Immunogold Localization of LHCP II Apoprotein in the Golgi of *Euglena*

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ABSTRACT. The light-harvesting chlorophyll a/b complex (LHCP II) of *Euglena gracilis* contains a 26.5-kDa polypeptide. This polypeptide is low or undetectable in wild-type cells grown in darkness or in dark-grown resting wild-type cells exposed to 7 ft-c of light (the threshold for chloroplast development). On exposure of dark-grown resting cells or cells at 7 ft-c to a normal intensity for development (80 ft-c), the 26.5-kDa apoprotein is rapidly formed. Using a highly specific antibody to the 26.5-kDa apoprotein, little reaction can be detected in electron micrographs of dark-grown resting cells or the same cells at 7 ft-c, as determined by deposition of protein A-gold. However, in the same cells after exposure to 80 ft-c of light, gold deposition over the Golgi apparatus as well as the thylakoids of the plastids can be seen. No deposition is seen if preimmune serum is substituted for immune serum.

The photosynthetic antenna system of *Euglena* contains a light-harvesting chlorophyll a/b complex (referred to as LHCP II) which yields a major polypeptide of 26.5-kDa. This polypeptide has been shown to be low or undetectable in a number of mutants low in *b* (G₁BU, Gr₁BSL and O₄BSL) and in wild-type cells grown in darkness (2) or in dark-grown nondividing wild-type cells exposed to low intensities of light at the developmental threshold (7 ft-c) (11). Even after prolonged exposure to light at the developmental threshold, development does not proceed further unless the cells are shifted to a more normal light intensity for development (80–150 ft-c) whereupon there is a rapid and selective accumulation of chlorophyll *b* and the 26.5-kDa LHCP II apoprotein (11). The LHCP II apoprotein is known to be transcribed in the nucleus and to be translated in the cytoplasm of a number of organisms including *Euglena* (4, 7, 8, 9), resulting in the formation of a precursor of the mature molecule which contains a transit sequence. This precursor is thought to attach to the and on entry, the transit sequence is removed and the resulting mature apoprotein is incorporated into the photosynthetic thylakoid membrane. In the course of this work on the photocontrol of the formation of the 26.5-kDa LHCP II apoprotein in *Euglena* using a

highly specific antibody, we observed a clear antibody reaction in the Golgi of *Euglena* using immunoelectron microscopy. This finding and its developmental implications are the subject of the present communication.

MATERIALS AND METHODS

Procedures based on previously described methods (11, 12) were used to prepare dark-grown resting cells of *Euglena gracilis* Klebs var. *bacillaris* Coli. Cells taken from cultures maintained on slants of Hutner's pH 3.5 medium (3) for many generations in complete darkness were used to inoculate liquid Hutner's pH 3.5 medium. These cultures were grown on shakers in darkness and were transferred weekly. For transfer to resting medium, the cell density was allowed to reach about 10⁶ cells/ml, and 200 ml of culture was added aseptically to 500 ml of sterile resting medium containing 0.5% (w/v) mannitol, 10 mM KH₂PO₄ and 10 mM MgCl₂ adjusted to pH 5.0 with KOH. After shaking for 3 days in darkness resting cultures of the *bacillaris* strain were exposed to low light intensity (7 ft-c) for up to 168 h. Some cultures at 7 ft-c were moved to normal saturating light (80 ft-c) after 72 h. Dark-grown resting cultures of the *bacillaris* or Z strain were exposed to 80 ft-c of light directly to induce normal development. Light was supplied by Toshiba daylight fluorescent lamps and was measured with a Photocell Illuminometer API-5 (Toshiba).

The preparation and properties of highly specific antiserum to the 26.5-kDa LHCP II apoprotein have been described previously (2). The purified antiserum and similarly purified preimmune serum obtained from the same rabbits before im-

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Abbreviation: LHCP II; light-harvesting chlorophyll a/b binding protein of photosystem II.

munization were used in the experiments to be described. The antiserum was found to contain only antibodies to the 26.5-kDa LHCP II apoprotein (2).

For electron microscopy, glutaraldehyde was added to the cell suspension to a final concentration of 2% (v/v), and after standing in an ice bath for 5 min the suspension was centrifuged at $500 \times g$ for 3–5 min. The cells were then resuspended in 0.1 M potassium phosphate buffer pH 7.2 containing 1.5% glutaraldehyde and were allowed to incubate for 45 min at 4°C. The cells were then washed three times with phosphate buffer alone by centrifugation and the pellet was embedded in 2% agarose which was then cut into small cubes. The cubes were passed through an ethanol series (50% to 90% v/v) and were then placed in acetone. The cubes were then embedded in Epon 812 resin (Tabb Lab.). Thin sections, cut with a diamond knife on a Sorvall MT-1 microtome, were placed on nickel slit grids which were floated section side down on drops of 0.01 M phosphate buffer (pH 7.0) containing 0.85% (w/v) sodium chloride (phosphate-buffered saline, PBS) and additionally, 0.5% (w/v) bovine serum albumin (BSA), for 30 min at room temperature. The grids were then floated on PBS with 0.5% BSA containing antiserum or preimmune serum at 100–400 fold dilution for 20 min at 37°C. The grids were then washed 5 times in PBS by flotation, and were then floated on PBS containing a 20-fold dilution of fifteen nm of protein A-gold (E•Y Labs, USA) for 20 min (6). The grids were subsequently floated on 3% (w/v) uranyl acetate for 10–20 min and after drying were viewed in a JEOL 100B electron microscope at 80 kV.

RESULTS AND DISCUSSION

Figure 1 illustrates that dark-grown resting cells of *Euglena gracilis* exposed to 7 ft-c of light for 72 h show little or no reaction with anti-LHCP II antibody as determined by deposition of particles from protein A-gold. Even if the incubation at 7 ft-c is prolonged to 168 h, there is little gold deposition (Fig. 2 arrowheads). This is in agreement with previous data which show that comparable cells do not accumulate LHCP II or the rest

of the antennas under these conditions (11). When cells that have been incubated for 72 h at 7 ft-c are shifted to 80 ft-c for 2 h, conditions that are known to induce antenna formation (11), considerable gold deposition is found over the Golgi apparatus and somewhat less over the plastids (Figs. 3 and 4). That the gold deposition is indeed in the Golgi apparatus is verified in Figs. 4 and 5. Figure 4 shows that gold deposition is localized over cisternae and certain dense areas. These results were also obtained using the freeze-substitution method (figures not shown). Dark-grown resting cells incubated for 72 h at 7 ft-c followed by 80 ft-c for 8 h yield serial sections in which the gold label is clearly present at all levels of two Golgi complexes regardless of their orientation (Fig. 5 A–D). Exposure of the cells to the higher light intensity after 72 h at 7 ft-c results in the formation first of “curly” thylakoids, i.e., thylakoids that are curved at their tips. There was some intimation of this in previous work with much more primitive fixation (1), but further work will be necessary to describe these structures completely and to follow their development into straight, stacked thylakoids. However, plastids incubated at the higher intensity for 8 h after 72 h at 7 ft-c clearly show gold particles over the thylakoids (Fig. 6). Indeed, in all cases, the gold particles, representing reaction with LHCP II antibody and protein A-gold over the plastids are always seen to be closely associated with the thylakoids themselves and not with the stroma or other structures (Fig. 9). This is to be expected for a polypeptide which is known to be part of an insoluble thylakoid antenna complex. The Golgi still shows some label at 8 h of illumination at the higher intensity, but no gold label is seen in the Golgi in the cell containing fully developed chlorophyll whose thylakoids are heavily labelled (Fig. 8). At all stages (see, for example, Fig. 7) substitution of preimmune serum for immune serum eliminates gold deposition over both the Golgi complex and the plastids.

We have also used LHCP II antibody and protein A-gold in dark-grown resting cells of *Euglena* undergoing

- Fig. 1.** Dark-grown resting cell of *Euglena gracilis* var. *bacillaris* incubated at 7 ft-c for 72 h. Arrow shows Golgi apparatus. $\times 21,600$. C, chloroplast; G, Golgi apparatus; M, mitochondrion; N, nucleus; P, plastid; Pm, paramylum; Py, pyrenoid; T, thylakoid and V, vacuole. All sections were treated with antiserum to LHCP II except those (as noted) which were treated with preimmune serum.
- Fig. 2.** Dark-grown resting cell of *E. gracilis* var. *bacillaris* incubated at 7 ft-c for 168 h. The arrowheads show gold particles (low immunoreaction with LHCP II antibody). $\times 30,000$.
- Fig. 3.** Dark-grown resting cell of *E. gracilis* var. *bacillaris* incubated at 7 ft-c for 72 h followed by 80 ft-c for 2 h. Note immunoreaction over plastid thylakoid and Golgi apparatus. Arrow shows Golgi apparatus. $\times 14,000$.
- Fig. 4.** Dark-grown resting cell of *E. gracilis* var. *bacillaris* incubated at 7 ft-c for 72 h followed by 80 ft-c for 2 h. An enlargement of the Golgi apparatus is shown. Note that gold particles (immunoreaction with LHCP II antibody) are concentrated over cisternae (arrow) and certain dense areas. $\times 172,500$.
- Fig. 5.** Dark-grown resting cell of *E. gracilis* var. *bacillaris* incubated at 7 ft-c for 72 h followed by 80 ft-c for 8 h. Four consecutive serial sections (A–D) through Golgi apparatus. G₁ and G₂ are two separate Golgi with different orientations. $\times 51,000$.
- Fig. 6.** Dark-grown resting cell of *E. gracilis* var. *bacillaris* incubated at 7 ft-c for 72 h followed by 80 ft-c for 8 h. An arrow shows Golgi apparatus. $\times 52,500$.
- Fig. 7.** Dark-grown resting cell of *E. gracilis* var. *bacillaris* incubated at 7 ft-c for 72 h followed by 80 ft-c for 8 h. The section was treated with preimmune serum. $\times 51,000$.

Fig. 8. Dark-grown resting cell of *E. gracilis* var. *bacillaris* incubated at 7 ft-c for 72 h followed by 80 ft-c for 96 h. An arrow shows Golgi apparatus. Note that no gold label is seen in the Golgi apparatus. $\times 18,000$.

Fig. 9. Dark-grown resting cell of *E. gracilis* var. *bacillaris* incubated at 7 ft-c for 72 h followed by 80 ft-c for 96 h. A magnification of part of a chloroplast. Note that gold deposition is localized over thylakoids (arrows show). Star mark shows stroma. $\times 48,000$.

normal light-induced plastid development (figures not shown). No deposition of gold is observed in electron micrographs of dark-grown resting cells in agreement with the previous finding that the LHCP II polypeptide is very low or undetectable in this material (11). On exposure to light, gold deposition over the Golgi and thylakoids of the plastids is seen that is extremely similar to that already documented in this paper for the cells shifted from low light (7 ft-c) to high light (80 ft-c). Thus the appearance of immunoreacting LHCP II polypeptide in the Golgi (and of course plastids) is not a peculiarity of the intensity control of LHCP II formation but is characteristic of normal chloroplast development.

Nuclear-coded, cytoplasmically synthesized chloroplast and mitochondrial proteins usually originate as messages which, on translation, yield a protein that is somewhat larger than the final mature polypeptide, (see Schmidt and Mishkind (10) and Keegstra and Olsen (5)

for discussion and references). The increased size is due to the presence of an additional polypeptide sequence (called a "transit sequence") which is thought to be involved in the uptake of the polypeptide by the appropriate organelle; in the process of uptake the transit sequence is removed and the mature protein finds its proper location within the organelle.

However, in *Euglena*, the LHCP II precursor produced is much larger than usual (110–207 kDa) (8) and presumably contains multiple copies of the LHCP II precursor (i.e., LHCP II plus transit sequence). The simplest hypothesis, based on the present work, is that the large polypeptide is cleaved to individual LHCP II precursor molecules (each containing a transit sequence) would then be taken up by the chloroplasts and processed in the usual way. However, other possibilities (such as addition of other LHCP II components in the Golgi and later cleavage in the chloroplasts) are not

ruled out.

It has also been shown that light does not change the level of translatable RNA for the large polypeptide (8) and, therefore, photocontrol is exerted at the translational level (7). Since we find little or no anti-LHCP II reactive material in the Golgi or plastids in dark-grown cells or in cells exposed to 7 ft-c for prolonged periods, light must exert its effect either at translation or during movement of the translation product from the ribosomes to the Golgi complex and plastids.

We do not know whether there is Golgi involvement in LHCP II accumulation in other species. In *Euglena*, however, the production of a very large precursor may necessitate a processing step in the Golgi to cleave this to the more normal size. Whether this is connected at all with the mode of control of LHCP II formation in *Euglena* and whether the Golgi may modify the nascent LHCP II material in other ways remain subjects for further study.

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