Acta Bot. Neerl. 26(2), April 1977, p.177–182.

NUCLEAR DIVISIONS AND CYTOKINESIS DURING UNICELL AND COENOBIA FORMATION IN SYNCHRONIZED CULTURES OF SCENEDESMUS QUADRICAUDA (TURP.) BREB. (CHLOROPHYCEAE)

C. L. M. STEENBERGEN and M. J. B. BÄR-GILISSEN

Limnologisch Instituut 'Vijverhof', Nieuwersluis

SUMMARY

Mitotic activity, cytokinesis and arrangement of daughter cells within the parental wall are studied in synchronized cultures of *Scenedesmus quadricauda*, which yield either unicells or coenobia. Irrespective of the formation of either unicells or coenobia, no essential differences occur in the nuclear patterns and the positions of the cleavage planes. Only the stages of daughter cell arrangement differ significantly in these two cycles. Daughter unicells are arranged within the parental wall more or less radially, whereas daughter coenobia become arranged with their axis parallel to the axis of the mother cell.

1. INTRODUCTION

Nuclear patterns and formation of coenobia in *S. quadricauda* have been described by SMITH (1914). SETLIK et al. (1972) have given an accurate account of the time course of nuclear divisions and protoplast fissions. Full details of stages in nuclear divisions, observed by light microscopy, have been presented by SULEK (1975). He points out that nuclear divisions in *S. quadricauda* have a mitotic character, showing phases that are typical of the mitosis of eukaryotic organisms. Analysis of mitosis and cytokinesis at the electron microscopic level have been published by NILSHAMMAR & WALLES (1974) and PICKETT-HEAPS & STAEHELIN (1975).

These observations have all been made on *Scenedesmus* cells producing coenobia only. However, the occurrence of unicellular stages in spiny *Scenedesmus* strains has been clearly established (e.g. TRAINOR 1971) and pictures of stages of the cleavages in unicell-yielding cells are given by SWALE (1967) and STEENBERGEN (1975). They observed that unicells can arise from coenobia and vice versa.

In the present study are compared the mitotic activity and the cytokinesis in two synchronized culture systems, which both have a daily production of predominantly eight daughter cells per mother cell. In one system the daughter cells are arranged in 8-celled coenobia and in the other system they are found as separate cells.

2. METHODS

Synchronized cultures of the pleomorphic *S. quadricauda* strain (STEENBERGEN 1976) are grown in 1000 ml glass vessels at a temperature of 30 °C in our S.22 nutrient medium (STEENBERGEN 1974) and continuously stirred and aerated with 400 ml air per minute enriched with 3.0 vol. % CO_2. In a synchronized culture all cells pass through the same stages of the life cycle more or less simultaneously. Synchronization is achieved by exposing the cultures to light-dark changes. Growth of the cells is restricted to the photoperiod, whereas division processes occur in the dark period.

Upon inoculation with a stock culture adapted to a temperature of 30 °C and exposed to light-dark changes consisting of 14 hours of light and 10 hours of darkness (LD:14,10) a synchronized coenobia-yielding culture system is established. Unicells arise in LD:12,12 cycles after inoculation with a stock culture adapted to a temperature of 15 °C or 20 °C (see STEENBERGEN 1975). A light intensity of 0.5×10^5 erg $cm^{-2}sec^{-1}$ from fluorescent tubes (Philips TL 33) is used. The cultures are diluted daily at the beginning of the light period to a standard cell number of 0.4×10^6 cells ml^{-1} with fresh nutrient medium. Details on culture methods are presented elsewhere (Steenbergen, in press).

Fixation and staining of the nuclei is carried out on a microscope slide using the HCl-Giemsa method as described by NORRIS & SWAIN (1971) (cf. SULEK 1975). As fixative either Lugol's solution (made up of 10 g of I_2, 20 g of KI, 200 ml H_2O and 20 g of glacial acetic acid) or Carnoy's fluid (consisting of ethanol:chloroform:glacial acetic acid 6:3:1 v/v) is used. The cells are hydrolyzed with 1 N HCl at a temperature of 60 °C for 8 minutes. After rinsing in phosphate buffer (pH 7.0), the cells are stained with Gurr's Improved Giemsa Stain R. 66 for 60 minutes at room temperature. The specimens are mounted in Euparal.

3. RESULTS AND DISCUSSION

To study the progress of nuclear divisions in the synchronized cultures the number of nuclei per cell is determined at half hourly intervals in stained preparations. The progress of cytokinesis is studied at half hourly intervals in live material using phase contrast and Nomarski optics. The numbers of the respective stages are converted into percentages of the total cell population (see *fig. 1A, B*). Cell division stages are clearly visible only when the respective cleavage is completed. In this way the end of the first, second and third cleavage respectively, is recorded. After cytokinesis in the coenobia-yielding cycle the daughter cells elongate and become arranged with their axis parallel to the axis of the mother cell, whereas in the unicell-yielding cycle the cells become ovoid and are arranged more or less radially within the parental wall (for details see STEENBERGEN 1975). The parallel and the radial arrangement of the daughter cells are the final stages before the liberation of daughter coenobia and daughter unicells respectively. These final stages are recorded also.

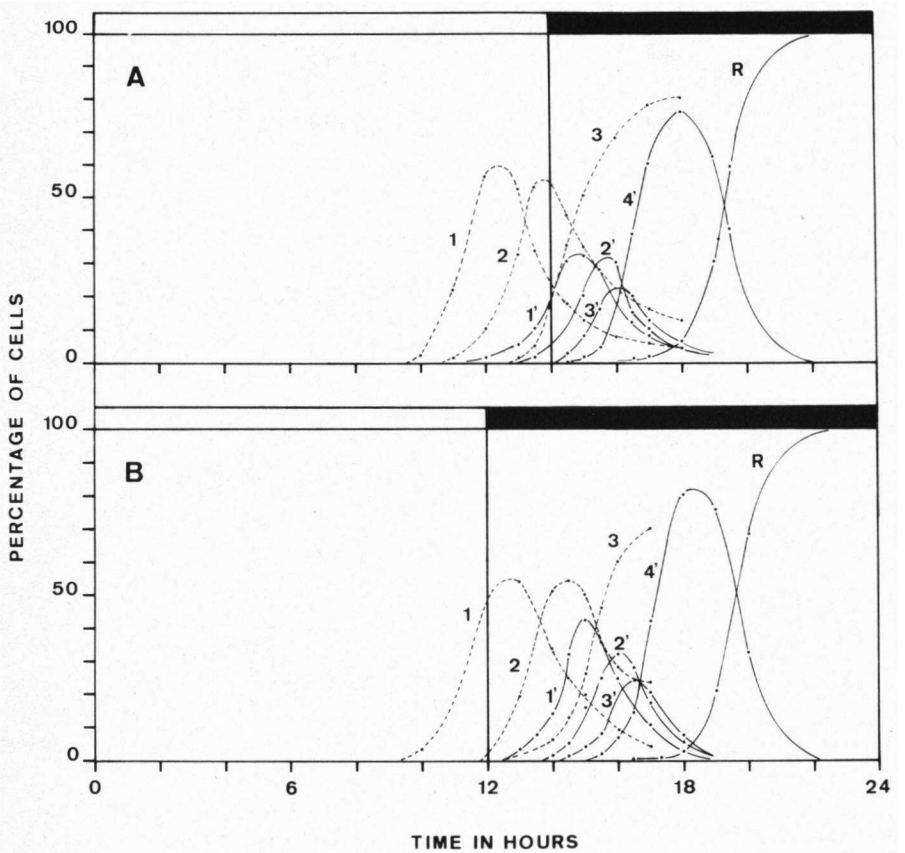

Fig. 1A. Time dependent mitotic activity and cytokinesis in a synchronized culture of *S. quadricauda* yielding a population consisting of 4% 2-celled coenobia, 14% 4-celled coenobia and 81% 8-celled coenobia. Curves 1, 2 and 3 give the percentage of cells in the population which have respectively 2, 4 and 8 nuclei. The curves 1', 2' and 3' indicate respectively the first, second and third cleavage. Curve 4' marks the final arrangement of the daughter cells and curve R indicates the course of the release of the daughter cells.
Fig. 1B. Time dependent mitotic activity and cytokinesis in a synchronized culture of *S. quadricauda* yielding a population consisting of 2% coenobia and 98% unicells (for symbols see Fig. 1A).

Furthermore the liberation of these coenobia and unicells in the cultures is registered.

A careful comparison of the time course of mitotic activity, protoplast fissions, daughter cell arrangements and release of daughter cells in both cycles does not reveal essential differences (see *fig. 1A, B*). The first cleavage is completed when 4 nuclei are present. This finding is in accordance with the observations of SMITH (1914) and NILSHAMMAR & WALLES (1974), but does not agree with the results of SETLIK et al. (1972). The latter authors observed

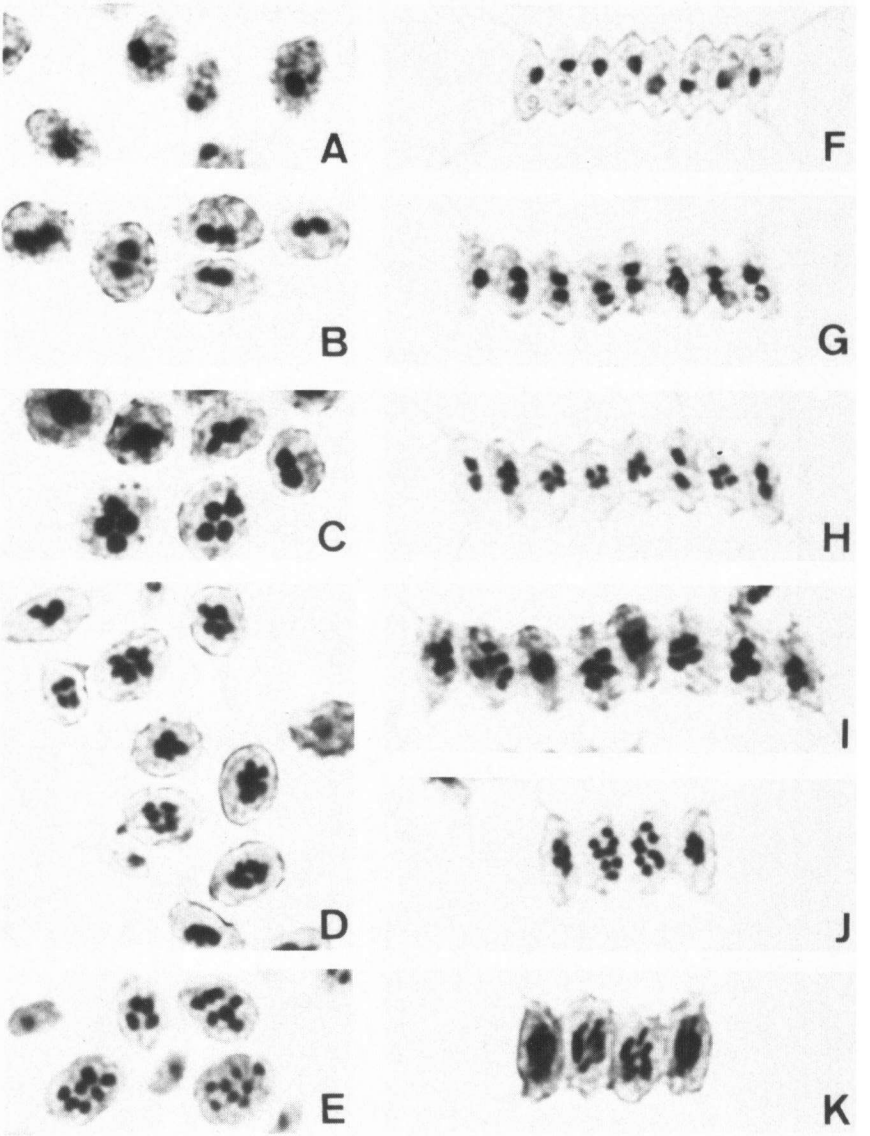

Fig. 2. Nuclear patterns at various stages of the unicell-yielding cycle (A-E) and the coenobia-yielding cycle (F-K) of *S. quadricauda*. A, F, mature cells just before the first nuclear division; B, G, cells containing 2 nuclei; C, H, cells containing 2 and 4 nuclei; D, I, J, stages of the third nuclear division; E, arrangement of ovoid daughter cells and some released unicells; K, cell elongation and arrangement of coenobial cells. (Cells stained by the HCl-Giemsa method, magnification 1280 ×).

that the protoplast fission starts in cells, which contain 8 nuclei.

The hypothesis that unicells arise because division processes occur so fast that there is no time for the daughter cells to become attached to one another (see SWALE 1967) is not supported by our results.

Finally nuclear patterns and the positions of the cleavage planes are compared (see *fig. 2*).

For both cycles, judged by the position of the nuclei, the first mitotic spindle is formed parallel to the long axis of the mother cell (*fig. 2B, G*). The second mitosis occurs perpendicular to the spindle of the first one. The two spindles of the second mitosis run parallel (*fig. 2C, H*) (cf. SULEK 1975). Usually the spindles of the third mitosis are at an angle of less than 90 degrees to the plane in which the spindles of the second mitosis lie. The spindles of the third mitosis do not run parallel (*fig. 2D, I, J*). It has been observed that the division of the nuclei takes place simultaneously. The above observations are in agreement with those of SMITH (1914), NILSHAMMAR & WALLES (1974) and SULEK (1975).

In both unicell- and coenobia-yielding mother cells the first cleavage is in transversal direction and the second one is longitudinal (STEENBERGEN 1975). The positions of the third cleavage planes vary in cells of both cycles. This fact may be due to variability in the positions of the spindles of the third mitosis. In both cycles we observed that in many mother cells the third cleavage planes are formed more or less diagonally. They do not appear simultaneously in the four daughter cells (cf. SMITH 1914). The stages of daughter cell arrangement differ significantly in both cycles (STEENBERGEN 1975) (cf. *fig. 2E, K*). The typical elongation of the daughter cells occurring when coenobia are formed seems to be inhibited in the formation of unicells. Evidence was obtained that the induction of the formation of unicells is restricted to a certain period in the cellular life cycle before the occurrence of the nuclear divisions (cf. STEENBERGEN 1975). Our conclusion is that the induction by itself has no bearing both upon the time course of division processes and on the morphology of mitosis and cytokinesis as observed by light optics.

ACKNOWLEDGEMENTS

The authors are thankful to Mr. E. M. J. Dekkers for his invaluable help in preparing the photographs.

REFERENCES

NILSHAMMAR, M. & B. WALLES (1974): Electron microscope studies on cell differentiation in synchronized cultures of the green alga Scenedesmus. *Protoplasma* **79**: 317–332.

NORRIS, J. R. & H. SWAIN (1971): Staining bacteria. In: *Methods in microbiology* (J. R. NORRIS & D. W. RIBBONS, ed.) **5A**, pp. 105–134. Academic Press, London and New York.

PICKETT-HEAPS, J. D. & L. A. STAEHELIN (1975): The ultrastructure of Scenedesmus (Chlorophyceae). II. Cell division and colony formation. *J. Phycol.* **11**: 186–202.

SETLÍK, E., E. BERKOVÁ, J. DOUCHA, S. KUBIN, J. VENDLOVA & V. ZACHLEDER (1972): The coupling of synthetic and reproduction processes in Scenedesmus quadricauda. *Arch. Hydrobiol. Suppl.* **41**: 172–213.

SMITH, G. M. (1914): The cell structure and colony formation in Scenedesmus. *Arch. Protistenk.* **32**: 278–297.

STEENBERGEN, C. L. M. (1974): *The role of light in the life cycle of Scenedesmus obliquus (Turp.) Kütz.* Thesis, University of Utrecht.

— (1975): Light-dependent morphogenesis of unicellular stages in synchronized cultures of Scenedesmus quadricauda (Turp.) Bréb. (Chlorophyceae). *Acta Bot. Neerl.* **24**: 391–396.

— (1976): Pleomorphism in Scenedesmus quadricauda (Turp.) Bréb. (Chlorophyceae). *Hydrobiol. Bull.* **10**: 14.

— (in press): Pleomorphism of Scenedesmus quadricauda (Turp.) Bréb. (Chlorophyceae) in synchronized cultures. *Proc. Int. Symp. Algal Cultures, Sandefjord.* SIL-Mitteilungen.

SULEK, J. (1975): Nuclear division in Scenedesmus quadricauda (Turp.) Bréb. *Arch. Hydrobiol. Suppl.* **46**: 224–258.

SWALE, E. M. F. (1967): A clone of Scenedesmus with Chodatella-stages. *Br. Phycol. Bull.* **3**: 281–293.

TRAINOR, F. R. (1971): Development of form in Scenedesmus. In: *Contributions in phycology* (B. C. PARKER & R. M. BROWN Jr. ed.) pp. 81–92. Allen Press Inc., Lawrence, Kansas.