A New Subunit of Cytochrome $b_{6}f$ Complex Undergoes Reversible Phosphorylation upon State Transition*

Patrice Hamel‡, Jacqueline Olive§, Yves Pierre¶, Francis-André Wollman, and Catherine de Vitry**

From the Department of Chemistry and Biochemistry, UCLA, Los Angeles, California 90095-1569, the Institut Jacques Monod, Université de Paris VII, 75005 Paris, France, the Laboratoire de Physico-Chimie Moléculaire des membranes Biologiques, CNRS UPR 9052, Institut de Biologie Physico-Chimique, 75005 Paris, France, and the Physiologie Membranaire et Moléculaire du Chloroplaste, CNRS UPR1261, Institut de Biologie Physico-Chimique, 75005 Paris, France

The Journal of Biological Chemistry Vol. 275, No. 22, Issue of June 2, pp. 17072–17079, 2000

A 15.2-kDa polypeptide, encoded by the nuclear gene PETO, was identified as a novel cytochrome $b_{6}f$ subunit in Chlamydomonas reinhardtii. The PETO gene product is a bona fide subunit, subunit V, of the cytochrome $b_{6}f$ complex, because (i) it copurifies with the other cytochrome $b_{6}f$ subunits in the early stages of the purification procedure, (ii) it is deficient in cytochrome $b_{6}f$ mutants accumulating little of the complex, and (iii) it colocalizes with cytochrome $f$, which migrates between stacked and unstacked membrane regions upon state transition. Sequence analysis and biochemical characterization of subunit V shows that it has a one transmembrane α-helix topology with two large hydrophilic domains extending on the stromal and luminal side of the thylakoid membranes, with a luminal location of the N terminus. Subunit V is reversibly phosphorylated upon state transition, a unique feature that, together with its topological organization, points to the possible role of subunit V in signal transduction during redox-controlled short term and long term adaptation of the photosynthetic apparatus in eukaryotes.

The cytochrome bc complexes such as bc$_1$ and bc$_{6}f$ are ubiquitous in energy-transducing membrane systems; their major metabolic function is to couple the oxidation of quinols to the translocation of protons across the membrane, resulting in the establishment of a transmembrane electrochemical proton gradient required to drive the synthesis of ATP (1). Beside its role in photosynthetic electron transport, the cytb$_{6}f$ complex is also recruited for redox sensing and signal transduction in chloroplasts (2, 3). The cytb$_{6}f$ complex plays a key role in the supramolecular reorganization of the photosynthetic apparatus upon state transitions: changes in the redox state of plastoquinones (PQs) that bind to cytb$_{6}f$ complexes allow a photosynthetic cell to adapt to changes in light quality as well as to changes in intracellular ATP levels (4). At the molecular level, plastoquinol binding at the $Q_r$ site of cytb$_{6}f$ complexes on the lumen side of the thylakoid membranes activates a kinase that phosphorylates the light-harvesting antenna on the stromal side of the membranes (5–9). How the plastoquinol binding signal is transduced across the membrane for kinase activation remains unknown. This reversible protein phosphorylation process controls the migration of the antenna and the cytb$_{6}f$ complex between the stacked membrane regions containing photosystem II (PSII) and the unstacked membrane regions containing PSI (10). Studies with Chlamydomonas reinhardtii in vivo have shown that state I corresponds to a low phosphorylation of the peripheral antenna proteins that are mainly associated with PSII, with the photosynthetic apparatus being set for a linear electron flow from PSII to PSI aimed at carbon fixation; in state II, the peripheral antenna proteins become heavily phosphorylated, are mainly associated with PSI and the photosynthetic apparatus is set for cyclic electron flow and supplies high ATP levels (4, 11).

C. reinhardtii displays the same photosynthetic apparatus as that of vascular plants but shows dispensable photosynthesis. Its cytb$_{6}f$ complex has been purified and comprises at least seven subunits (12). cyt$_f$, cyt$_b$, and subunit (suV) are identified, respectively, by the chloroplast genes petA, petB, and petD (13), whereas the Fe$_3$S$_2$ Rieske protein is encoded by the nuclear gene PETC (14). In addition, there are three small 4-kDa hydrophobic subunits, each forming a single transmembrane α-helix, products of the chloroplast genes petG (15) and petL (16), and the nuclear gene product PETM (17). Recently, a putative fourth small subunit has been identified as a product of a chloroplast gene petN in higher plants (18). This gene is located in the nucleus in Volvox (19) and in C. reinhardtii as suggested by homologies to expressed sequence tags (20).

Last, a nucleus-encoded 19-kDa polypeptide, termed suV, had been proposed to associate with the rest of the cytb$_{6}f$ subunits in C. reinhardtii, based on its presence in cytb$_{6}f$-enriched fractions and its absence or low representation in cytb$_{6}f$-deficient mutants (21).

We report here the biochemical, molecular, and topological characterization of suV, encoded by the gene termed PETO and identified just after PETN, and provide evidence that it is conserved in other photosynthetic eukaryotes. The potential roles played by this cytb$_{6}f$ phosphoprotein in photosynthetic electron transport, redox sensing, and signal transduction are discussed.
C. reinhardtii Strains—Wild-type and mutant strains ΔpetB and ΔpetD (22), petC-Δ1 (6), and ATP synthase mutant F54 (23) were grown on Tris acetate-phosphate medium, pH 7.2, at 25 °C under dim light (5–6 μmol of photons m\(^{-2}\) s\(^{-1}\)).

**suV Protein Microsequencing**—Thylakoid membranes (1.5 mg of chlorophyll/ml) from the F54 mutant, deficient in ATP synthase, were solubilized 15 min at 4 °C in the presence of 2.2% Mega-8 (w/v), 20 mM Tricine, pH 8.0, 3 mM KCl, and 3 mM MgCl\(_2\). The solubilized supernatant, which is enriched in cytb\(_f\) complex was separated on 12–18% SDS-polyacrylamide gels in the presence of 8 M urea, and electrottransferred onto polyvinylidene difluoride membranes in a semidry system as described previously (24). Sequencing of the band containing suV and of a tryptic fragment was performed according to Edman degradation by J. d’Alayer (Laboratoire de Mise en Œuvre des Protéines, Institut Pasteur, Paris).

**Data and RNA Analyses**—C. reinhardtii cDNA library in phage λgt10 (25) was generously provided by L.-G. Franzén (Department of Plant Physiology, Botanical Institute, Göteborg University, Sweden). Phage DNA of the cDNA library was prepared out of 10\(^9\) phage particles from liquid Escherichia coli culture as described previously (26). Desalted oligonucleotides were purchased from Oligo Express (Paris, France). Polymerase chain reaction procedure followed was as in Ref. 6, and the annealing temperature was 55 °C. Two phage-specific primers (5'-TGAGCAAGTTCCAGCTTGAAGTC-3'; 5'-GCTTATTTCTTCCAGGGTA-3') were added to thylakoid membranes at a final chlorophyll concentration of 10 μg/ml. Primers were amplified using Ampli-Taq DNA polymerase (Perkin-Elmer Cetus) solubilization of thylakoid membranes (12). Desalted oligonucleotides were purchased from Oligo Express (Paris, France). Polymerase chain reaction (PCR) procedure followed was as in Ref. 25 with 10 μm NaF and 10 μm EDTA added prior to 4 °C. After 30 min, the prelabeled cells were placed for 30 min in either state I or state II conditions. For state I, cells were illuminated at 50 μmol of photons m\(^{-2}\) s\(^{-1}\) in the presence of 10 \(-5\) M 3,4-dichlorophenyl-1,1-dimethylethylamine to get oxidation of the PQ pool. For state II, the PQ pool was reduced by placing dark-adapted cells in anaerobic conditions using 20 mM glucose and 2 mg/ml glucose-oxidase as described previously (31). State I and II cells were rapidly broken at 4 °C with a French pressure cell at 4000 psi after adding 10 μm NaF, 10 μm EDTA, 0.1 μm sucrose, and protease inhibitors to the suspension medium. Thylakoid membrane proteins were prepared as in Ref. 25 with 10 μm NaF and 10 μm EDTA added in all buffers.

**suV Immunoprecipitation**—In vivo \(^{32}P\)-labeled thylakoid membrane proteins were solubilized in the presence of 2% SDS at 100 °C for 50 s and diluted 10-fold in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1 mM EDTA, 2% Triton X-100, 100 μm NaF, 200 μM phenylmethylsulfonyl fluoride (buffer A). Protein A-Sepharose CL4B (Amersham Pharmacia Biotech) was incubated 30 min in 1 ml of distilled water, and washed three times in 1 ml of buffer A. To 1 ml of protein A-Sepharose CL4B in buffer A 30 μl of suV antisera was added or none as a control and samples were incubated 1 h at 4 °C and washed once in 1 ml of buffer A. The diluted solubilized thylakoid membrane proteins were mixed with 2 μl of protein A-Sepharose CL4B, which had not been in presence of suV antibodies; samples were incubated 1 h at 4 °C, and washed three times in 1 ml of buffer A. Samples were resuspended in 100 mM 1,4-dithiothreitol and 100 mM Na\(_2\)CO\(_3\) and solubilized in the presence of 2% SDS at 100 °C for 50 s for polypeptide analysis.

**RESULTS**

**Sequence and Topology of the PETO Gene Product**—We have previously identified a nucleus-encoded polypeptide of 19-kDa apparent molecular mass that was absent from the thylakoid membranes of cytb\(_f\) mutants (21). To avoid its copurification with some ATP synthase subunits that display a similar apparent molecular mass, we recovered suV (PetO) from the ATP synthase-deficient mutant F54, using a thylakoid membrane solubilization with Mega-8, which selectively extracts cytb\(_f\) complex. After gel electrophoresis and transfer onto nitrocellulose membranes, we were able to microsequence 31 residues of the N terminus and one internal tryptic fragment of purified suV (Fig. 1).

The cDNA for suV was cloned by PCR amplification of a C. reinhardtii cDNA library with a degenerate primer derived from the N-terminal microsequence and phage-specific primers. The nucleotide and amino acid sequences (GenBank™ accession number AA222893) are shown in Fig. 1 and are aligned with the sequence obtained from N terminus sequencing. These sequences are homologous to ones found within a higher plants, i.e., AACAAUGGCGCC. The bipartite transit peptide is 51 residues long and shows features typical of a luminal-targeting sequence. The first domain is analogous to stroma-targeting peptides: it comprises an alanine in the second position, a short uncharged N-terminal region, a central region rich in residues with basic (R, K) or small (A, S) sidechains.
chains, a consensus sequence VXA just before the putative intermediate processing site. This latter feature is observed in many transit peptides for nuclear-encoded chloroplast proteins.

suV was recovered together with the other subunits of the cytb\(_f\) complex in the supernatant after solubilization with Hecameg of the thylakoid membranes (Fig. 4). Upon centrifugation of the supernatant on a sucrose gradient, part of suV spread over fractions of higher density, most likely due to membrane-associated Phosphoprotein17074.

Northern blots probed with the entire open reading frame of suV revealed a 1.3-kb transcript that corresponds to the size of the insert amplified from the cDNA library (data not shown). We also detected a few longer transcripts in low amounts, which might correspond to splicing intermediates of the primary PETO transcript.

The PETO Gene Product, suV, Behaves as a Genuine Subunit of cytb\(_f\) Complex—The antiserum raised against the N terminus of suV recognized only suV in the thylakoid membrane of C. reinhardtii (Fig. 3A). The steady-state level of suV was greatly diminished in whole cell protein extracts and in purified thylakoid membrane proteins from cytb\(_f\)-deficient mutants that do not accumulate significant levels of any of the cytb\(_f\) complex subunits (see \(\Delta_{petB}\) or \(\Delta_{petD}\) mutants, Fig. 3, A and B, which lack, respectively, cytb\(_b\) and suV). In contrast, suV still accumulated together with the other transmembrane subunits of cytb\(_f\) in mutants lacking specifically the Rieske protein, such as the petC- mutants (see also Fig. 8) or at alkaline pH. Neither treatments (Table I). Membranes were treated with chaotropic agents such as KSCN (see also Fig. 8) or at alkaline pH. Neither treatments caused any dissociation from the Rieske protein: Although it was not released from cytb\(_f\) subunits during the next purification steps that consisted of a chromatography on a hydroxyapatite column followed by a desorption step in the presence of high concentrations of ammonium phosphate.

suV Is a Transmembrane Protein with Its N Terminus Facing the Lumen of the Thylakoid Membranes—We investigated the binding of suV to the thylakoid membranes using various dissociating treatments (Table I). Membranes were treated with chaotropic agents or incubated at high ionic strength or alkaline pH. Neither cytf, cytb\(_b\), nor PetG were extracted by these treatments, as expected from their transmembrane anchoring. In contrast, typical peripheral membrane polypeptides (OEE1) were released in the supernatant. As reported earlier, the Rieske protein showed an intermediate susceptibility to dissociating conditions (28), although it has been clearly identified as a transmembrane protein in the three-dimensional structure of bc\(_1\) complexes (33, 34). suV behaved similarly to the Rieske protein: Although it was not released from the membranes at high ionic strength, it readily dissociated with chaotropic agents such as KSCN (see also Fig. 8) or at alkaline pH.

![FIG. 2. Putative transmembrane topology of suV.](http://www.jbc.org/)

Northern blots probed with the entire open reading frame of suV revealed a 1.3-kb transcript that corresponds to the size of the insert amplified from the cDNA library (data not shown). We also detected a few longer transcripts in low amounts, which might correspond to splicing intermediates of the primary PETO transcript.

The PETO Gene Product, suV, Behaves as a Genuine Subunit of cytb\(_f\) Complex—The antiserum raised against the N terminus of suV recognized only suV in the thylakoid membrane of C. reinhardtii (Fig. 3A). The steady-state level of suV was greatly diminished in whole cell protein extracts and in purified thylakoid membrane proteins from cytb\(_f\)-deficient mutants that do not accumulate significant levels of any of the cytb\(_f\) complex subunits (see \(\Delta_{petB}\) or \(\Delta_{petD}\) mutants, Fig. 3, A and B, which lack, respectively, cytb\(_b\) and suV). In contrast, suV still accumulated together with the other transmembrane subunits of cytb\(_f\) in mutants lacking specifically the Rieske protein, such as the petC-\(\Delta_1\) mutant (Fig. 3C).

suV was recovered together with the other subunits of the cytb\(_f\) complex in the supernatant after solubilization with Hecameg of the thylakoid membranes (Fig. 4). Upon centrifugation of the supernatant on a sucrose gradient, part of suV remained associated with the cytb\(_f\) complex. However, some suV spread over fractions of higher density, most likely due to aggregated forms of the isolated subunit. suV fully dissociated from the other cytb\(_f\) subunits during the next purification steps that consisted of a chromatography on a hydroxyapatite column followed by a desorption step in the presence of high concentrations of ammonium phosphate.

suV Is a Transmembrane Protein with Its N Terminus Facing the Lumen of the Thylakoid Membranes—We investigated the binding of suV to the thylakoid membranes using various dissociating treatments (Table I). Membranes were treated with chaotropic agents or incubated at high ionic strength or alkaline pH. Neither cytf, cytb\(_b\), nor PetG were extracted by these treatments, as expected from their transmembrane anchoring. In contrast, typical peripheral membrane polypeptides (OEE1) were released in the supernatant. As reported earlier, the Rieske protein showed an intermediate susceptibility to dissociating conditions (28), although it has been clearly identified as a transmembrane protein in the three-dimensional structure of bc\(_1\) complexes (33, 34). suV behaved similarly to the Rieske protein: Although it was not released from the membranes at high ionic strength, it readily dissociated with chaotropic agents such as KSCN (see also Fig. 8) or at alkaline pH.

![FIG. 2. Putative transmembrane topology of suV.](http://www.jbc.org/)
Biochemical evidence for a transmembrane orientation of suV, with the N terminus facing the lumen, was provided by analysis of proteolysis experiments. Thylakoid membrane vesicles were incubated in the presence of protease V8 (endoprotease Glu-C) and then subjected or not to sonication. Before sonication, most of the thylakoid vesicles are in a right-side-out position and exogenous proteases have no access to the lumen side of the membranes. In contrast, upon sonication, the vesicles burst and exogenous proteases have access to both sides of the membranes. suV was degraded by proteases in the absence of sonication, in contrast to the OEE2 protein used here as a control for a luminal resident protein (Fig. 5). Thus, suV exposes some protein motifs to the stromal side of the thylakoid membranes. However, the N-terminal-directed antibody detected a partly protease-protected suV fragment in unsonicated vesicles. This fragment was no longer detected after sonication. Similar observations were made using trypsin as an exogenous protease (experiments not shown). The selective detection of this fragment in unsonicated thylakoid vesicles shows that the N-terminal domain of suV extends in the lumen. The V8-produced fragment of 11-kDa apparent molecular mass should correspond to a truncated product of suV at its stromal C terminus, at E96, E109, or E115, leaving together the transmembrane and N terminus domains that have a predicted size of 10–12 kDa. However, its limited accumulation argues for the high susceptibility of the truncated product to endogenous proteases, whether they are membrane-associated or lumen-located. This high protease susceptibility of suV is also substantiated by in vivo experiments, which show a drastic decrease in suV content in strains that do not accumulate cytbf complexes (Fig. 3). This behavior is in marked contrast with that of OEE2, which is stable in the thylakoid lumen in the absence of PSI assembly (28). Therefore, we conclude from these assays with exogenous proteases that, in agreement with the sequence data, suV behaves as a transmembrane protein with the N terminus facing the thylakoid lumen and the C terminus extending into the stroma.

We then compared the immunogold labeling of thylakoid membrane vesicles that were exposed either to the anti-suV antibody, which recognizes the N-terminal sequence of the mature protein, or to a monoclonal antibody that recognizes a lumen-located motif of cytbf. Evidence for the lumen location of this cytbf epitope came from its immunodetection in mutant cells expressing only a soluble form of cytbf that lacks both the C-terminal transmembrane α-helix and thestromal stretch of the polypeptide chain (data not shown). Immunogold labeling showed an unambiguous colocalization of these two antibodies on the same inside-out vesicle (Fig. 6), giving further support to the localization of the N terminus of suV on the membrane luminal side.

A Protein Phosphorylated in State II—When the intersystem electron carriers of the photosynthetic electron transport chain switch from an oxidized (state I) to a reduced (state II) steady state, the photosynthetic apparatus undergoes a change in its supramolecular organization such that most of light-harvesting complex II (LHCCI) and some cytbf complex gather next to PSI in the stromal lamellae membrane regions (10). The supramolecular reorganization of the thylakoid membranes can be detected by immunocytochemistry (Table II). In this experiment, thin sections of broken cells of C. reinhardtii, pretreated in either of the two states, were incubated with colloidal gold-coupled antibodies specific for PSII, LHCCI, or cytbf antigens. The movement of LHCCI or cytbf complexes toward PSI-enriched unstacked membrane domains in state II is demonstrated in Table II by the increase in the ratio of immunogold labeling of unstacked versus stacked membrane region in state II as compared with state I. In contrast PSI remained in the stacked membrane regions in the two states. suV displayed the same behavior as cytbf, being enriched in the unstacked regions in state II as compared with state I. This change in lateral distribution between the two states further supports the association of suV with the rest of the cytbf complex, which shows lateral displacement upon state transitions.

State transitions are accompanied by reversible changes in the phosphorylation of several thylakoid membrane polypeptides. The major phosphoproteins that were previously identified in C. reinhardtii (31) belong either to the light-harvesting complex proteins (LHCP) family or to PSI (Fig. 7A). In particular, phosphorylation of the LHCP polypeptides increases in state II conditions, this process being dependent upon the presence of the cytbf complex (5). Therefore, this increased phosphorylation is not observed in mutants lacking cytbf complexes (Fig. 7A). The pattern of phosphoproteins detected in state II showed the presence of an additional component that has the same electrophoretic mobility as suV. This phosphorylated protein is absent in cytbf mutants that show very little accumulation of the cytbf complex subunits and do not undergo state transition (Fig. 7A). Fig. 7B shows that this change in phosphorylation was not accompanied by a change in the steady-state level of membrane-bound suV between states I and II. That this phosphoprotein is indeed a phosphorylated form of suV was further confirmed by its behavior upon extraction with chaotropic agents or detergents (Fig. 8A and data not shown). Furthermore, the phosphorylated protein can be immunoprecipitated by the anti-suV antiserum (Fig. 8B). Thus suV is a cytbf subunit that can be reversibly phosphorylated upon state transitions.

Conservation of suV in Other Photosynthetic Eukaryotes—We found no homologues of suV within the fully sequenced cya-
nobacterial genomes available in data banks. However, a homologue to suV (GenBank™ accession number AF110791) was identified among cDNAs from Volvox (19), which is another green alga closely related to C. reinhardtii. A BLASTP 2.0 alignment (35) showed 147 identities out of the 198 residues of suV from C. reinhardtii. In particular, the consensus processing sites, hydrophobic segments, and putative phosphorylation motifs are conserved.

Because the N-terminal peptide sequence that we have used to prepare antibodies to suV is well conserved between C. reinhardtii and Volvox, with 12 identities out of 15 residues, we attempted to see whether the antipeptide would crossreact with suV candidates from other photosynthetic eukaryotes. A protein of similar apparent molecular weight as suV was indeed recognized in thylakoid membranes from spinach (Fig. 9). This cross-reaction argues for the presence of suV in higher plant cyt_{b_6}f complexes.

**DISCUSSION**

A Transmembrane Protein Associated with the cyt_{b_6}f Complex—We first identified suV as a nucleus-encoded polypeptide of apparent molecular mass of 19 kDa that was present in cyt_{b_6}f-enriched fractions and deficient in thylakoid membranes of C. reinhardtii mutants lacking the cyt_{b_6}f complex (21). We therefore proposed that suV was a genuine subunit of the cyt_{b_6}f complex. This conclusion was subsequently challenged on the basis that suV was not recovered in highly purified and active cyt_{b_6}f preparations (12, 36). Indeed, we show here that suV dissociates from cyt_{b_6}f complex during the purification steps. Thus suV behaves as a loosely bound partner of the protein complex and is not required for plastoquinol/plastocyanin oxidoreductase activity. However, it should be considered as a
suV and labeled antennae (CP26, CP29, LHCP13, LHCP11, in state I and in state II.

A hydrophobic stretch of 17–19 residues suggested that suV terminus location of suV in the lumen was supported by proteolysis experiments and immunogold labeling. The presence of a hydrophobic stretch of 17–19 residues suggested that suV displayed a typical bipartite transit sequence with an ANA motif before the N terminus of the mature protein, we expected suV would have its N terminus domain located on the lumen side of the membrane. Indeed, the N terminus location of suV in the lumen was supported by proteolysis experiments and immunogold labeling. The presence of a hydrophobic stretch of 17–19 residues suggested that suV formed one transmembrane α-helix connecting two hydrophilic domains of about 65 residues each. The hydrophobicity of the transmembrane α-helix, 1.9 kcal/residue on the Goldman-Engelman-Steitz scale, is in the range of the other predicted transmembrane α-helices of cytb6 subunits (17). The release of suV from the membranes with chaotropic agents or in alkaline pH, when the Rieske protein but not the rest of the cytb6 complex is extracted, suggests a peripheral location of its transmembrane span with respect to the helix bundle of suV and cytb6. This distal position of the α-helix of suV within the cytb6 complex is consistent with its progressive release during cytb6 purification.

What is the Function of suV?—suV is required neither for electron transfer nor for the dimerization of the isolated cytb6 complex, because a Hecameg-based purification procedure yields suV-depleted protein complexes that are fully active and dimeric (12). However, we cannot exclude a role of suV in the electron transfer in vivo or in the supramolecular organization of the photosynthetic apparatus. For example, PufX has only an indirect role in electron transfer in Rhodobacter sphaeroides: this small transmembrane polypeptide absence retards quinone exchange between the reaction center and the bc1 complex probably due to the PufX role in the supramolecular organization of the photosynthetic apparatus (37).

Searches of the GenBank showed no sequence homologies between suV and proteins of known function, and no specific motif speak for any obvious biochemical activity. Therefore, the function of suV can only be speculated upon. There are neither histidines nor cysteines in the sequence of the mature protein.
Thus suV is the only known cyt\textsubscript{b\textsubscript{6}}-associated subunit that is reversibly phosphorylated upon state transitions in \textit{C. reinhardtii}. It suggests that suV is a possible key partner of the phosphorylation-mediated state transition process in photosynthetic eukaryotes. The \textit{PETO} gene is absent from cyanobacterial chromosomes is consistent with such a function because cyanobacteria use a different, although presently poorly understood, mechanism to perform state transitions. State transitions in photosynthetic eukaryotes encompass the redox-controlled activation of a kinase and the subsequent redistribution of LHCCI and cyt\textsubscript{b\textsubscript{6}} next to PSI, once LHCCI proteins are phosphorylated. We found no evidence that suV could act as a kinase by itself, because there are no known kinase motifs in its amino acid sequence. Moreover, the peaks of kinase activity that are detected along the sucrose gradient loaded with the cyt\textsubscript{b\textsubscript{6}}-enriched supernatant do not match suV distribution. The available data rather suggest that suV could play a role in the activation of the LHCCI kinase. There is a need for signal transduction upon kinase activation by reduced plastoquinol: the redox sensor for kinase activation is the Q\textsubscript{6} site in the cyt\textsubscript{b\textsubscript{6}} complex, which is located on the lumen side of the membranes (6–9), whereas the catalytic site of the kinase is on the stromal face of the thylakoid membranes where reside all of the target sites for protein phosphorylation. The redox activation of the kinase via changes at the Q\textsubscript{6} site of cyt\textsubscript{b\textsubscript{6}} complexes is most likely accompanied by significant conformational changes of the Rieske protein in the thylakoid lumen (7–9). suV transmembrane topology with two large hydrophilic domains extending on both sides of the membrane has features of a typical signal-transducing protein. The extended N-terminal domain of suV in the lumen is well suited to sense the structural changes of the Rieske protein. The single transmembrane helix of suV, whose flexibility is supported by the suV sensitivity to chaotrophic agents, could transduce conformational changes to the phosphorylatable stromal C-terminal domain that interacts with the LHCCI kinase. It would then remain phosphorylated as long as the kinase is activated. The need for some signal-transducing protein in thylakoid membranes also stems from the recent finding that an immunophilin-like lumenal membrane protein (46) regulates the stromal activity of a membrane-bound phosphatase (47). The transducer protein has not yet been identified.

Redox sensing at the plastoquinone pool level has also been advocated for long term adaptation of the photosynthesis apparatus involving changes in the stoichiometry of the reaction centers and/or antenna proteins (48). In particular, the ratio of PSI to PSII centers has been proposed to be controlled by the plastoquinone pool redox state (49, 50). cyt\textsubscript{b\textsubscript{6}} is also thought to be the sensor for the up-regulation of the nuclear chlorophyll a/b binding protein genes in oxidizing conditions (51, 52). The cyt\textsubscript{b\textsubscript{6}}-associated suV is a reasonable candidate for these various signal transduction processes that control gene expression. In particular, its stromal C-terminal domain is highly basic and might interact with negatively charged mRNAs or proteins. A search for mutants showing altered expression of the \textit{PETO} gene products should provide an answer as to the possible role of suV in signal transduction.

Acknowledgments—We thank D. Bernard for immunodetection experiments, D. Drauper for RNA analysis, L.-G. Franzen for the cDNA library, T. Kallas for comments on the manuscript, J.-L. Popot for his interest and support during the early phase of the project, and O. Vallon for anti-cyt\textsubscript{b\textsubscript{6}} monoclonal antibody. We are greatly indebted to S. Merchaut (UCLA) for her financial support to P. H.

REFERENCES

1. Soriano, G. M., Ponnamare, M. V., Carrell, C. J., Xia, D., Smith, J. L., and Cramer, W. A. (1999) Bioenerg. Biocrim. 31, 201–212
2. Wollman, F.-A. (1999) \textit{The Molecular Biology of Chloroplasts and Mitochon- dria} in \textit{Chlamydomonas} (Rochaix, J.-D., Goldschmidt-Clermont, M., and Merchant, S., eds) pp 459–476. Kluwer Academic Publishers, Dordrecht, the Netherlands
3. Keren, N., and Ohad, I. (1999) \textit{The Molecular Biology of Chloroplasts and Mitochon-dria in Chlamydomonas} (Rochaix, J.-D., Goldschmidt-Clermont, M., and Merchant, S., eds) pp 569–596. Kluwer Academic Publishers, Dordrecht, the Netherlands
4. Bulte, L., Gans, P., Rebelle, F., and Wollman, F.-A. (1992) Biochim. Biophys. Acta 1092, 72–80
5. Wollman, F.-A., and Lemaire, C. (1988) Biochim. Biophys. Acta 933, 85–94
6. de Vitry, C., Finazzi, G., Baymann, F., and Kallas, T. (1999) \textit{Plant Cell} 11, 2031–2044
7. Zito, F., Finazzi, G., Delosme, R., Nitschke, W., Picot, D., and Wollman, F.-A. (1999) EMBO J. 18, 2961–2969
8. Vener, A.-V., van Kan, P. J., Rich, P. R., Ohad, I., and Anderson, B. (1997) \textit{Curr. Opin. Plant Biol.} 2, 227–233
9. Vallon, O., Bulte, L., Dainese, P., Olive, J., Bassi, R., and Wollman, F.-A. (1999) \textit{Proc. Natl. Acad. Sci. U. S. A.} 94, 1585–1590
10. Vener, A. V., Ohad, I., and Anderson, B. (1998) \textit{Curr. Opin. Plant Biol.} 1, 217–223
11. Breyton, C., de Vitry, C., Pierre, Y., and Popot, J.-L. (1995) \textit{J. Biol. Chem.} 270, 29342–29345
12. Bischlen, S., Choquet, Y., Kuras, R., and Wollman, F.-A. (1999) \textit{FEBS Lett.} 284, 257–262
13. de Vitry, C. (1994) \textit{J. Biol. Chem.} 269, 7603–7609
14. Besthold, D. A., Schmidt, C. L., and Malik, R. (1995) \textit{J. Biol. Chem.} 270, 29292–29298
15. Takashashi, Y., Rahire, M., Breyton, C., Popot, J.-L., Joliot, P., and Rochaix, J.-D. (1996) \textit{EMBO J.} 15, 2498–2508
16. de Vitry, C., Breyton, C., Pierre, Y., and Popot, J.-L. (1996) \textit{J. Biol. Chem.} 271, 10667–10671
17. Hager, M., Biehler, K., Illerhaus, J., Ruf, S., and Bock, R. (1999) \textit{EMBO J.} 18, 5834–5842
18. Meissner, M., Stark, K., Crennar, B., Kirk, D. L., and Schmitt, R. (1999) \textit{Curr. Genet.} 36, 363–370
19. Nakamura, Y., Sato, S., Fukuzawa, H., and Tabata, S. (1999) \textit{DNA Res.} 6, 369–373
20. Lemaire, C., Girard-Bascou, J., Wollman, F.-A., and Bennoun, P. (1986) \textit{Biochim. Biophys. Acta} 851, 229–238
21. Kuras, R., and Wollman, F.-A. (1994) \textit{EMBO J.} 13, 1019–1027
22. Lemaire, C., and Wollman, F.-A. (1989) \textit{J. Biol. Chem.} 264, 10235–10242
23. Atteia, A., de Vitry, C., Pierre, Y., and Popot, J.-L. (1994) \textit{J. Biol. Chem.} 269, 2564–2568
24. Atteia, A., and Franzen, L.-G. (1996) \textit{Biochim. Biophys. Acta} 2031, 792–799
25. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) \textit{Molecular Cloning: A Laboratory Manual}, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
26. Drauper, D., Girard-Bascou, J., and Wollman, F.-A. (1992) \textit{Plant Cell} 4, 283–285
27. Bischlen, S., de Vitry, C., and Popot, J.-L. (1994) \textit{J. Biol. Chem.} 269, 7597–7602
28. de Vitry, C., Olive, J., Drauper, D., Recouvreur, M., and Wollman, F.-A. (1989) \textit{J. Cell Biol.} 109, 991–1006
29. Vallon, O., Tee, G. S., Cramer, W. A., Simpson, D., Hoye-Hansen, G., and Borrud, L. (1989) \textit{Biochim. Biophys. Acta} 975, 132–141
30. Wollman, F.-A., and Delepeule, P. (1984) \textit{J. Cell Biol.} 98, 1–7
31. Franzen, L.-G., Rochaix, J.-D., and von Heijne, G. (1990) \textit{FEBS Lett.} 269, 165–168
32. Zhang, Z., Huang, L., Shulmeister, M. V., Chi, Y. I., Kim, K. H., Hung, L. W., Crofts, A. R., Berry, E. A., and Kim, S. H. (1998) \textit{Nature} 392, 677–684
33. Kim, H., Xia, D., Yu, C. A., Xiao, J., Kazunori, A. M., Zhang, L., Yu, L., and
Cytochrome $b_{6}$-associated Phosphoprotein

Deisenhofer, J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8026–8033
35. Atlschul, S. T., Madden, T. L., Schaeffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402
36. Wynn, R. M., Bertsch, J., Bruce, B. D., and Malkin, R. (1988) Biochim. Biophys. Acta 935, 115–122
37. Verméglio, A., and Joliot, P. (1999) Trends Microbiol. 7, 435–440
38. Laverne, J. (1983) Biochim. Biophys. Acta 725, 25–33
39. Joliot, P., and Joliot, A. (1988) Biochim. Biophys. Acta 933, 319–333
40. Bennett, J., Shaw, E. K., and Michel, H. (1988) Eur. J. Biochem. 171, 95–100
41. Coughlan, S. J. (1988) Biochim. Biophys. Acta 933, 413–422
42. Gal, A., Herrmann, R. G., Lottspeich, F., and Ohad, I. (1992) FEBS Lett. 298, 33–35
43. Rintamäki, E., Salonen, M., Suoranta, U.-M., Carlberg, L., Andersson, B., and Åro, E.-M. (1997) J. Biol. Chem. 272, 30476–30482
44. Testi, M. G., Croce, R., Polverino-De Lautero, P., and Bassi, R. (1996) FEBS Lett. 399, 245–250
45. Bruce, D., Brimble, S., and Bryant, D. A. (1989) Biochim. Biophys. Acta 974, 66–74
46. Fulgosi, H., Vener, A. V., Altschmied, L., Herrmann, R. G., and Andersson, B. (1998) EMBO J. 17, 1577–1587
47. Vener, A. V., Holka, A., Fulgosi, H., Andersson, B., and Herrmann, R. G. (1999) Biochemistry 38, 14955–14963
48. Allen, J. F., and Nilsson, A. (1997) Physiol. Plant. 100, 863–868
49. Chow, W. S., Melis, A., and Anderson, J. M. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7502–7506
50. Pfannschmidt, T., Nilsson, A., and Allen, J. F. (1999) Nature 397, 625–628
51. Escoubas, J. M., Lomas, M., LaRoche, J., and Falkowski, P. G. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10237–10241
52. Maxwell, D. P., Lautenbach, D. E., and Huner, N. P. A. (1995) Plant Physiol. 109, 787–795
A New Subunit of Cytochrome $b_6f$ Complex Undergoes Reversible Phosphorylation upon State Transition

Patrice Hamel, Jacqueline Olive, Yves Pierre, Francis-André Wollman and Catherine de Vitry

J. Biol. Chem. 2000, 275:17072-17079. 
doi: 10.1074/jbc.M001468200 originally published online March 21, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M001468200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 49 references, 20 of which can be accessed free at http://www.jbc.org/content/275/22/17072.full.html#ref-list-1