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Circadian variation in ATP content in the chloroplasts of *Acetabularia mediterranea*

In the unicellular alga *Acetabularia mediterranea*, several functions vary with the time of the day: photosynthesis[1–4], chloroplastic carbohydrate content[5], chloroplastic RNA synthesis[6] and chloroplast shape[7]. All these functions are located in the same organelle, the chloroplast. Since ATP is an essential product of photosynthesis, the ATP content of the chloroplasts has been investigated at two different times of the day, both in the chloroplasts and in the cytoplasm.

It has been found that there is a variation in the ATP content of the chloroplasts in Acetabularia, the level being higher 1 h or 0.5 h after the beginning of the light period than it is at the middle of the light period. This difference is maintained in constant light.

The algae were grown under standard conditions, which have been described elsewhere in detail[8]. The algae were not completely sterile, but no antibacterial treatment has been carried out, since it is known that bacteria do not display circadian rhythmicity under normal conditions[9,10]. The lighting regime was 12 h:12 h. The intensity of the light was 1400 lux. Tests for endogenicity were carried out by skipping one or three dark periods and keeping the algae in constant light of 1400 lux.

The determination of the ATP content was done according to SCHRAM[11]. The measurements were performed with a scintillation counter, using the luminescent reaction produced by the luciferin–luciferase reaction. The determinations were made on the chloroplast pellet and on the supernatant obtained after centrifuging for 5 min at 2000 $\times$ *g* manually homogenized samples of algae according to GOFFEAU AND BRACHET[12]; after rinsing, the fractions were rapidly brought to 100° for 2 min before the measurements. Although there is no indication of leakage of ATP from the chloroplasts during the preparation, any leakage would reduce their ATP content and consequently reduce the observed difference. ATP added to samples of algae at the beginning of the procedure has been recovered in addition to that of the supernatant, showing that no measurable degradation had been occurring.

The determination of the chlorophyll content was made according to ARNON[13] with a Beckman spectrophotometer.

The results of the experiments conducted under light–dark (L:D) conditions are reported in Table I. The ATP content of the chloroplasts is significantly higher at the beginning of the light period (whether the measurements were made after 30 or 60 $\pm$ 15 min after illumination) than in the middle of the light period (after 6 h). When a statistical analysis is made of the values obtained at these two different moments of the light period, the difference is found to be significant with a probability of 0.01 in four cases, and 0.05 and 0.10 in the two other cases.

Arhythmicity sometimes happens in the cultures. In two experiments with arhythmic algae, no significant difference between means has been found.

The rhythm is truly endogenous: the alternation of light and dark is not a prerequisite for the variation in ATP content. Determinations were made at the beginning or at the middle of the subjective light period (that would have been the light period, were not one or several dark periods being missed). The results of the

TABLE I

ATP CONTENT OF THE CHLOROPLASTS OF ACETABULARIA AT THE BEGINNING AND AT THE MIDDLE OF THE LIGHT PERIOD

The mean values were obtained from 3 or 4 samples of algae (S.D. in parentheses), except in the cases where no S.D. has been calculated (for which 2 samples have been available). Lighting regime, L:D = 12:12. Light period, from 9 to 21 h. Time of measurements: t_1 (± 15 min) 9.30 a.m. in Expts. I–V, 10.00 a.m. in the other experiments. t_2 (± 20 min) 3.00 p.m.

Expt. No.	*ATP content of chloroplasts (pmoles of ATP per μg of chlorophyll)*			*ATP content of supernatant (pmoles/20 algae)*		
	Time t_1	*Time* t_2	*Ratio* t_1/t_2	*Time* t_1	*Time* t_2	*Ratio* t_1/t_2
I	6.30 (1.90)	3.55 (1.23)	1.8	—	—	—
II	7.26 (1.33)	4.41 (—)	1.6	640	318	2.0
III	4.37 (—)	2.88 (—)	1.5	600	898	0.7
IV	4.49 (0.36)	2.99 (0.69)	1.5	724	860	0.8
V	3.78 (—)	2.88 (—)	1.3	—	—	—
VI	7.88 (0.33)	5.13 (0.19)	1.5	1031	861	1.2
VII	6.18 (0.07)	4.22 (0.09)	1.5	806	1196	0.7
VIII	6.76 (0.28)	3.72 (0.49)	1.8	591	390	1.5
IX	6.20 (0.08)	4.71 (0.24)	1.3	1188	995	1.2

experiments carried out under constant conditions are recorded in Table II. In four cases, one dark period has been missed, and in one case, three dark periods have been missed. The difference between means has been found to be significant with a probability of 0.01 in all cases.

The supernatant also contains ATP the variation of which has been measured. This variation with the time of the day can be quite large but in one or the other direction; this situation stands in striking contrast with the regular decrease in ATP content in the chloroplasts when time passes from 10 a.m. to 3 p.m.

These experiments lead to the following conclusion: in the chloroplasts of Acetabularia, the ATP content is higher in the early morning than it is in the middle

TABLE II

ATP CONTENT OF THE CHLOROPLASTS OF ACETABULARIA AT THE BEGINNING AND AT THE MIDDLE OF THE SUBJECTIVE LIGHT PERIOD (1 OR 3 DARK PERIODS HAVE BEEN MISSED)

Mean value calculation, lighting regime prior to L:L and time of measurements, as in Table I. S.D. in parentheses.

Expt. No.	*ATP content of chloroplasts (pmoles of ATP per μg of chlorophyll)*			*ATP content of supernatant (pmoles/20 algae)*		
	Time t_1	*Time* t_2	*Ratio* t_1/t_2	*Time* t_1	*Time* t_2	*Ratio* t_1/t_2
XI	6.23 (0.99)	2.26 (0.66)	2.8	803	692	1.2
*	5.58 (0.92)	2.19 (0.26)	2.5	801	633	1.3
XII	8.75 (0.34)	6.20 (—)	1.4	747	976	0.8
XIII	6.11 (0.68)	3.78 (0.80)	1.6	644	504	1.3
XIV	5.58 (0.24)	3.73 (0.32)	1.5	1342	999	1.3

* Case in which 3 dark periods have been missed.

of the day (or in the middle of the subjective light period). Its decrease is tentatively correlated with the greater need for energy-rich bonds when carbohydrate and RNA synthesis are increasing in rate. A similar situation has been encountered by BOMSEL AND PRADET[14]: studying the ATP, ADP and AMP balance in the wheat leaf, they observed a decrease in the number of $\sim P$ under conditions in which the carbohydrate synthesis is higher.

The irregularity in the variation in the ATP contained in the supernatant is tentatively ascribed to differences in physiological or morphological states of the algae.

Knowledge of the existence of variations in ATP content of the chloroplasts of Acetabularia is essential to furthering our understanding of the metabolism of the chloroplasts and the relations between the various circadian rhythms: decrease in ATP content of the chloroplasts integrates the variation of ATP resulting from photosynthesis (an ATP-generating process) and the variation resulting from the rhythms in carbohydrate synthesis and RNA synthesis (ATP-consuming processes); photosynthesis, carbohydrate synthesis and RNA synthesis are circadian rhythms.

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