to divide into 4 and 8 daughter cells, respectively.

was accumulated to the same final amount as in the cells grown in a complete medium (compare Figs. 2D and 3). Since starch synthesis was accompanied by only very poor growth of cells, the ratio of starch to protein content (or dry matter) was much higher than in the control culture.

In the daughter cells originated from the second population (started from the more developed cells — see the preceding section) the protein content was slightly higher. Accordingly, the commitment to divide into four cells appeared in a noticeable fraction of the population (Fig. 5B, curve 2i) and the commitment to eight cells in a small but significant percentage of the cells (Fig. 5B, curve 3i). The corresponding division processes were accomplished only in the darkened samples (used for estimating the commitment) but no division

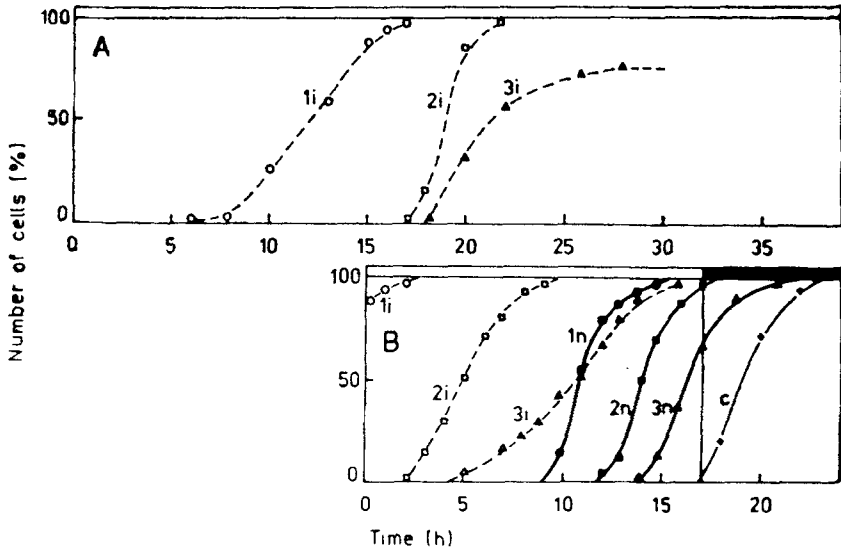


Fig. 6. Time of commitment to nuclear and cellular division and termination of division processes in sulphur-refed populations of *Scenedesmus quadricauda*. A — 1i, 2i, 3i — fraction of cells which attained the commitment to the first, second and third nuclear divisions, respectively, if deprived of light and re-fed by sulphur. B — culture was transferred to the complete medium at the end of the second light period for control culture. Light and dark periods are indicated by the white and black strips at the top of panels. Culture conditions and labelling of the curves: see Fig. 1.

whatever occurred in the population exposed further to light. Thus, the commitments assessed in the dark samples attained a certain maximum and then decreased again, a phenomenon unique in our experience. Another unusual feature was that even in the dark a certain proportion of the cells committed was unable to complete the cellular division after their nuclei had divided, and they were tetranuclear after a period sufficiently long for cell division to take place (Fig. 5B, curve 2'i).

Recovery From Sulphur Starvation

In populations described in the preceding section the only reproductive process occurring during the second cell cycle was the division of a certain fraction of nuclei. The starch reserves in the cells remained high. Subcultures

derived from the sulphur starved culture in intervals of two hours, were supplied with sulphur in the dark. The starch reserves present in the cells at the time of light deprivation then determined the extent of growth and reproductive processes performed during the dark period in a complete medium (Fig. 6A). The course of nuclear divisions and their commitments during the dark period in one subculture is illustrated in Fig. 6B. This subculture was supplied with sulphur and deprived of light at the end of the second light period, when the starch reserves were so great that the same

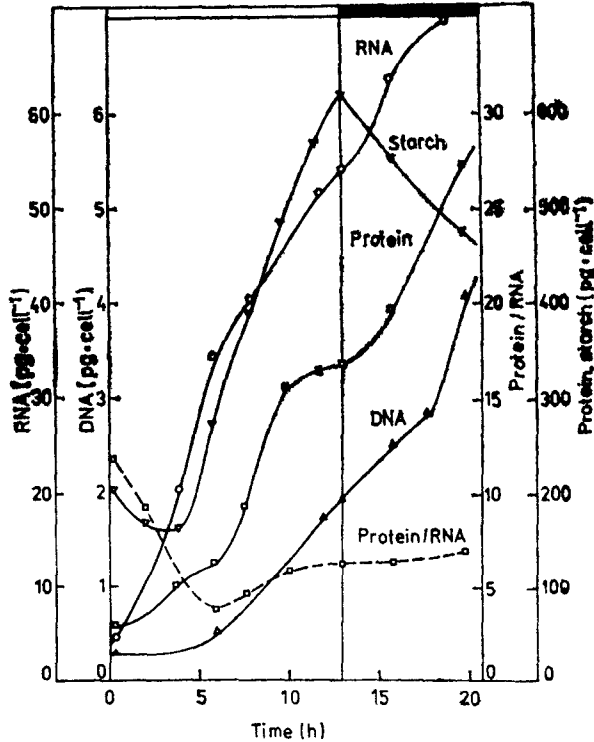


Fig. 7. Variation in RNA, protein, DNA, and starch contents and in the protein to RNA ratio during the cell cycle in a sulphur-refed synchronous population of *Scenedesmus quadricauda*. Sulphur was added at the end of the second dark period of the control culture. Culture conditions: see Fig. 1. Light and dark periods are indicated by white and black strips at the top of the panel

number of cell reproductive sequences occurred in the dark as in an illuminated control culture. The cells of this population divided therefore into eight daughter cells (Fig. 6B).

The course of macromolecular syntheses upon restoration of the sulphur supply (Fig. 7) corresponded entirely to that in a culture cycling on the complete medium. In contrast to the previous experiments with nitrogen and phosphorus starvation (BALLIN *et al.* 1988, ZACHLEDER *et al.* 1988), RNA synthesis started immediately at a high rate in a sulphur refed culture.

In another experiment, sulphur was added to the starved culture in light without the intervening dark period. Again, in the light (Fig. 8A) RNA synthesis started immediately at the rate comparable with that in a control

culture. In this case, a parallel refeeding experiment was performed with a subpopulation that was darkened simultaneously with sulphate addition (Fig. 8B). Even here the start of RNA recovery was rather steep. This experiment again shows how much progress can the cells make at the expense of starch reserves accumulated during the period when growth was suppressed.

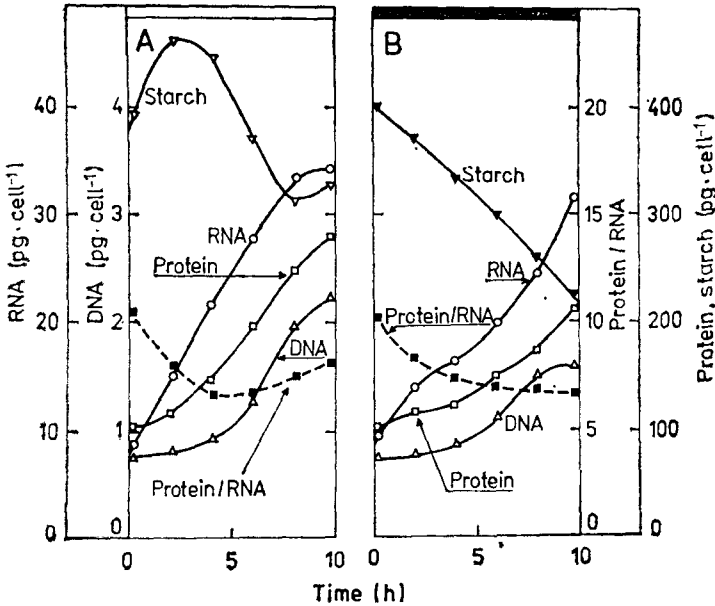


Fig. 8. Variation in RNA, protein, DNA, and starch contents and in the protein to RNA ratio in light- (A) and dark-growing (B) sulphur-refed synchronous populations of *Scenedesmus quadricauda*. Sulphur was added to the nutrient medium at the end of the second light period of the control culture. The duration of the control dark period (10 h) is shown in the Figure. Culture conditions: see Fig. 1. Light and dark periods are indicated by white and black strips at the top of panels.

DISCUSSION

Our results did not support the view of the authors who suggested that sulphur deficiency would primarily and specifically affect nuclear or cellular division in *Scenedesmus quadricauda*. Instead, the response to sulphur withdrawal was in principle very similar to that observed when nitrogen or phosphorus compounds were omitted from the nutrient solution.

Thus, under three nutrient limitations studied, the strategy adopted by the cells facing exhaustion of one of the essential mineral nutrients seems to be the same. The pivotal event in the cell's defense is the arrest of RNA synthesis. This occurs as soon as some, so far unknown, mechanism is able to detect the absence of the component in the outer environment. It happens, however, before the internal reserves of the component in question are exhausted. Upon arrest of RNA synthesis an appreciable amount of protein can still be synthesized. Its relative amount compared with the amount present in the cell at the moment when the supply of the nutrient was cut

off, differs for the various limitations, but it is usually large enough to allow nuclear and cellular divisions which correspond to the DNA copies present.

In this way daughter cells are formed which in the absence of the respective nutrient cannot develop further. In various types of cell cycles this so-called G1-arrest, the most important control point, occurs generally close to the end of the G1 phase and before DNA replication starts. The withdrawal of various nutrients arrests the development of many microorganisms close to this control point (HARTWELL 1974). As our results show, chlorococcal algae are no exception.

A most interesting question is how the different primary signals announcing a lack of light or mineral nutrients produce a single response — the arrest of RNA synthesis. Unfortunately, our results do not provide any satisfactory explanation.

Although the general response to sulphur deficiency of *Scenedesmus* cells is to bring about a G1 arrest, under specific conditions we also found the occurrence of the G2 arrest. The division processes under sulphur deficiency were triggered and terminated probably only if the cells were deprived of light, but not in continuous light. This can be interpreted in line with HASE *et al.* (1959) who assumed a competition between the growth and division processes for limiting nutrient in *Chlorella* cells. Our results indicated that under sulphur starvation the growth processes were preferred over division in the light and the reverse was true in the dark.

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