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# The molecular organization of chlorophyll-protein complexes in the Xanthophycean alga *Pleurochloris meiringensis*

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**Chlorophyll-protein complexes were isolated from the xanthophycean alga *Pleurochloris meiringensis*, using gel electrophoresis as well as sucrose-density gradient centrifugation. Using gel electrophoresis four different pigmented zones were obtained. They were characterized as Photosystem I, Photosystem II, light-harvesting complex and free pigment, respectively. The light-harvesting complex lost most of the xanthophylls and the efficiency of energy transfer to chlorophyll *a* was reduced. The sucrose-density separation yielded three chlorophyll-protein complexes without any free pigments. The two heaviest complexes could be identified to represent Photosystem I proteins by their fluorescence emission maxima at 715 nm in liquid nitrogen. The most abundant chlorophyll-protein complex was characterized as light-harvesting protein with a predominant polypeptide subunit of 23 kDa. In this light-harvesting complex, the pigments chlorophyll *a*, *c*, heteroxanthin, diadinoxanthin and vaucheriaxanthin ester are present in a ratio of 1000:224:148:264:129. On the basis of the fluorescence spectrum of a partially deconnected light-harvesting complex chlorophyll *c* is considered to function mainly in the mediation of energy transfer from xanthophylls to chlorophyll *a*.**

## Introduction

All photosynthetically active carotenoids and chlorophylls are non-covalently bound in so-called chlorophyll-protein complexes. In eukaryotic algae the reaction center complexes were found to be uniform with respect to their pigments or polypeptides, whereas the light-harvesting systems differ widely in their pigmentation and polypeptide composition. It was suggested that the different light-harvesting systems reflect both taxonomic relations and evolutionary vestiges. Three different lines of light-harvesting have been postulated: the phycobiliprotein-containing 'biliphyta', the chlorophyll *c*-containing 'chromophyta' and the chlorophyll *b*-containing 'chlorophyta' [1,2]. Intensive studies in the last years have elucidated a multiplicity of light-capturing carotenoids in the antenna systems not only in chlorophyll *c* but also in chlorophyll *b*-containing algae [3,4].

Nevertheless, there are some alga groups, whose molecular organization of the photosynthetic pigments is poorly understood. Especially, the antenna systems of yellow-geen algae, including the

Abbreviations: Chl, chlorophyll; CPa, chlorophyll-protein complex of PS II; CPI, P-700 chlorophyll *a*-protein complex of PS I; LHC, chlorophyll *a*/*c* diadinoxanthin light-harvesting complex; FP, free pigments; PAGE, polyacrylamide gel electrophoresis; PS, Photosystem.

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Eustigmatophytes and the Xanthophytes are less known. Because of a very strong instability of their chlorophyll proteins against detergent-based solubilization, the isolation of intact antenna complexes is strongly hindered. There are only three reports about the chlorophyll proteins of these algae [5–7]. Because the lack of chlorophyll *b* and the low content of chlorophyll *c*, the xanthophylls are expected to play an important role in light-harvesting. Up to now, there is no reliable information about the nature and the stoichiometric ratio of the xanthophylls to chlorophyll *a* in the chlorophyll-proteins of Xanthophytes.

The present study continues our work about xanthophycean algae giving more detailed information about the stoichiometry of pigments in the protein complexes, the polypeptide pattern and the molecular basis of energy transfer.

## Material and Methods

*Pleurochloris meiringensis* (Culture Collection of Göttingen, No. 860-3) was cultivated under low light conditions (3.5 $W/m^2$) as previously published for xanthophycean algae [5].

For the isolation of the chlorophyll-protein complexes the cells were harvested by centrifugation, homogenized and prepared as previously described [4]. The thylakoids were solubilized with SDS/Chl $a = 10:1$ for PAGE and with digitonin/Chl $a = 40:1$ for sucrose-density centrifugation, respectively. The sucrose-density centrifugation was adopted from Berkaloff et al. [8] using a stepwise gradient (55%, 50%, 40%, 30%, 20%, 15%, 10% sucrose). The gel electrophoresis of the denaturated polypeptides was done according to Laemmli [9]. The molecular size of the polypeptides was determined on the basis of standard

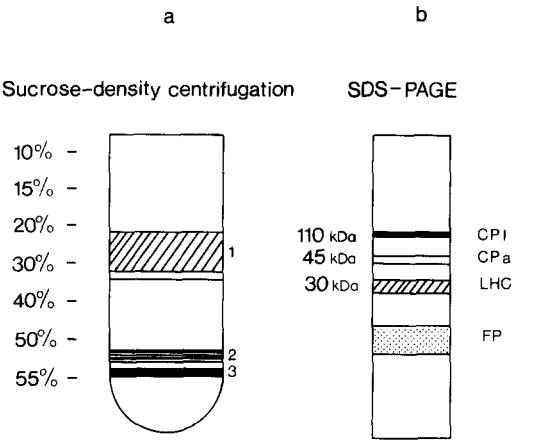


Fig. 1. Schematic representation of the chlorophyll-protein complexes from *Pleurochloris meiringensis* obtained by sucrose-density centrifugation (a) and by gel electrophoresis (b).

curves by logarithmical plotting the $R_f$ values of protein bands versus the molecular weight of the protein standards.

The absorption spectra of the eluted protein complexes were carried out by a Shimadzu MPS-2000 photometer. The fluorescence spectra were recorded at 77 K with a Hitachi F-3000 fluorometer at 0.5 μg Chl/ml. The excitation spectra were performed with a band pass of 3 nm for the excitation and 20 nm for the emission. The emission spectra were monitored with a band pass of 20 nm for the excitation and of 3 nm for the emission. The wavelength of excitation and emission is given in the figure legends. The pigment separations were performed by an HPLC system, which was earlier described in detail [10].

## Results

Fig. 1 gives the separation pattern of the chlorophyll-protein complexes isolated by sucrose-

TABLE I

DISTRIBUTION AND SPECTRAL CHARACTERISTICS OF THE CHLOROPHYLL-PROTEIN COMPLEXES FROM *PLEUROCHLORIS MEIRINGENSIS* ISOLATED BY SDS-PAGE

| Fraction | % of total chlorophyll | Apparent MG (kDa) | Absorption maxima (nm) | Fluorescence emission maxima (nm) | Polypeptides1 (kDa) |
|---|---|---|---|---|---|
| CP I | 19.9 | 110 | 420, 436, 674 | 684, 716 | 80.2 |
| CPa | 14.8 | 45 | 420, 435, 671 | 686 | 35.6 |
| LHC | 11.0 | 30 | 420, 440, 483, 671 | 680 | 29.3; 23.4 |
| FP | 54.3 | – | 440, 482, 670 | – | – |

density centrifugation (fig. 1a) and by gel electrophoresis (fig. 1b). Using sucrose-density centrifugation three pigmented bands can be observed. The two heaviest fractions (number 2 and 3 in Fig. 1a) do not show any differences with respect to their absorption or fluorescence spectra (data not shown). Therefore, only fraction 2 was used for further investigation. The upper band contains more than 60% of total chlorophyll. It must be emphasized that this separation procedure does not produce free pigments.

The gel electrophoretic method yields three chlorophyll-proteins with apparent molecular weights of 110 kDa (CP I), 45 kDa (CPa), 30 kDa (LHC) and a free pigment zone. This latter fraction contains nearly 60% of total pigments as a result of the instability of the chlorophyll-proteins in the presence of ionic detergents. Table I gives the distribution of the PAGE-isolated chlorophyll-protein complexes and their spectroscopical characteristics. Because of the high losses of pigments from the protein during gel electrophoresis the examination was focussed on the sucrose-density separated chlorophyll complexes.

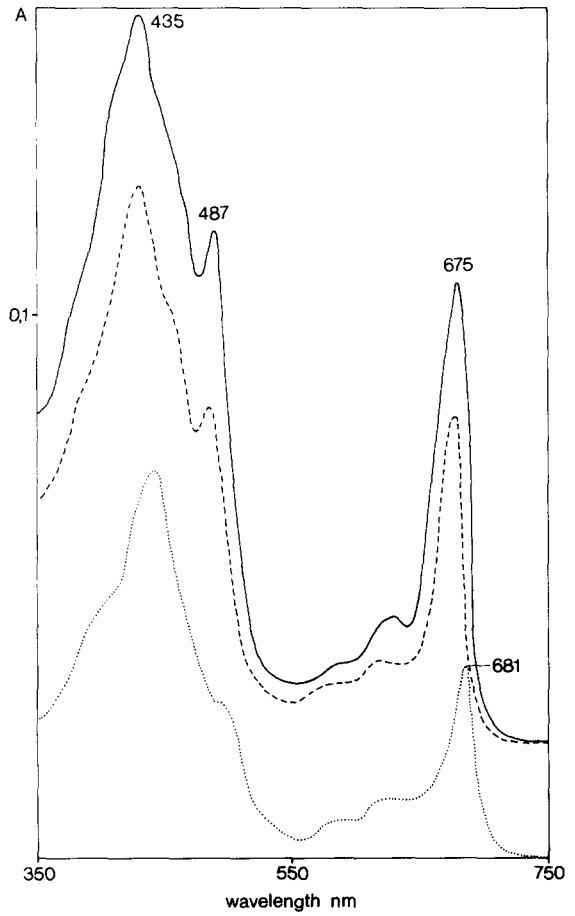


Fig. 2. Absorption spectra of total cells (solid line), LHC (broken line) and of the Photosystem I complex (dotted line) from *Pleurochloris meiringensis* isolated by sucrose-density centrifugation.

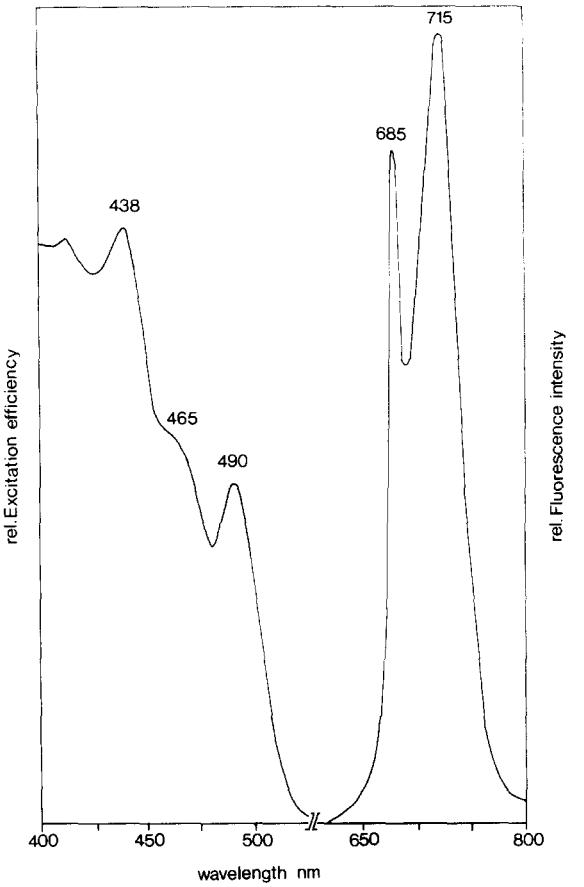


Fig. 3. Fluorescence excitation and emission spectra at 77 K of total cells from *Pleurochloris meiringensis*. For the excitation spectrum: emission wavelength, 685 nm. For the emission spectrum: excitation wavelength, 490 nm.

In order to characterize the two predominant pigment proteins from sucrose gradient, the absorption and fluorescence spectra as well as the pigment composition of both fractions were analyzed. Fig. 2 gives the absorption spectra of total cells and the two major bands (no. 1 and 2 in Fig. 1a) obtained by sucrose-density centrifuga-

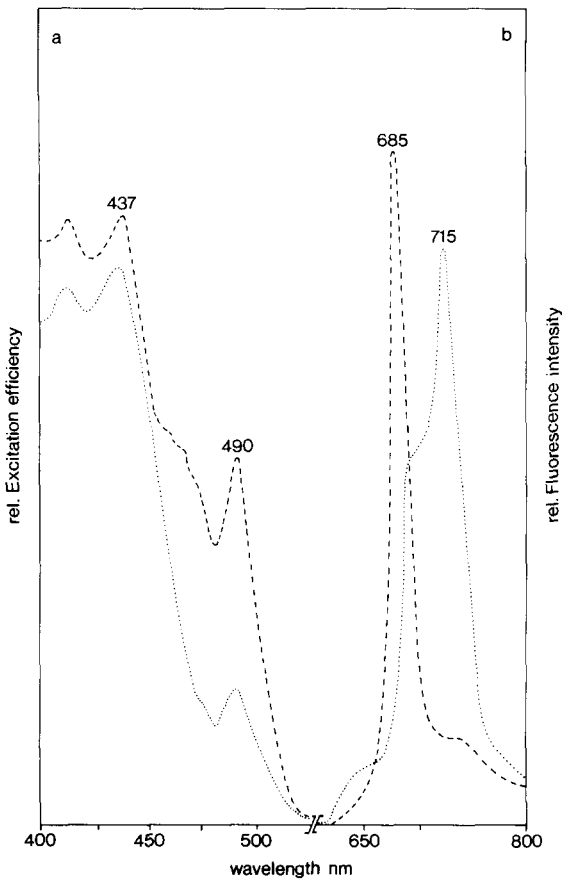


Fig. 4. (a) Fluorescence excitation spectra at 77 K of the LHC (broken line) and of the Photosystem I complex (dotted line) from *Pleurochloris meiringensis*. Emission wavelength, 685 nm. (b) Fluorescence emission spectra at 77 K of LHC (broken line) and of Photosystem I complexe (dotted line) isolated from *Pleurochloris meiringensis*. Excitation wavelength, 490 nm.

tion. In total cells (solid line) the red region of the spectrum is dominated by chlorophyll *a*, whereas chlorophyll *c* is not clearly detectable. In the blue wavelength region, however, a shoulder at 465 nm and a distinct absorption maximum at 487 nm becomes obvious referring to chlorophyll *c* and to high amounts of xanthophylls. The absorption spectrum of the upper fraction (equilibrating at 20% sucrose) exhibits a strong absorption at 487 nm, very similar to total cells. In the heavy fraction this maximum is less pronounced.

In Fig. 3 the fluorescence spectra of intact cells at 77 K show that the carotenoids absorbing at 490 nm transfer very efficiently the energy to chlorophyll *a*. The fluorescence emission spectrum of total cells suggests the existence fo the light-harvesting antenna (at 685 nm) and of the PS I-antenna complex (at 715 nm).

The fluorescence excitation spectrum of the upper fraction from the sucrose gradient (broken line in Fig. 4a) reveals a very effective energy transfer from the xanthophylls to chlorophyll *a*. The very strong fluorescence intensity induced by wavelength between 460–490 nm indicates that most of the accessory pigments remained well connected during isolation procedure. In the heavy complex (dotted line) the small amounts of accessory pigments revealed by absorption spectroscopy are also present in the excitation spectrum; these pigments are found to be well connected with chlorophyll *a*, too. The fluorescence

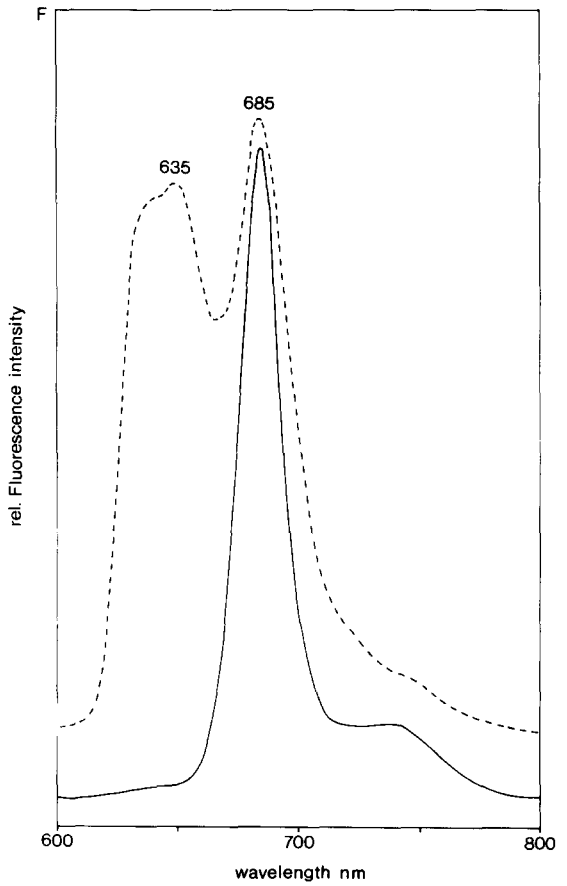


Fig. 5. Fluorescence emission spectra at 77 K of the freshly isolated LHC (solid line) and after 'aging' (broken line). Excitation wavelength, 490 nm.

emission spectrum reveals an emission maximum at 685 nm for the upper complex and at 715 nm for the lower one (Fig. 4b). In the latter case a shoulder at 695 nm can be observed. It must be emphasized that only chlorophyll *a* emission is detectable, although the excitation wavelength was adjusted at 490 nm. This indicates an intact energy transfer from the xanthophylls to chlorophyll *a* without any deconnected pigments. On the basis of these data it can be concluded that the upper fraction is the major light-harvesting antenna (LHC), whereas the second one is related to PS I.

If the LHC is stored for 1 day in a refrigerator (aged LHC) or if SDS is added, the emission spectrum shows a clear chlorophyll *c* emission at 635 nm (Fig. 5). This emission can be induced by the excitation for chlorophyll *c* (at 465 nm, band pass 3 nm) and for the xanthophylls (at 490 nm, band pass 3 nm).

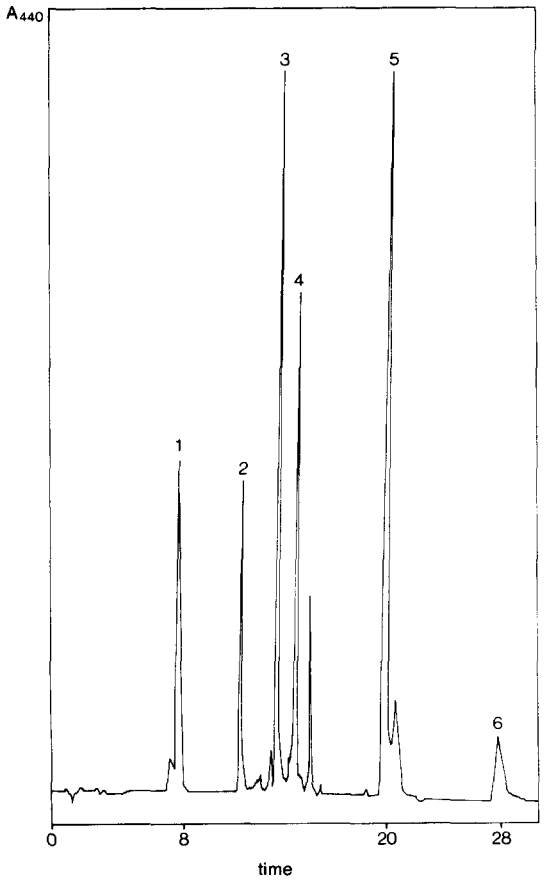


Fig. 6. HPLC scan of pigments of LHC isolated by sucrose-density centrifugation. 1, chlorophyll *c*; 2, heteroxanthin; 3, diadinoxanthin; 4, vaucheriaxanthin ester; 5, chlorophyll *a*; 6, *β*-carotene.

TABLE II

PIGMENTS PER 1000 CHLOROPHYLL *a* IN THYLAKOIDS, PS I AND LHC ISOLATED BY SUCROSE DENSITY CENTRIFUGATION

| mol pigment per 1000 Chl *a* | Total thylakoids | LHC | PS I |
|---|---|---|---|
| Chl *c* | 53±13 | 224±69 | 10± 4 |
| Heteroxanthin | 40±14 | 148±43 | 34±14 |
| Diadinoxanthin | 117±22 | 264±40 | 98±14 |
| Vaucheriaxanthin ester | 63±11 | 129±23 | 51±11 |
| *β*-Carotene | 15± 0,5 | n.d. | 23± 4 |

Fig. 6 gives the HPLC scan of the pigments isolated from total thylakoids. The cell contain as accessory pigments small amounts of chlorophyll *c* and the xanthophylls, heteroxanthin, diadinoxanthin and vaucheriaxanthin ester. There is no evidence for violaxanthin. This pattern can be considered to be typical for xanthophyte algae. Table II gives the molar amounts of the pigments from total thylakoids, LHC and PS I purified by sucrose density centrifugation. Comparing the LHC and the PS I complex with total thylakoids it becomes obvious that the LHC is strongly enriched in chlorophyll *c*, diadinoxanthin and vaucheriaxanthin ester; the molar ratio of xanthophyll per chlorophyll *a* is double as high as in the thylakoids. By contrast, the PS I complex contains less of accessory pigments in comparison to the whole thylakoid fraction.

Because the chlorophyll proteins isolated by sucrose-density centrifugation are usually not as pure as done by gel electrophoresis, the protein fractions were run on a denaturating gel system to check their purity and to measure the molecular weights of their apoproteins. Fig. 7 shows the result that the LHC contains only one predominant subunit with a molecular mass of 22–23 kDa. The polypeptide pattern of the PS I fraction is more complex. It contains beside the PS I subunits of 60 kDa some other polypeptides at 25 kDa, 22 kDa, 18 kDa. Obviously, the PS I fraction is strongly contaminated with other membrane

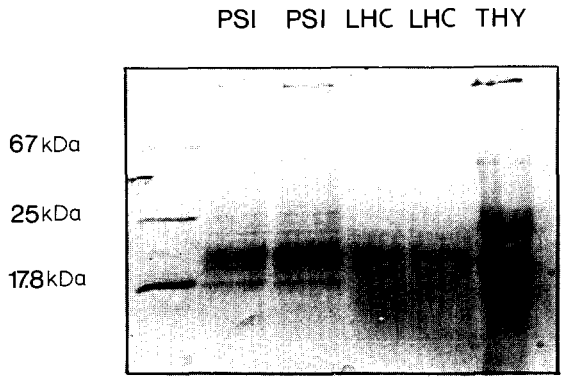


Fig. 7. Polypeptide pattern of thylakoids (THY), PS I complex (PSI) and LHC isolated by sucrose-density centrifugation.

components, especially with PS II and/or light-harvesting antenna units.

## Discussion

The yellow-green algae consist in various taxa, which contain only small amounts of chlorophyll *c*. They lack fucoxanthin, a pigment usually present in heterocontic algae. Within this group the Xanthopyceae and the Eustigmatophyceae can be distinguished by their carotenoids. The Eustigmatophyceae possess as main xanthophyll violaxanthin which is replaced by diadinoxanthin in the xanthophytes [11,12]. The present work shows that under mild separation conditions using a sucrose-density centrifugation two different chlorophyll proteins can be isolated without any losses of pigments. The resulting LHC is considerably pure, whereas the PS I complex contains some non specific polypeptides. The method of SDS-PAGE reveals three complexes with very high losses of pigments.

The features of the PS I complex from Pleurochloris are very similar to those of chlorophycean alga [10,13,14]. It exhibits a long-wavelength absorption at 682 nm and a long-wavelength emission at 715 nm in liquid nitrogen. The origin of the 695 nm emission could be caused either by a Photosystem II impurity or by an altered chlorophyll species of Photosystem I. There is no variability in the existence of long-wavelength emission as reported from other chlorophyll *c*-containing algae [15]. It seems that the PS I complex is associated with a 'peripheric' antenna system. There is no clear evidence that this antenna system is specifically associated with PS I like the LHC I from higher plants and green algae [16].

The pigment composition of the LHC shows the xanthophylls, heteroxanthin, diadinoxanthin and vaucheriaxanthin ester, which amount in total to 500 molecules per 1000 chlorophyll *a*. The ratio chlorophyll *a*/chlorophyll *c* is found to be 8 : 1. These numbers can be considered to reflect in-vivo conditions because no pigment was lost. Brown [7] reports similar xanthophyll/chlorophyll *a* ratios in the yellow-green alga Nannochloropsis, that is a member of the Eustigmatophytes, lacking measurable amounts of chlorophyll *c*. It is not clear yet, if all the three mentioned xanthophylls act as true light-harvesting antenna pigments. But on the basis of the very efficient energy transfer from blue-green light to chlorophyll *a* in yellow-green algae the xanthophylls likely play the same role as chlorophyll *b* in green algae. The absorption capacity of chlorophyll *c* is only little even in the blue-wavelength region, if one considers its low concentration.

The partial deconnection of the pigments by adding SDS or by aging the protein leads to an intensive chlorophyll *c* fluorescence, especially if the xanthophylls are excited. Therefore, chlorophyll *c* mediates the energy transfer from xanthophylls to chlorophyll *a*.

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