

## Coordination Between Thylakoid Differentiation and Cell Division of *Cyanidium caldarium*

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### Abstract

*Cyanidium caldarium* was transferred from solid heterotrophic agar culture to autotrophic fluid culture medium. During 17 days under permanent light the cell number of this algal culture increased from  $3 \times 10^5$  cells/ml to  $6 \times 10^8$  cells/ml. Two periods of different cell division rates can be distinguished: a first one with high rates of cell division between the 1st and the 5th day after the transfer and a second one with lowered rates of cell division between the 5th and the 17th day after transfer. This change in cell division rates is accompanied by a drastic decrease of the chlorophyll content of *Cyanidium caldarium* from  $3 \times 10^{-9}$  mg/cell to about  $10^{-10}$  mg/cell. Sometimes the whole culture becomes yellow for at least four or five days, but regreening occurs subsequently. This temporary bleaching of *Cyanidium caldarium* is not caused by a lack of nutritional components or by enrichment of carbohydrates in the cytoplasm. The protein spectrum of the thylakoids from *Cyanidium caldarium* was modified during the bleaching period. The chlorophyll-protein complex of photosystem I (CPI) was no longer detectable, whereas the amount of the proteins of 79, 70, 64 and 51 kDa was increased. These results were compared with the effect of heterotrophic nutrition without or with light. The disharmony between cell division and thylakoid differentiation is discussed in relation to the evolutionary stage of *Cyanidium caldarium*.

Key words: *Cyanidium caldarium*, endocyanelles, thylakoid differentiation, cell division, symbiosis

### 1. Introduction

The systematic affiliation of the unicellular bluish-greenish organism, *Cyanidium caldarium* is the subject of controversy. Its biochemical and ultrastructural organization shows the following features: the mode of cell division into endospores (Allen, 1959; Brock, 1978) resembles that of one of the chlorophyceae, *Chlorella*; the pigment composition (Allen, 1959; Chapman et al., 1968; Brock, 1978) corresponds with cyanobacteria and with some rhodophytes; the cellular ultrastructure

(Chapman, 1974; Mercer et al., 1962; Seckbach, 1972; Edwards and Mainwaring, 1973) corresponds with well-recognized red algae such as *Porphyridium cruentum* (Gantt and Conti, 1966); the fatty acid composition (Ikan and Seckbach, 1972; Holton et al., 1968) corresponds with cyanobacteria. Based on studies on sterol content (Seckbach and Ikan, 1972; Ikar and Seckbach, 1972), storage glucans (Seckbach and Fredrick, 1981), amino acid sequencing of ferredoxin (Andrews et al., 1981) and primary photosynthetic products (Nagashima and Fukuda, 1981) *Cyanidium caldarium* was regarded as an intermediate between cyanobacteria and rhodophyta. Fredrick (1976) and Kremer et al. (1978), however, have classified *Cyanidium caldarium* as a symbiosis based on a cyanobacterium and an apochlorotic eukaryote.

All these systematic affiliations of *Cyanidium caldarium* have in common an evolutionary aspect of the coordination between the prokaryotic photosynthetically active compartments and their eukaryotic environmental cells. In this publication a disharmonious physiological interplay between both partners is shown which will give evidence for only a weak regulation of the prokaryotic entity by the eukaryotic cell. These prokaryotic pigmented entities with photosynthetic activities in *Cyanidium caldarium* are called 'chloroplasts' in this paper without any classification.

## 2. Materials and Methods

*Cyanidium caldarium*, strain 108.79 (Sammlung von Algenkulturen, Pflanzenphysiologisches Institut, Universität Göttingen) was cultivated autotrophically (Allen 1959) at 42°C and a light intensity of 6.000 Lux. The culture was supported with a mixture of air and CO<sub>2</sub> (5%).

The amount of chlorophyll *a* was determined by the method of McKinney (1941) and the amount of carbohydrates by the method of Roe (1955). For preparation of thylakoids, cells were harvested and broken in a Bühler Zelmühle. Fragments of thylakoids were isolated on a Ficoll/saccharose/glycerol gradient (Brandt, 1980) and then resuspended in a medium comprising 0.33 M sorbitol/1 mM MgCl<sub>2</sub>/2 mM EDTA/4 mM mercaptoethanol/50 mM Tricine-KOH, pH 8.4 (Brandt, 1980). The fractionation of the thylakoids by treatment with deoxycholate was done by the method of Brandt et al. (1982). The proteins were separated without heat denaturation in 15% acrylamide gels (Brandt, 1980). Absorption spectra in the spectral region between 400 and 750 nm (Shimadzu spectrophotometer UV-200, Shimadzu Seisakusko Ltd., Kyoto, Japan) of the chlorophyll-protein complexes were recorded directly in slab-gel segments without elution.

## 3. Results

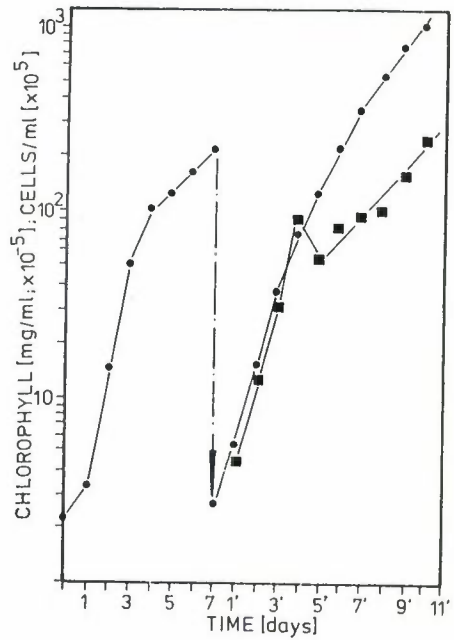
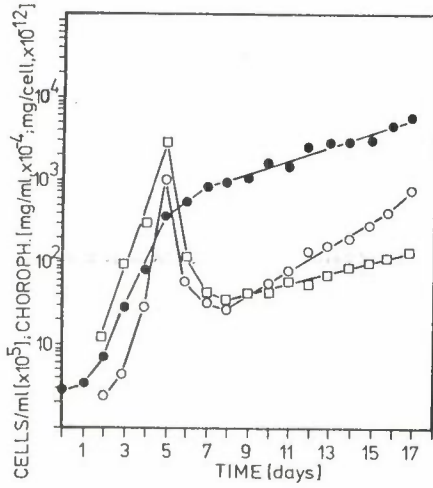
After transfer from slow growing agar cultures of *Cyanidium caldarium* to fast growth conditions in autotrophic liquid medium the cell number increases rapidly with a doubling time of 12 hours during the first five days (Fig. 1). Afterwards this doubling time is diminished by about 80 per cent. A decrease of the chloro-

phyll content of the chloroplasts precedes this change of the doubling time of the *Cyanidium* cells for one day at the 4th day. The cell number/ml as well as the chlorophyll content of the cells increase slowly during the following 20 days. The change of growth behaviour at about the 5th day cannot be prevented by dilution of the culture. Only the decrease of the chlorophyll content is somewhat diminished (Fig. 2). It is therefore evident that this phenomenon is not caused by the lack of some growth factors of the liquid medium after the first five days.

Another explanation for the 'bleaching-period' of the *Cyanidium* culture can be the temporary change of the 'environmental physiological conditions' of the chloroplasts in their cells from autotrophic to photoheterotrophic conditions caused by their own photosynthetic activity and CO<sub>2</sub>-fixation during the first five days. Addition of glucose, however, at the 4th day after transfer to the liquid medium does not intensify the 'bleaching-period' but prevents the decrease of the pigment content for further two days (Fig. 3).

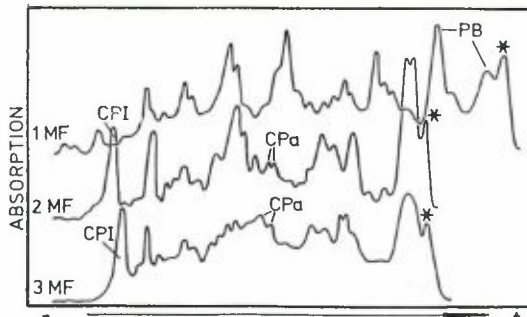
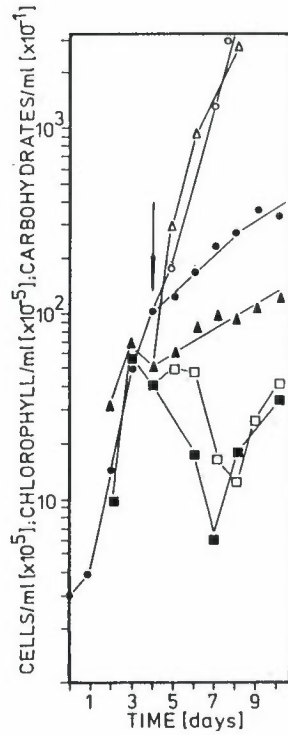
All the chlorophyll and the phycobilines are located within the chloroplasts and are organized as pigment-protein complexes with specific proteins (Fig. 4). After treatment of isolated thylakoids with two different concentrations of deoxycholate (Brandt et al., 1982) the chlorophyll-protein complex of the photosystem I CPI and two chlorophyll-protein complexes CPa<sub>1</sub> and CPa<sub>2</sub>, both related to photosystem II were identified in the second and third membrane-fraction. Therefore they are intrinsic (Guidotti, 1980) such as known also from *Euglena gracilis* (Brandt, 1980), *Chlamydomonas stellata* (Brandt et al., 1982), *Cyanophora paradoxa* (Marten et al., 1983) and other organisms (Thorner et al. 1979). Two phycobilines are present in the first membrane fraction. The maxima of the absorption spectra of CPI, CPa<sub>1</sub> and CPa<sub>2</sub> are different in the 670 nm and 435 nm regions (Fig. 5) which demonstrates the association of chlorophyll *a* with one of three different apo-proteins in each case.

The protein spectrum of the combined second and third membrane fractions of *Cyanidium* thylakoids is markedly changed during the 'bleaching-period' (Fig. 6). At the 7th day after transfer to liquid medium the CPI of the 120 kDa-, the 105 kDa-, the 58 kDa-, the 19kDa- and the 18 kDa-protein are no longer detectable, whereas the amount of proteins with molecular weights of 70,000, 64,000 and 51,000 increase. In contrast the modification of the protein spectrum by heterotrophic nutrition with or without light is not comparable with these alternations under autotrophic conditions during the 'bleaching-period' (Fig. 7). In particular the 105 kDa- and the 51 kDa-protein decrease. Consequently the 'bleaching-period' of the *Cyanidium* culture demonstrates a temporary disharmony between chloroplasts and eukaryotic environment and does not reflect a temporary heterotrophic situation in the cytoplasm for the chloroplasts.



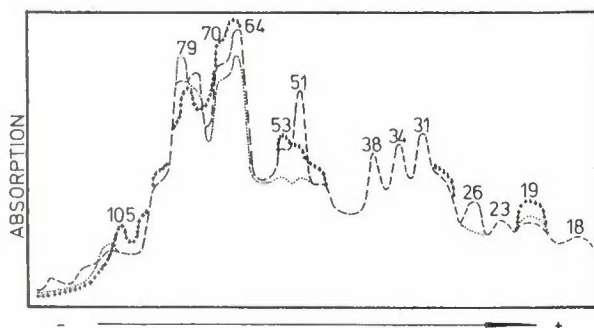
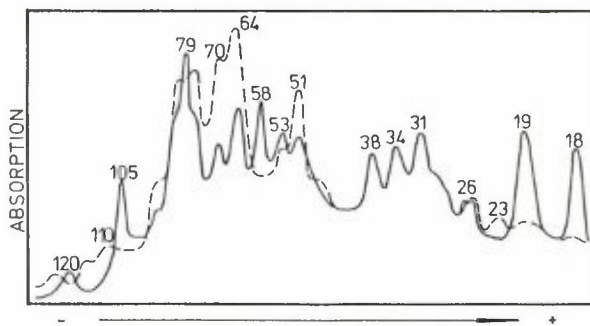
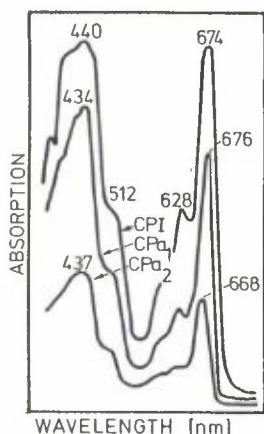
**Figure 1.** Growth of *Cyanidium caldarium* after transfer from an agar culture into an autotrophic liquid medium. —●—●— cells/ml, —○—○— chlorophyll/ml, —□—□—□— chlorophyll/cell.

**Figure 2.** Growth of *Cyanidium caldarium* after transfer from an agar culture into an autotrophic liquid medium and dilution (---→) at the 7th day. —●—●— cells/ml, —■—■—■— chlorophyll/ml.



**Figure 3.** Growth of *Cyanidium caldarium* after transfer from an agar culture into an autotrophic liquid medium and addition of glucose (final concentration 1%) at the 4th day. —○— cells/ml under autotrophic conditions, —●— cells/ml under heterotrophic conditions, —■— chlorophyll/ml under autotrophic conditions, —□— chlorophyll/ml under heterotrophic conditions, —▲— carbohydrates/ml under autotrophic conditions, —△— carbohydrates/ml under heterotrophic conditions.

**Figure 4.** Protein patterns of the three membrane fractions of thylakoid proteins (1MF-3MF) of chloroplasts from *Cyanidium caldarium*.



**Figure 5.** Absorption spectra of the chlorophyll-protein complexes CPI, CPa<sub>1</sub> and CPa<sub>2</sub> separated by polyacrylamide gel electrophoresis of thylakoids of *Cyanidium caldarium*.

**Figure 6.** Protein pattern of the combined 2nd and 3rd membrane fractions (see methods) of thylakoids from *Cyanidium caldarium* at the 3rd day (—) and the 7th day (---) after transfer from an agar culture into an autotrophic liquid medium.

**Figure 7.** Protein pattern of the combined 2nd and 3rd membrane fractions (see methods) of thylakoids from *Cyanidium caldarium* at the 7th day (---) after the transfer from an agar culture into an autotrophic liquid medium, at the 7th day in the light after addition of glucose at the 4th day (·····) and at the 7th day after addition of glucose at the 4th day and darkness between the 4th and 7th day (+++++).

#### 4. Discussion

Bleaching phenomena occur in several unicellular organisms such as *Chlorella protothecoides* and *Euglena gracilis* after heterotrophic nutrition (Aoki and Hase, 1967; Oh-hama and Hase, 1976; Koll et al., 1980; Schuler et al., 1981) or by cultivation at higher temperature (Pringsheim and Pringsheim, 1952; Brandt, 1976). The mechanism of these two methods for bleaching have been already analyzed in detail (Monroy and Schwartzbach, 1984; Brandt and Wiessner, 1977). They both have in common the inhibition of the transcription or the translation of the chloroplast genome. The temporary bleaching of *Cyanidium caldarium*, however, is based on another kind of mechanism. After transfer into liquid medium the growth of *Cyanidium* cultures shows a fast logarithmic growth phase for 4-5 days and afterwards a slow logarithmic growth phase. The change from the first to the second phase of logarithmic growth is initiated by a decrease in chlorophyll content one day earlier. This is similar to the growth behaviour of *Cyanophora paradoxa* (Marten et al. 1981). In *Cyanidium caldarium*, however, the decrease of the chlorophyll content is also the beginning of the reversible 'bleaching-period' of the chloroplast. It demonstrates a drastic regularity defect between chloroplasts and nucleocytoplasm in *Cyanidium caldarium*, which may be attributed to the regulation of both translation systems. A hint to the weak regulatory connection of the two partners is also the fact that *Cyanidium caldarium* cannot be synchronized by the normally used light-dark-changes (data not shown). In comparison cultures of *Cyanophora paradoxa* show no temporary 'bleaching-period' after the change of logarithmic growth behaviour (Marten et al., 1981) and can be synchronized by suitable light-dark-cycles (Marten et al., 1983). Therefore we conclude that *Cyanidium caldarium* represents an earlier evolutionary stage of the chloroplast organization within a eukaryotic cell.

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