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ENERGY TRANSFERS FROM PHOTOSYSTEM II TO PHOTOSYSTEM I IN *CRYPTOMONAS RUFESCENS* (CRYPTOPHYCEAE)

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Summary

In *Cryptomonas rufescens* (Cryptophyceae), phycoerythrin located in the thylakoid lumen is the major accessory pigment. Oxygen action spectra prove phycoerythrin to be efficient in trapping light energy.

The fluorescence excitation spectra at −196°C obtained by the method of Butler and Kitajima (Butler, W.L. and Kitajima, M. (1975) Biochim. Biophys. Acta 396, 72—85) indicate that like in Rhodophyceae, chlorophyll *a* is the exclusive light-harvesting pigment for Photosystem I.

For Photosystem II we can observe two types of antennae: (1) a light-harvesting chlorophyll complex connected to Photosystem II reaction centers, which transfers excitation energy to Photosystem I reaction centers when all the Photosystem II traps are closed. (2) A light-harvesting phycoerythrin complex, which transfers excitation energy exclusively to the Photosystem II reaction complexes responsible for fluorescence at 690 nm.

We conclude that in Cryptophyceae, phycoerythrin is an efficient light-harvesting pigment, organized as an antenna connected to Photosystem II centers, antenna situated in the lumen of the thylakoid. However, we cannot afford to exclude that a few parts of phycobilin pigments could be connected to inactive chlorophylls fluorescing at 690 nm.

Introduction

Phycobiliproteins and chlorophyll c_2 are the accessory pigments for photosynthesis in Cryptophyceae [1—4]. In contrast with Cyanobacteria and Rhodo-

Abbreviations: PS I, Photosystem I; PS II, Photosystem II.

phyceae, these algae possess only one single type of phycobiliproteins [5—7] which is contained within the intrathylakoidal space [8,9]. The first investigations of Haxo and Fork [10] suggest that these pigments trap light energy. In our work, by means of low temperature spectroscopic studies [11,12] and action spectra for oxygen evolution [13], we confirm the antenna composition of Photosystem II, the role played in light capture by the intrathylakoidal phycoerythrin, and the transfer of the trapped light energy to the Photosystem II reaction centers under nonsaturating conditions.

Materials and Methods

The axenic cultures of *C. rufescens* were grown on S_2T_2 medium at room temperature in Erlenmeyer flasks [9]. The control cultures (T) were exposed to daylight (about $4\ J \cdot s^{-1} \cdot m^{-2}$) and darkness (12 h/12 h). The algae grown at high light intensity (FL) were continuously exposed to fluorescent tubes (ITT Claude 40 RS) at approx. $10\ J \cdot s^{-1} \cdot m^{-2}$.

Concentrations of chlorophylls *a* and c_2 were calculated by using the equations of Jeffrey and Humphrey [24]. Phycoerythrin content was estimated with the extinction coefficient communicated by Mac Coll: $E^{1\%}_{565}$ (mg/ml) = 9.9.

The techniques to obtain low temperature absorption spectra and fluorescence emission spectra were previously described [14,15].

The rate of oxygen evolution was measured by the modulated polarographic technique of Joliot and Joliot [16] according to Lemasson et al. [13].

The fluorescence excitation spectra were obtained by the method of Butler and Kitajima [11]. After 15 min in darkness, the alga suspension was layered on a Millipore filter (Ref. Ap20), with an absorbance less than 0.2 at 680 nm. The modulated 300 Hz monochromatic exciting light (1 nm passband) was transmitted from a tungsten lamp (6 V, 15 A, Philips) to the first fiber-optic light pipe, by means of a Jobin and Yvon HRS monochromator. The transmitted light was eliminated by Wratten filters (24, 45 and 89 B), and a Matra interference filter (690 nm or 730 nm). The remaining fluorescent light measured with a RTC 1003 photomultiplicator was amplified with a lock-in amplificator (Tekelec TC 9700), and then transmitted to a Tektronic computer (TEK 31) with a DM 501 interface. The intensity of the incident light was measured with a photodiode (BPY 13) corrected with a thermopile CA1 (Kipp and Zonen). The frozen algae were flashed by a Stroboslave generator in order to change the state of PS II reaction centers.

Results and Discussion

The typical absorption spectrum at −196°C of *C. rufescens* control cultures (T) shown in Fig. 1: (1) Two peaks at 678 nm and 670 nm which represent at least two chlorophyll forms, (2) two shoulders at 640 nm and 630 nm which correspond to the satellite forms of chlorophyll *a* and to chlorophyll c_2, and (3) one peak at 568 nm and a shoulder at 605 nm specific of the *C. rufescens* phycoerythrin.

As shown in Fig. 1 and Table I, the pigment content of this alga is modified

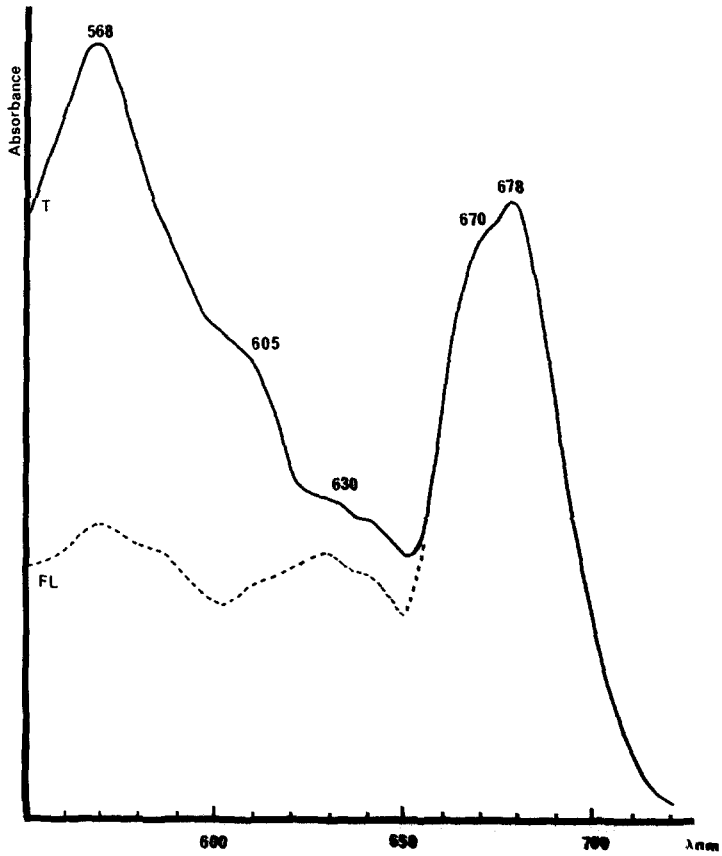

Fig. 1. Absorption spectra at −196°C of *C. rufescens*. ———, absorption of control cultures (T); - - - - - -, absorption of high light intensity cultures (FL).

by the culture conditions. In high light intensity (FL), the pigment content is lowered.

The fluorescence emission spectrum at −196°C of control cultures (T) indicates two peaks at 730 nm and 690 nm attributed respectively to PS I and PS II [17–19], and a 630 nm emission band related to phycoerythrin (Fig. 2). In high light intensity (FL), when phycoerythrin content is low, the 630 nm and 690 nm peaks decrease, therefore we can think that phycoerythrin is involved in the 690 nm emission band and transfers excitation energy to the chlorophyll forms fluorescing at 690 nm.

TABLE I

PIGMENT CONTENT OF THE DIFFERENT SAMPLES OF *C. RUFESCENS*

Amounts in μg pigment/10^6 cells; 10^6 cells = 1.04 mg dry weight. Chl, chlorophyll; PE, phycoerythrin.

Samples	Chl *A*	Chl C_2	% Chl C_2/Chl *A*	PE	PE/Σ Chl
T	11	0.6	5.45	42	3.6
SN	2.4	0.15	6.25	5	2

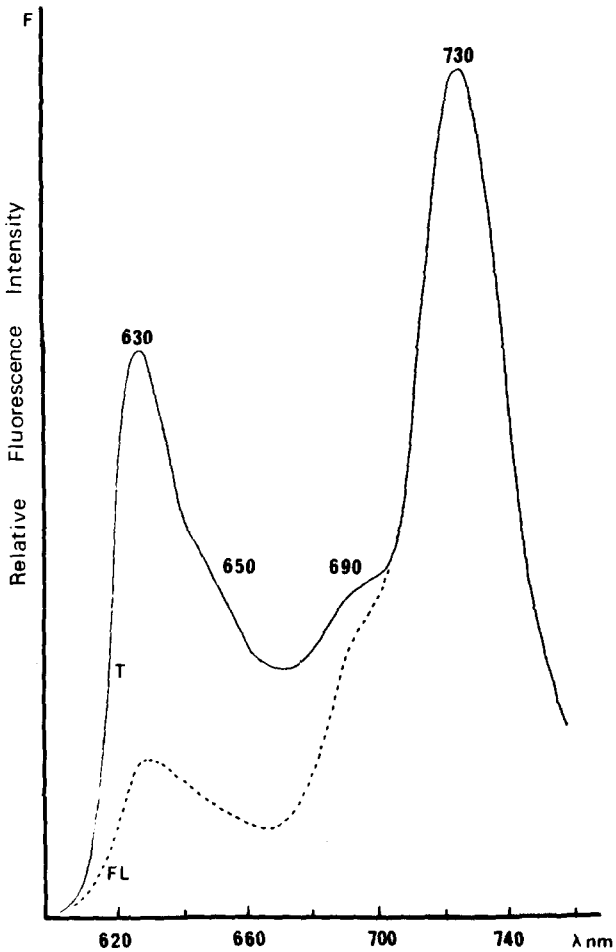

Fig. 2. Fluorescence emission spectra at −196°C of *C. rufescens*. ———, control cultures emission (T); - - - - - -, high light intensity cultures (FL). (Excitation light: quartz iode lamp with a Schott BG 23 filter).

The fluorescence excitation spectra at −196°C according to the method of Butler and Kitajima [11] are utilized to study the pigment distribution between the two photosystems (Figs. 3 and 4). The 690 nm fluorescence emission band comes from the PS II antenna [12]. The 730 nm fluorescence emission band is more complex [20] and comes from the antennae of the two photosystems and from the light-harvesting pigment complex (Fig. 4). The excitation spectrum of the 730 band (F_{730}) represents the weighted sum of the excitation spectra of the two photosystems [12]. The variable part $F_{730(V)}$ (difference spectrum between $F_{730(M)}$ and $F_{730(O)}$) represents the spectrum of the light-harvesting pigment of PS II which transfers energy to PS I centers when all the PS II reaction centers are closed. In higher plants, like in Rhodophyceae, the $F_{690(M)}$,$F_{690(V)}$,$F_{690(O)}$ are proportional to $F_{730(V)}$ and represent the absorption spectrum of PS II antenna [21—23]. In *C. rufescens*, the $F_{730(V)}$ and $F_{690(V)}$ spectra are very different from $F_{690(O)}$ and $F_{690(M)}$. In the latter spectra a great participation of phycobiliproteins is manifest, while in the

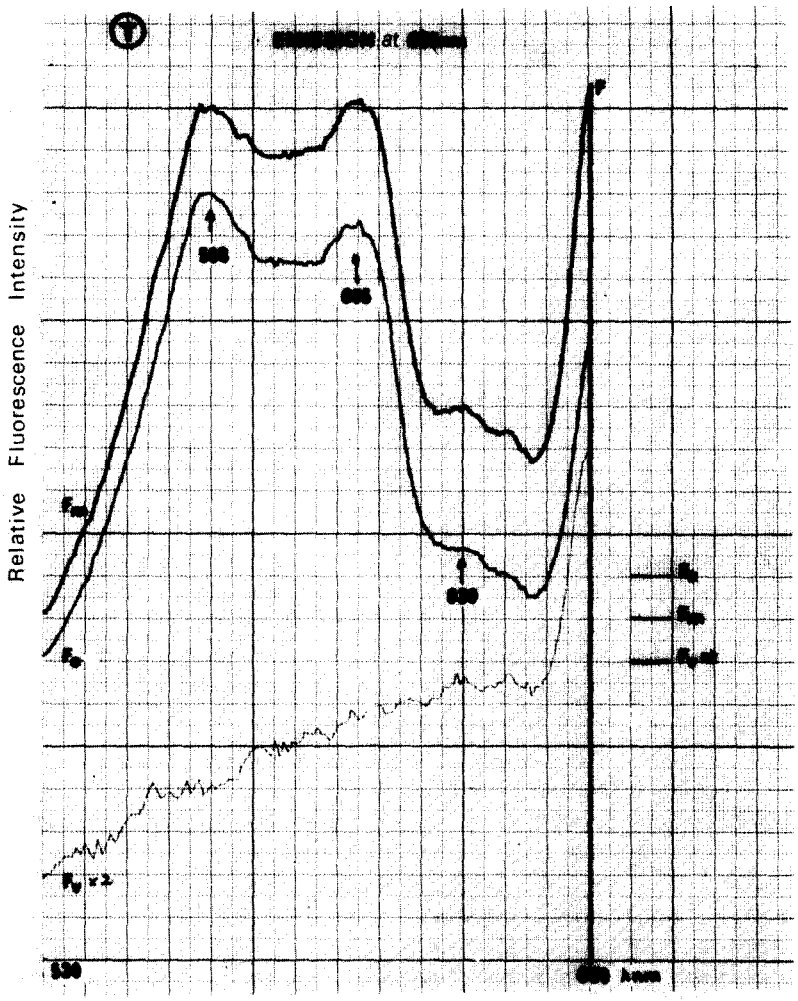

Fig. 3. Excitation spectra for 685 nm fluorescence from *C. rufescens* control cultures (T) (nomenclature of Butler and Kitajima [11]); the variable part $F_{(V)}$ was multiplied by 2.

former, the participation of phycoerythrin is non-existent.

We conclude from these facts, that when all the traps of PS II are closed, the excitation energy collected by the chlorophyll antenna of PS II is transmitted to PS I, while the energy collected by phycobilin pigments is not transferred to PS I centers. A light-harvesting pigment complex, identical with those of higher plants and Rhodophyceae connects the antennae of PS I and PS II, but this pigment complex does not contain phycoerythrin.

The role of phycoerythrin in Cryptophyceae is then original. (1) We can suppose that phycoerythrin is linked to inactive chlorophylls fluorescing at 690 nm, not connected to PS II centers, and then, energy collected by these pigments is not utilized for oxygen evolution. The investigations of Haxo and Fork [10] indicated that phycoerythrin is utilized as a light-harvesting pigment in Cryptophyceae. In *C. rufescens*, the O_2 action spectrum is nearly similar to

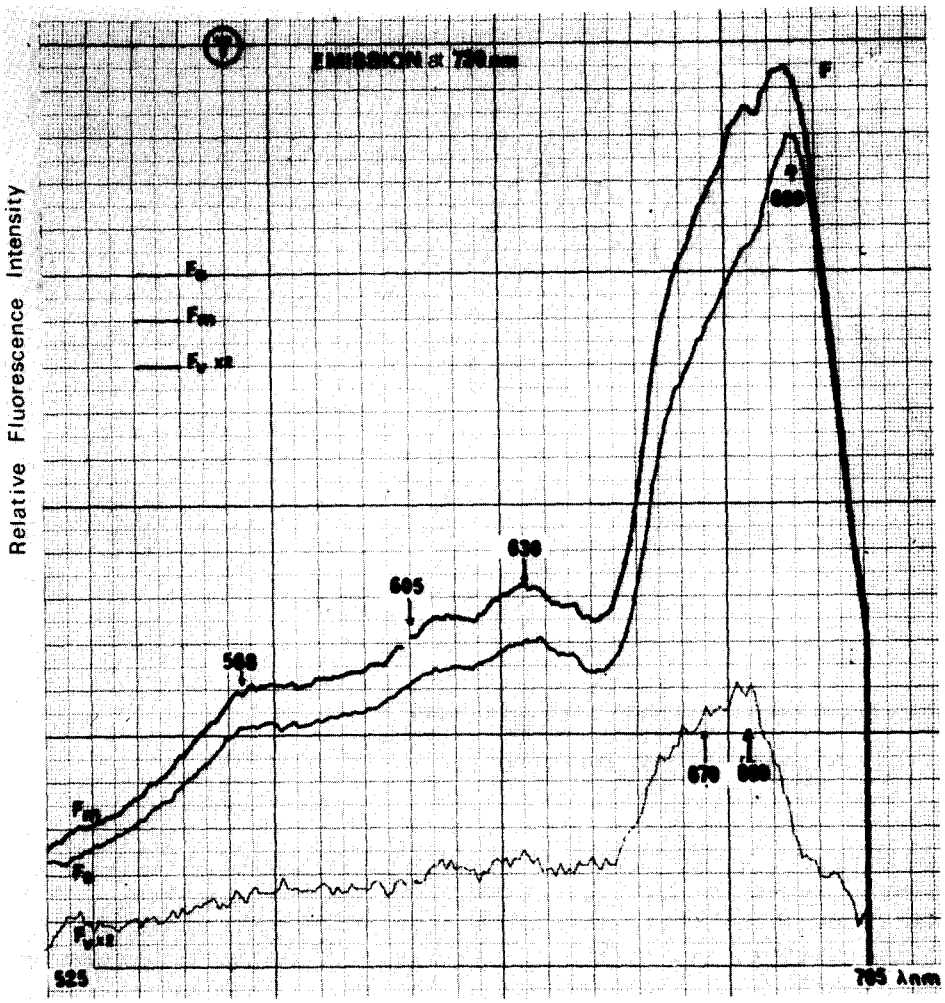

Fig. 4. Excitation spectra for 730 nm fluorescence from *C. rufescens* control cultures (T) (nomenclature of Butler and Kitajima [11]); the variable part $F_{(V)}$ was multiplied by 2.

absorption spectrum, and shows a peak at 565 nm corresponding to phycobiliprotein pigments (Fig. 5). Thereby, the light-harvesting role played by phycoerythrin is clearly resolved in this alga, and we can then exclude this first hypothesis.

(2) We suppose that phycoerythrin is linked to the PS II centers in such a way that it did not communicate with the major part of chlorophyll. A first chlorophyll antenna is able to transfer energy to PS I when the PS II reaction centers are all closed via a light-harvesting chlorophyll pigment complex; a second independent phycobiliprotein-chlorophyll antenna is connected to the PS II reaction centers (Fig. 6). We cannot exclude however, that some part of phycobiliprotein pigments could be connected to inactive chlorophylls fluorescing at 690 nm.

The action spectra of PS I antenna F(α) and of PS II antenna F(β) could be

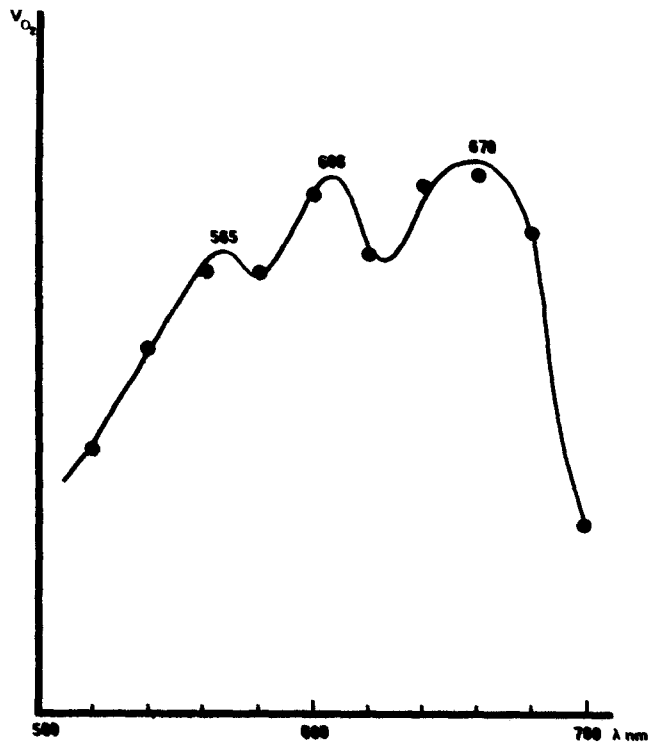

Fig. 5. O_2 action spectrum of *C. rufescens* control cultures.

determined by the method of Butler and Strasser [12] from the F_{730} and F_{690} fluorescence excitation spectra (Fig. 7). The ratio $F_{690(V)}/F_{690(M)}$ was estimated in the 'chlorophyllous area' of the spectra (650—670 nm) to be 0.25.

A precise weighted sum of $F(\alpha)$ and $F(\beta)$ proportional to the absorption spectrum is difficult to obtain because of the 'noisy' background of fluo-

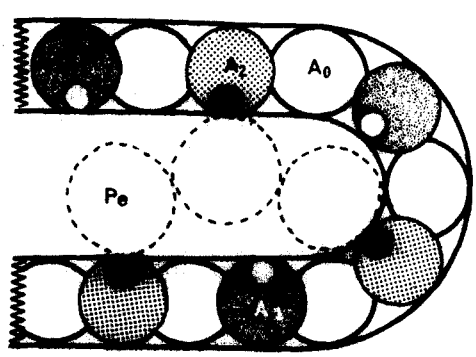

Fig. 6. Schematic interpretation of the phycobiliprotein and chlorophyll antennae in the thylakoid of *C. rufescens*. A_1, PS I chlorophyll antenna; ○, PS I reaction center. A_2, PS II chlorophyll antenna; ●, PS II reaction center. A_0, chlorophyll antenna between A_1 and A_2. Pe, PS II phycobiliprotein antenna. The intrathylakoidal representation of phycoerythrin units does not anticipate the ultrastructural organization of this pigment.

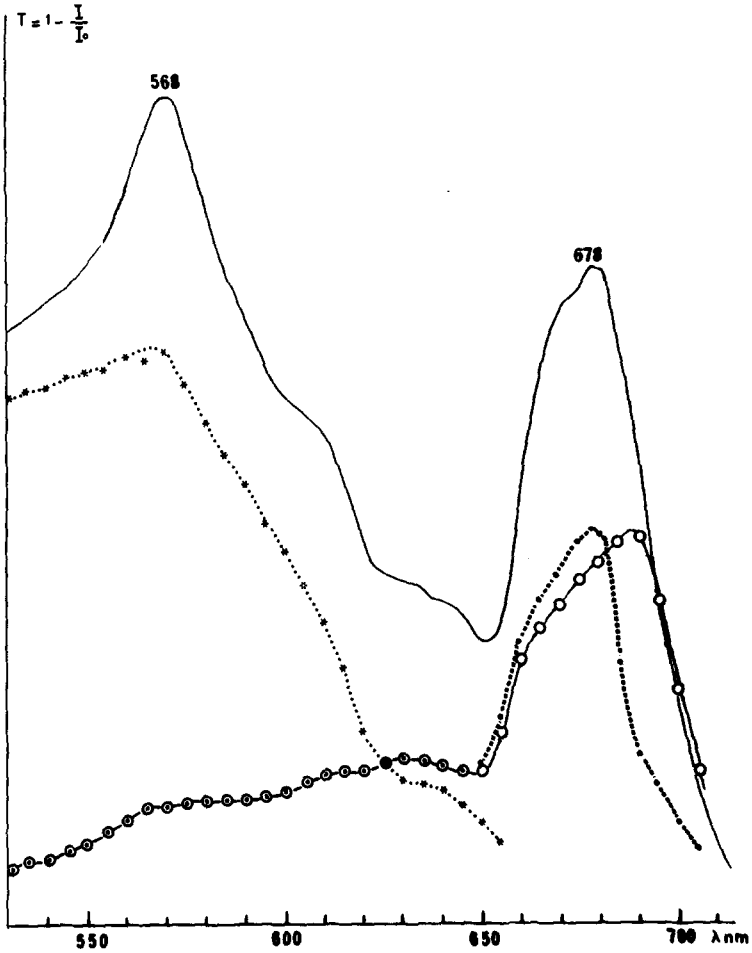

Fig. 7. Action spectra of PS II phycobiliprotein and chlorophyll antennae (Fβ) and action spectrum of PS I chlorophyll antenna (Fα) (Butler and Kitajima [11]). *·····*, PS II phycobiliprotein antenna (Pe = T − Fα − Fβ); ●-----●, PS II chlorophyll antenna (Fβ = $F_{730(V)}$); ○———○, PS I chlorophyll antenna (Fα = $F_{730(M)} - (F_{690(M)}/F_{690(V)})\ F_{730(V)}$); ———, absorption spectrum at −196°C of control cultures (T) (T = 1 − (I/I_0)).

rescence excitation spectra. So we have supposed in first approximation that chlorophyll *a* is equally distributed between the two antennae (Fig. 7). The action spectra of phycobiliprotein antenna was calculated from the absorption spectrum.

The curious system of light-harvesting pigment in Cryptophyceae, demonstrated by spectral method agrees with the intrathylakoidal localization of phycobiliproteins in these algae [8,9], localization being particularly original in phycobiliprotein organisms.

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