# SELECTIVE INHIBITION BY CYCLOHEXIMIDE OF NUCLEAR DNA SYNTHESIS IN SYNCHRONOUS CULTURES OF *CHLORELLA*

F. Wanka and J. Moors

Laboratory of Chemical Cytology,
University of Nijmegen,
The Netherlands

**Received August 21, 1970**

SUMMARY

In synchronous cultures of *Chlorella pyrenoidosa* the synthesis of nuclear DNA was inhibited by more than 90% in the presence of 15 µM cycloheximide. Incorporation of externally applied $^{14}$C-uracil into nuclear DNA was inhibited to about the same extent, while the inhibitor had only little effect on satellite DNA synthesis. The results suggest that continuous protein synthesis is required for nuclear DNA replication.

Cycloheximide (Actidione) has been shown to inhibit the incorporation of radioactive precursors into protein and nucleic acids of *Chlorella* (1,2). The inhibiting effect on protein synthesis has been supported by the observation that the increase of several enzyme activities ceases in the presence of 15 µM cycloheximide (3,5). Direct evidence of the inhibition of protein synthesis has been obtained in cell free systems of yeast (5) and reticulocytes (6). The inhibition of DNA synthesis seems to be a secondary effect because of the resistance of DNA polymerase activity *in vitro* to cycloheximide concentrations up to 600 µM (7). It might be due to interference with specific regulation processes, in which case synthesis of nuclear and cytoplasmic DNA are likely to respond in different ways to the inhibitor.

Synchronous cultures of Chlorella pyrenoidosa are suitable to study this possibility because of the presence of two satellite DNAs which, in contrast to the nuclear DNA, incorporate $^{14}$C-uracil continuously during the entire life cycle (8).

Chlorella pyrenoidosa strain 211/8b Göttingen was grown autotrophically and synchronized by alternating periods of 16 hours light and 8 hours dark (9). DNA was labeled by use of 2-$^{14}$C-uracil and, after extraction and purification, banded in a preparative CsCl density gradient as described in detail recently (8).

Under the experimental conditions used, nuclear DNA undergoes usually 4 successive duplications between the 10th and 18th hour after the beginning of the light period (10). High amounts of label are incorporated into the pyrimidine moieties of the major DNA component during this time (8). This incorporation became inhibited for more than 90% in the presence of 15 µM cycloheximide (Fig.1). The labeling of satellite DNA seems to be less affected but an unambiguous result cannot be obtained in the presence of strong nuclear DNA synthesis. Therefore, another experiment was carried out lasting from the 19th until the 23rd hour after the beginning of the light period. During this time label is incorporated only into the satellite DNA which consists of a larger component banding at a buoyant density of 1.689 g/cm$^3$ and a smaller one appearing as a shoulder at a density of about 1.700 g/cm$^3$ of the CsCl gradient (8). The inhibitor had only little effect on both of them (Fig.2.). The decrease of the labeling caused by 15 µM cycloheximide varied between 0 and 40%. This can

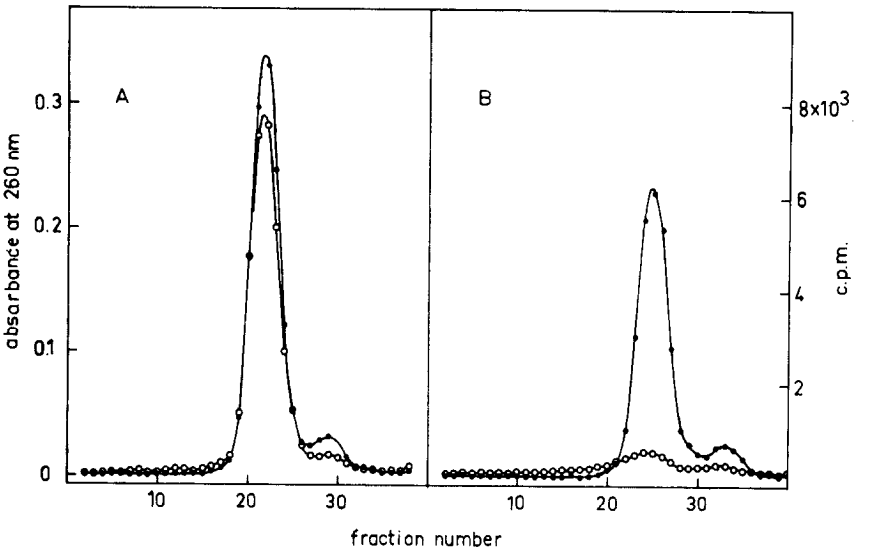


Fig. 1. Inhibition by cycloheximide of labeling of nuclear DNA. A sterile solution of 20 μC 2-$^{14}$C-uracil and 4.5 μmoles of cycloheximide in 2 ml dist. water was added to 300 ml of Chlorella culture 15 hours after the beginning of the light period. The cells were collected two hours later, and DNA was extracted and dissolved in 4.4 ml of 0.02 M TrisHCl buffer pH 8. 5.5 g of CsCl were added and the solution was centrifuged for 65 hours in a Spinco angle rotor type 65 at 35,000 r.p.m. and 4°. Fractions of 8 drops were collected and 2 ml of 0.02 M TrisHCl buffer were added and the optical density (●——●) and the radioactivity (o——o) were measured.
A. Control without cycloheximide.
B. Cycloheximide present.

be mainly ascribed to dilution of the label by endogenous pyrimidine nucleotides which were found to increase up to 140% of the control in the presence of the inhibitor (11).

As reported by Morris (1,2), the inhibiting effect of cycloheximide depends on the precursor used for labeling of the DNA. Consequently, conclusions about inhibition of DNA synthesis based on labeling experiments require additional support concerning the quantitative changes of the DNA content. This was obtained by another experiment in which

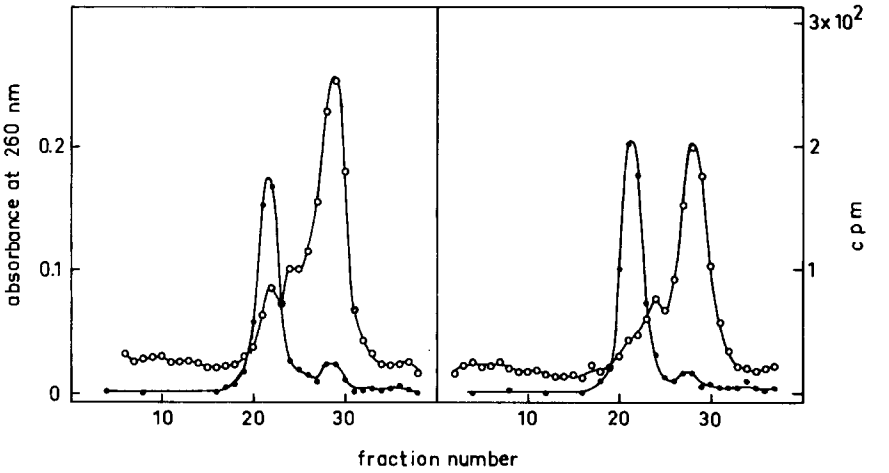


Fig. 2. Resistence of satellite DNA synthesis to cycloheximide. Cycloheximide and $^{14}$C-uracil were added to 300 ml of Chlorella culture 19 hours after the beginning of the light period. The cells were collected 4 hours later. Further details as in Fig. 1.

aliquots of 100 ml were withdrawn from a synchronous culture at different times during the replication period and supplied with 15 µM cycloheximide. After growth for another 4 hours in the presence of the inhibitor, the DNA content had increased by 5 to 15%. Compared to the DNA synthesis in the untreated control culture the inhibition was about 95% at any time during the replication period (Fig.3).

The results reported here are in agreement with the apparent failure of nuclear DNA replication in the absence of protein synthesis in yeast (14). The almost complete cessation of the DNA increase caused by cycloheximide at all times during the replication period suggests that protein formation is required concomitantly with DNA synthesis. It seems unlikely that these proteins are enzymes involved in the synthesis of DNA or DNA precursors, because all

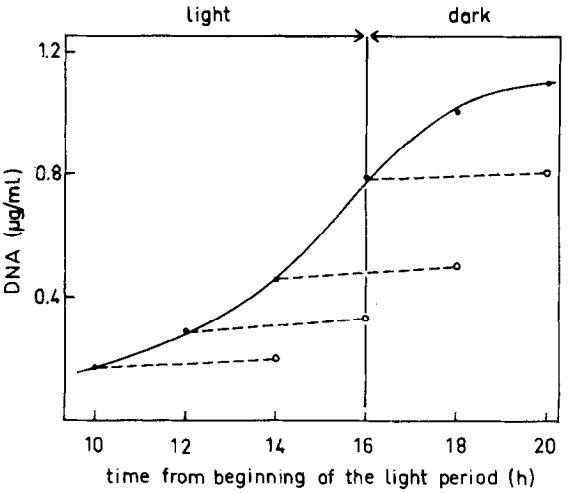


Fig. 3. Effect of cycloheximide on the net increase of DNA. Cells were collected at the given times and the DNA was extracted as described previously (12) and determined by the diphenylamine reaction according to Burton (13). ●——● DNA content of the control culture, o----o DNA found after growth for 4 hours in the presence of 15 uM cycloheximide. For experimental details see the text.

enzyme activities investigated so far in Chlorella remain high for several hours after addition of cycloheximide (3,4,7). The possibility has to be considered that these are specific proteins which are formed only during DNA synthesis. Such is the case with certain histone fractions as shown by Gallwitz and Mueller (15). The investigations are being continued with regard to the regulatory processes involved in nuclear DNA synthesis.

ACKNOWLEDGEMENTS

We are much indebted to Mr. J.M.A. Aelen for technical assistance and to Dr. I.K. Vasil for kindly checking the manuscript. The investigations were supported in part by the Netherlands Foundation for Chemical Research with financial aid of the Netherlands Organization for the advancement of pure Research.

REFERENCES

1. Morris, I., Nature 211, 1190 (1960).
2. Morris, I., J.Exp.Bot. 18, 54 (1967).
3. Wanka, F., and Poels, C.L.M., Europ.J.Biochem. 9, 478 (1969).
4. Wanka, F., Joppen, M.M.J., and Kuyper, Ch.M.A., Zeitschr.f. Pflanzenphysiol. 62, 146 (1970).
5. Siegel, M.R., and Sisler, H., Biochim.Biophys.Acta 103, 558 (1965).
6. McKeehan, W., and Hardesty, B., Biochem.Biophys.Res. Comm. 36, 625 (1969).
7. Schönherr, O.Th., Dissertation of the Faculty of Science, University of Nijmegen, (1969).
8. Wanka, F., Joosten, H.F.P., and de Grip, W.J., Arch.f. Mikrobiol., in press.
9. Wanka, F., Arch.f.Mikrobiol. 52, 305 (1965).
10. Wanka, F., and Mulders, P.F.M., Arch.f.Mikrobiol. 58, 257 (1967).
11. Vereijken, P.H., unpublished results.
12. Wanka, F., Planta 58, 594 (1962).
13. Burton, J., Biochem.J. 62, 315 (1956).
14. Grossman, L.I., Goldring, E.S., and Marmur, J., J.Mol.Biol. 46, 367 (1969).
15. Gallwitz, D., and Mueller, G.C., J.Biol.Chem. 244, 5947 (1969).