Distribution of nucleic acids among subcellular fractions of *Chlorella**

In a previous study[1] it was observed that the base compositions of RNA and DNA fractions obtained from *Chlorella* cells by the SCHMIDT-THANNHAUSER[2] method change significantly in the course of synchronous growth. To pursue this observation further, cells of *Chlorella ellipsoidea* were disrupted and fractionated into three subcellular components, and the nucleic acids were isolated from these three fractions. The distributions and some characteristics of the nucleic acids are reported here.

Chlorella cells were disrupted with a French pressure cell (Amercian Instrument Co., Inc., Silver Spring, Md.) at 0°–4° in a medium containing 70 m*M* KCl, 2 m*M* Ca^{++}, and 5 m*M* Mg^{++}. Over 95 % of the cells were disrupted by being put through the apparatus twice. The ruptured cell material was centrifuged at 0° for a short period to eliminate the remaining whole cells and cell debris. Then it was centrifuged successively at 20,000 × *g* for 10 min and at 105,000 × *g* for 90 min to give pellets R-I and R-II, respectively. The first pellet (R-I) was dark green and consisted mainly of broken chloroplasts. The second pellet (R-II) was pale green and the final supernatant fluid was clear and amber. An example of chemical analyses on the three fractions is shown in Table I. RNA is distributed in all three fractions, but DNA is found almost exclusively in R-I and S. R-II consists mainly of ribonucleoprotein particles. The ribonucleoprotein particles obtained in a sucrose medium (0.20 *M* sucrose, 2 m*M* Ca^{++}) and which can be degraded with RNase had sedimentation coefficients ($s_{20,w}$) of about 40, 55 and 75 S. Preliminary assays showed that the relative amount of RNA in the three fractions and of DNA in the two fractions that contained it varied in the course of synchronous growth.

TABLE I

CHEMICAL ANALYSES ON THE THREE CELL FRACTIONS OF *Chlorella ellipsoidea*

Cell materials: 3.6 g in dry weight.

Material	*Amount of material in cell fraction (mg)*			*Whole homogenate*
	R-I	*R-II*	*S*	
Chlorophyll*	50	0.03	0.00	55
RNA**	22	27	8.4	61
DNA**, ***	3.1	0.36	3.5	7.5
Protein§	510	85	200	820

* Methanol extractions.
** SCHMIDT-THANNHAUSER method[2].
*** DISCHE diphenylamine reaction[3].
§ Biuret reaction.

The same distribution of DNA into two fractions as shown in Table I was also found in homogenates produced by alumina grinding[3] and by the Hughes press[4], or when other disrupting media were used (*e.g.*, 0.1–0.5 *M* sucrose or 0.02–0.15 *M* salts in combination with 0, 10^{-4}, and $2 \cdot 10^{-3}$ *M* Ca^{++} and 0, 10^{-3}, and $5 \cdot 10^{-3}$ *M* Mg^{++}).

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

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A fractionation of the disrupted cell material with a sucrose gradient (from 10 to 60 %) at 1700 rev./min for 30 min[5] gave the same result and no DNA was detected in cell components heavier than the broken chloroplasts (R-I). The broken chloroplasts purified by repeated differential centrifugation contained DNA as well as RNA, and they were stained a diffuse reddish purple with the Feulgen reagent, while the whole cell was stained by the dye in one or several locations.

The nucleic acids were isolated by detergent extraction (a modification of the KAY AND DOUNCE method[6,7]) with Duponol C (E. I. DU PONT DE NEMOURS AND CO., INC., Wilmington, Del.) (see ref. 4). About 60 % of the total nucleic acids was usually obtained as ethanol-precipitable, nondialysable material. The nucleic acids thus obtained were hydrolyzed with alkali, and the DNA was separated from the ribonucleotides of the RNA by precipitation with acid and solubilization with alkali three times. The results are shown in Table II. The RNA in the supernatant contains more cytosine and less adenine than the RNA of the particulate fractions, and the DNA in R-I (DNA-I) contains more thymine and adenine than the DNA in the supernatant (DNA-II).

TABLE II

BASE COMPOSITIONS OF THE NUCLEIC ACIDS ISOLATED FROM THE THREE CELL FRACTIONS OF *Chlorella ellipsoidea*[1]

Analyses from several samples obtained by using different methods, each run in duplicate: RNA (5 samples) by ion-exchange and paper chromatography of ribonucleotides[8,9]; DNA (4 samples) by paper chromatography of bases after digestion with 72 % $HClO_4$ (3 samples)[9] and ion-exchange chromatography of deoxyribonucleotides[8] after DNAse and venom digestion (1 sample)[10].

Nucleic acid	*Base composition*	*Range of mole per cent in*		
		R-I	*R-II*	*S*
RNA	Uracil	23–24	23–24	21–24
	Adenine	24–27	23–27	18–24
	Cytosine	23–25	22–24	26–30
	Guanine	24–30	27–30	25–32
DNA	Thymine	22–28		19–21
	Adenine	24–28		21–23
	Cytosine	24–28		27–30
	Guanine	20–28		28–30

The phosphorus metabolism of the two DNA fractions was examined with ^{32}P tracer. An inoculum for the synchronous culture[1] prepared in unlabeled medium was grown at 16° with $^{32}PO_4$ present under a saturating light intensity, and the cells were harvested at 17 h when they were increasing in mass but were not synthesizing DNA (Expt. 1). Another inoculum that had grown in a ^{32}P medium for three generations was grown synchronously in an unlabeled medium, and the cells were harvested at 52 h when they had just begun to synthesize DNA[1] (Expt. 2). In both cases the two fractions containing DNA were isolated and digested with DNAase and snake venom phosphodiesterase (kindly supplied by Dr. L. ASTRACHAN of this Laboratory), and the specific activity of the separated (individual) deoxyribonucleotides was measured. The results are shown in Table III. Here it can be seen that DNA-I turns over faster than DNA-II in terms of phosphorus.

TABLE III

LABELING AND DELABELING OF DNA-I AND DNA-II WITH ^{32}P

In specific activity: counts/min/μmole P.

Expt.	*DNA-I*	*DNA-II*
1*	3700 (1610–5300)**	1200 (560–1870)
2***	390 (100–700)	1800 (1100–2440)

* A nonlabeled inoculum grown synchronously in a ^{32}P medium. The specific activities of the whole cells and of the culture medium at the harvest were 21,000 and 130,000 counts/min/μmole P, respectively.

** Figures in parentheses are the maximum and minimum specific activities for the four constituent nucleotides, the average of which appears above them.

*** A labeled inoculum grown synchronously in the unlabeled medium. The specific activities of the whole cells at the start of the culture and at harvest were 1200 and 280 counts/min/μmole P, respectively. At harvest, the DNA content per cell was 1.3 times as great as that at the start.

From all these findings, we conclude that DNA exists in two physically separable subcellular components in *Chlorella* cells and that the two DNA's differ from each other in metabolic activity as well as in base composition. It is also possible that DNA-I exists in or on the chloroplasts, whereas DNA-II is in the cytoplasmic portion, probably in a nucleus-like body in the cytoplasm of *Chlorella* cells. (Shortly after we had completed the work reported here, STOCKING AND GIFFORD presented a paper in which they demonstrated the uptake of labeled thymidine by the chloroplasts of the alga *Spirogyra*[10]).

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