Translation of cytochrome *f* is autoregulated through the 5′ untranslated region of *petA* mRNA in *Chlamydomonas* chloroplasts

(chloroplast gene expression/cytochrome *b₆f* complex assembly/translational autoregulation/epistasy/*Chlamydomonas reinhardtii*)

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ABSTRACT A process that we refer to as control by epistasy of synthesis (CES process) occurs during chloroplast protein biogenesis in *Chlamydomonas reinhardtii*: the synthesis of some chloroplast-encoded subunits, the CES subunits, is strongly attenuated when some other subunits from the same complex, the dominant subunits, are missing. Herein we investigate the molecular basis of the CES process for the biogenesis of the cytochrome *b₆f* complex and show that negative autoregulation of cytochrome *f* translation occurs in the absence of other complex subunits. This autoregulation is mediated by an interaction, either direct or indirect, between the 5′ untranslated region of *petA* mRNA, which encodes cytochrome *f*, and the C-terminal domain of the unassembled protein. This model for the regulation of cytochrome *f* translation explains both the decreased rate of cytochrome *f* synthesis *in vivo* in the absence of its assembly partners and its increase in synthesis when significant accumulation of the C-terminal domain of the protein is prevented. When expressed from a chimeric mRNA containing the *atpA* 5′ untranslated region, cytochrome *f* no longer showed an assembly-dependent regulation of translation. Conversely, the level of antibiotic resistance conferred by a chimeric *petA-aadA-rbcL* gene was shown to depend on the state of assembly of cytochrome *b₆f* complexes and on the accumulation of the C-terminal domain of cytochrome *f*. We discuss the possible ubiquity of the CES process in organellar protein biogenesis.

The major oligomeric protein complexes found in organellar energy transducing membranes display a concerted accumulation of their constitutive subunits: a number of yeast respiratory mutants and *Chlamydomonas* photosynthetic mutants display a pleiotropic loss of most subunits from a given complex, although their mutations were characterized as affecting primarily only a single subunit (1–7). In most cases, these phenotypes result from a posttranslational degradation of the unassembled subunits, with no alteration in their rates of synthesis (8–10).

Studies of *Chlamydomonas* mutants have revealed another contribution to the stoichiometric accumulation of subunits during chloroplast protein biogenesis that we have referred to as a control by epistasy of synthesis (CES; refs. 11 and 12). Some chloroplast-encoded subunits display a much lower rate of synthesis in the absence of their assembly partners, as detailed in Table 1 (6, 13–17). Thus, chloroplast protein synthesis is to some extent hierarchical. We define CES subunits as subunits whose rate of synthesis appears assembly-dependent and define dominant subunits as those whose absence results in reduced synthesis of CES proteins. A variety of mechanisms can account for these observations. The em-

ployed mechanism may actually differ from one CES subunit to another.

Herein we address the molecular basis of the CES process for the biogenesis of the cytochrome *b₆f* complex. Mutant strains lacking cytochrome *b₆* or subunit IV of the cytochrome *b₆f* complex (SUIV), two major chloroplast-encoded subunits of this complex, show an apparent 90% decrease in the rate of cytochrome *f* synthesis, which is not accompanied by a decreased half-life of the protein. Therefore, a 10% accumulation of unassembled cytochrome *f* occurs in the membrane. In contrast, SUIV and cytochrome *b₆* are rapidly degraded in the absence of cytochrome *f*, with no alteration of their synthesis rate (6). SUIV and cytochrome *b₆*, therefore, are dominant over cytochrome *f* in the hierarchical organization of subunit synthesis for cytochrome *b₆f* complex biogenesis.

Cytochrome *f* is encoded by the chloroplast *petA* gene. It is a *c*-type cytochrome, synthesized as a precursor protein with a lumen-targeting peptide that drives the translocation of most of the apoprotein through the thylakoid membrane. Preapocytochrome *f* then matures to holocytochrome *f* on the luminal side of the thylakoid membrane, by covalent heme attachment and cleavage of the targeting peptide (for reviews, see refs. 18 and 19). Mature cytochrome *f* remains membrane-associated through a C-terminal-located α-helix. Interestingly, two mutant strains in which a cytochrome *f* variant was obtained by site-directed mutagenesis showed a 3-fold increase in the rate of cytochrome *f* synthesis. In one case, the protein, truncated at its C terminus, lacked its transmembrane anchor and its stromal extension of 15 residues. The resulting mutant overexpressed a soluble form of cytochrome *f* that accumulated in the thylakoid lumen (20). In the other case, a highly protease-sensitive polypeptide was created by substituting the cysteinyl residues involved in the covalent binding of the *c*-type heme. This hemeless cytochrome *f* was synthesized at a much higher rate than wild-type cytochrome *f*, although the modified polypeptide accumulated to less than 0.5% of the wild-type level (21).

Herein, we provide conclusive evidence that the contrasting effects on cytochrome *f* synthesis—underexpression in absence of SUIV versus overexpression in strains lacking significant accumulation of the C-terminal (C_ter) domain of the protein—originate from a common molecular mechanism: a negative feedback regulation of cytochrome *f* translation, governed by the steady-state concentration of a structural protein motif present in the C_ter domain of the unassembled protein (12); this motif, which is shielded upon assembly but totally or virtually absent in the truncated or hemeless cytochrome *f* mutants, is readily accumulated and highly exposed

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Table 1. The CES subunits in *C. reinhardtii*

Photosynthetic protein complex	Subunits		
	Dominant	CES	Ref.
Cytochrome b_6f	SUIV	cyt. *f*	6
Photosystem I	PsaB	PsaA	15
Photosystem II	D2	D1	14
	D1	apoCP47	13
ATP synthase	Su. β	Su. α	16
RUBP	SS	LS	17

Absence of the indicated dominant subunits results in attenuated synthesis of the corresponding CES subunit.

RUBP, ribulose bisphosphate carboxylase; cyt. *f*, cytochrome *f*; Su., subunit; SS, small subunit; LS, large subunit.

in mutants failing to synthesize an assembly partner such as cytochrome b_6 or SUIV. We show that the *petA* 5′ untranslated region (UTR) contains all of the target information required for this regulation, thus ruling out posttranslational regulation and arguing for a control at the level of translation initiation.

MATERIALS AND METHODS

Strains, Media, and Genetic Methods. A wild-type *Chlamydomonas reinhardtii* strain (mt$^+$) derived from strain 137c and a *petA* (mt$^+$) deletion strain (6) were used for chloroplast transformation experiments. The other mutant strains used in this study were the deletion strain Δ*petD* (mt$^+$), previously named ΔQ in ref. 6, the nuclear mutant strains *mcd1-F16* (mt$^-$) (22, 23) and *ccs1-ac206* (mt$^-$) (24), and the F52L-55V (mt$^+$) transformant (21). Wild-type and mutant strains were grown on Tris–acetate–phosphate (TAP) medium (pH 7.2) at 25°C under dim light (5–6 μE per m^2 per s) (25). Crosses were performed as described (25). Characterization of cytochrome b_6f mutant phenotypes was carried out by using their fluorescence induction kinetics (26, 27).

Antibiotic-Resistance Tests. Cells initially grown on TAP medium were transferred to selective medium by streaking them continuously with a platinum loop from a zone at high concentration down to a zone where individual clones could be recovered. After 8–10 days, their sensitivity to antibiotics was detected by the simultaneous observation of two phenomena: a change from a dark-green to a yellow-brown color in the region of high cell density and the absence of division figures from individual cells in the region of low cell density, as determined by light microscopy.

Nucleic Acid Manipulations. Plasmid pF52L-55VΔ*petD* with the heme-attachment-defective cytochrome *f* sequence, a deletion of the *petD* gene, and the *aadA* cassette (conferring spectinomycin and streptomycin resistance) was constructed by replacing the entire *petA-petD* intergenic region located downstream of the *aadA* cassette in plasmid pAF52L-55V (21) with the 3′ UTR of the *petD* gene, using the strategy as described (20). Previous experiments have shown that this replacement does not modify the expression of the *pet* genes (ref. 28 and R.K., unpublished results).

Oligonucleotides FMETDIR (5′-TCGCGA<u>CATGTC</u>CTA-ACCAAGTATTTACTACT-3′) and FSTOPINV (5′-ACG-GC<u>TGCAG</u>TTAGAAGTTCATTTCTGCTA-3′) were used as primers with plasmid piWF (6) as a template to amplify by PCR the 950-bp cytochrome *f* coding sequence. The amplified fragment, after digestion with *Afl*III and *Pst*I (two restriction sites underlined in the sequences of the oligonucleotides above), was cloned into the vector pdFBE (6) that had been digested with *Nco*I and *Pst*I to create plasmid pAFRF. This substituted the *aadA* coding region from plasmid pdFBE with the *petA* coding region fused in-frame to the first 25 amino acids of the *atpA* gene, which were present in plasmid pdFBE (6, 29, 30). pAFRF was then digested with *Acc*I and *Sca*I, and

the resulting 3.7-kbp fragment was ligated to the 4.5-kbp fragment obtained from digestion of piWF with the same enzymes to yield plasmid pAFFF.

Oligonucleotides PETAPROM (5′-GCG<u>AATTC</u>GCAG-GCAGTGGCGGTACC-3′) and PETAATG (5′-GC<u>GGATC-C</u>ATGGACATAATTTTATTAATCTTAAAAC-3′) were used as primers to amplify the 690-bp 5′ region of the *petA* gene containing the *petA* promoter and the *petA* 5′ UTR, including the methionine initiation codon and the second amino acid (serine) of cytochrome *f*. The amplified fragment was digested with *Eco*RI and *Bam*HI (two restriction sites underlined in the sequence of the oligonucleotides above) and cloned into the vector pBKS$^-$ to yield plasmid p5F. p5F was digested with *Nco*I (boldface type in the oligonucleotide sequence above) and *Alw*NI, which cleave immediately downstream of the *petA* initiation codon and at the *ori* locus of the pBKS$^-$ vector, respectively. The resulting 1,550-bp fragment was ligated to the 3.1-kbp fragment obtained by digesting PUC-ATPX-AAD (29) with the same enzymes to create plasmid pFKR. The cassette FKR was then removed from the plasmid by digestion with *Eco*RV and *Sma*I and cloned into the unique *Stu*I site of the plasmid pR12–23 (31) to create plasmid pFKR12.

Transformation of *C. reinhardtii*. Wild-type and Δ*petA* cells were transformed by tungsten-particle bombardment as described (6). Phototrophic transformants were selected on minimum medium at 5–6 μE per m^2 per s. Transformants containing the *aadA* cassette were selected on TAP/spectinomycin (100 μg/ml)-containing plates. Resistant clones were then screened by fluorescence for defective cytochrome b_6f activity and subcloned on spectinomycin-containing plates until they reached homoplasmy, as determined by DNA filter hybridizations. At least three transformants were analyzed for each construct. Pulse-labeling experiments, protein isolation, separation, and analysis were carried out as in ref. 6.

RESULTS

Strains That Express a Short-Lived Form of Cytochrome *f* Escape the CES Process. Fig. 1 shows a pulse-labeling study comparing strains that underexpress (Δ*petD*) or overexpress (F52L-F55V) cytochrome *f* as compared with the wild-type control. The Δ*petD* strain lacks the chloroplast *petD* gene encoding SUIV of the cytochrome b_6f complex. Consequently, cytochrome *f*, being a CES subunit, displays a marked decrease in its rate of synthesis, resulting in the accumulation of only 10% of the wild-type level (8) but all in an unassembled
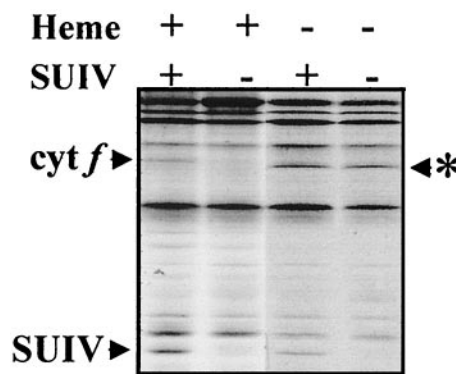

FIG. 1. Synthesis of hemeless cytochrome *f* escapes the CES process. Chloroplast translates from wild-type, Δ*pet*D, F52L-55V, and F52L-55VΔ*petD* strains (lanes from left to right). Whole-cell polypeptides were pulse-labeled for 5 min with [^{14}C]acetate in the presence of an inhibitor of cytoplasmic translation and separated in SDS/12–18% polyacrylamide gels in the presence of 8 M urea. Arrowhead, positions of cytochrome *f* and SUIV deduced from comparison with known polypeptide patterns; *, position of apocytochrome *f*, which migrates slightly faster than holocytochrome *f* (20).

configuration. The F52L-55V strain, because of the substitution of the two cysteinyl residues involved in covalent heme binding to apocytochrome *f*, expresses a highly protease-sensitive variant of cytochrome *f* that does not accumulate *in vivo* (<0.5% of the wild-type level of cytochrome *f*). The rate of synthesis of hemeless cytochrome *f* is approximately 3-fold higher than that of wild-type cytochrome *f* (2).

We wished to determine whether the increased rate of cytochrome *f* synthesis in F52L-55V reflected a regulatory mechanism that was similar to that responsible for the decreased rate of cytochrome *f* synthesis in Δ*petD*, namely, a feedback mechanism governed by the concentration of the C_{ter} domain of unassembled cytochrome *f*. Strains expressing the unstable hemeless cytochrome *f* lack significant accumulation of the C_{ter} domain and should bypass the CES process: thus, synthesis of hemeless cytochrome *f* should be insensitive to the presence or absence of its assembly partners. We therefore transformed the chloroplast genome of wild-type *C. reinhardtii* with plasmid pF52L-55VΔ*petD* carrying the F52L-55V mutations altering heme binding to cytochrome *f*, a deletion of the *petD* gene to prevent SUIV synthesis and a *aadA* cassette conferring spectinomycin resistance to allow selection of the desired double mutants.

We used pulse labeling to determine the rate of synthesis of hemeless cytochrome *f* in these transformants, which lack SUIV. Fig. 1 shows that its rate of synthesis was 3-fold higher than that of wild-type cytochrome *f*, whether SUIV was made (Fig. 1, F52L-55V) or not (Fig. 1, F52L-55VΔ*petD*). Therefore, this strain, which fails to accumulate the C_{ter} domain of the protein *in vivo*, no longer exhibits the CES process.

The *petA* 5′ UTR Is Required for the CES Process. The decrease of cytochrome *f* synthesis in the absence of its assembly partners could operate at the level of translation or by the susceptibility of newly synthesized polypeptide chains to proteolytic degradation. To discriminate between these hypotheses, we replaced the *petA* promoter and the *petA* 5′ UTR, where cis-acting signals for translation initiation are located, by those of the *atpA* gene (Fig. 2*A*). The *atpA* gene encodes the α subunit of the ATP synthase complex, whose expression is independent of the expression of the *pet* genes. The resulting plasmid pAFFF was introduced by biolistic transformation into the chloroplast of the Δ*petA* deletion strain. Phototrophic transformants, hereafter termed AFFF, were recovered on minimal medium, demonstrating that the promoter and 5′ UTR of *atpA* were able to drive cytochrome *f* synthesis at rates high enough to sustain phototrophic growth.

We next investigated whether the synthesis of cytochrome *f*, now under the control of the *atpA* 5′ UTR, was still regulated by the presence of its assembly partners. To this end, we crossed AFFF (mt+) with the nuclear mutant *mcd1-F16* (mt−), a strain that does not synthesize SUIV because *petD* mRNA is unstable (23). In this cross, all the daughter cells of each tetrad inherited the chimeric chloroplast gene AFFF, uniparentally transmitted by the mt+ parent. In contrast, only two members of the tetrad inherited the nuclear *mcd1-F16* mutant allele transmitted by the mt− parent, the two other members having a wild-type nuclear genome. We analyzed the expression of the *pet* genes in tetrad progeny by protein pulse labeling (Fig. 2*B*) or by immunoblotting using specific antibodies raised against cytochrome *f* or cytochrome b_6 (Fig. 2*C*). The *mcd1-F16* mutant members (the first and third members of the representative tetrad shown Fig. 2) failed to synthesize SUIV (Fig. 2*B*) and, consequently, also failed to accumulate cytochrome b_6, as did the parental *mcd1-F16* strain (Fig. 2*C*). The two other members synthesized SUIV (Fig. 2*B*) and accumulated wild-type levels of cytochrome b_6 (Fig. 2*C*).

As shown in Fig. 2*B*, the rate of synthesis of cytochrome *f* was similar in the four daughter cells of the tetrad, irrespective of the presence of SUIV. Therefore, at variance with cytochrome *f* expressed from the wild-type *petA* gene, whose
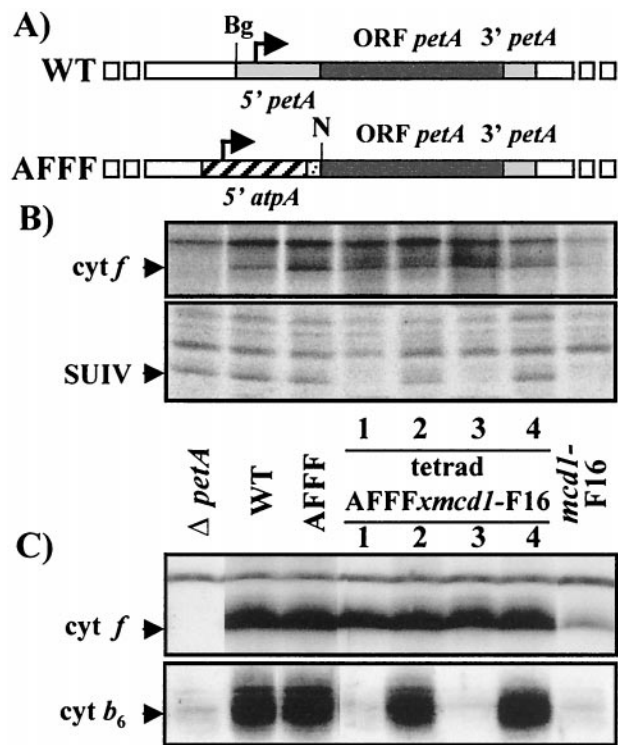

FIG. 2.   Cytochrome *f* synthesis under control of the *atpA* 5′ UTR escapes the CES process. (*A*) Maps of the *petA* gene in wild-type and AFFF strains (Bg, *Bgl*II; N, *Nco*I). The heavily hatched box in the 5′ region of *atpA* denotes the sequence encoding the 25 first amino acids of the α subunit of the ATP synthase complex. (*B*) Newly synthesized cytochrome *f* and SUIV detected by pulse-labeling experiments in Δ*petA*, wild-type, parental strains, and progeny of a representative tetrad from the cross AFFF × *mcd1-F16*. (*C*) Accumulation of cytochromes *f* and b_6, detected with specific antibodies in the same strains.

synthesis and accumulation decreased in the absence of SUIV in the parental *mcd1-F16* strain (Fig. 2, compare lanes *mcd1-F16* and WT), cytochrome *f* expressed from the chimeric *petA* gene was no longer regulated by the presence of its assembly partners. Accordingly, cytochrome *f*, being a stable protein, accumulated to the wild-type level in the four daughter cells, even though those two bearing the *mcd1-F16* mutation lacked SUIV and cytochrome b_6. No changes in *petA* mRNA levels were detected among the tetrad progeny by RNA filter hybridizations (data not shown). In summary, we conclude that translation initiation of cytochrome *f*, rather than posttranslational degradation of the polypeptide, is involved in this CES process.

A Reporter Gene, Driven by the *petA* 5′ UTR, Mimics the CES Behavior of Cytochrome *f*. If the *petA* 5′ UTR contains all the target information required for the autoregulation of cytochrome *f* synthesis, it should confer the same regulatory properties to a reporter gene translated under its control. The product of such a reporter gene should be underexpressed in an assembly-deficient strain lacking SUIV and overexpressed in strains unable to accumulate cytochrome *f*, for example, those defective in heme binding to cytochrome *f*.

We introduced the chimeric gene *petA*-5′-UTR-*aadA*-*rbcL*-3′-UTR, hereafter referred to as FKR into a neutral site of the chloroplast genome (Fig. 3*A*). The *rbcl* 3′ UTR confers stability to chimeric RNAs. As expected, the FKR cassette conferred spectinomycin and streptomycin resistance to the resulting FKR12(mt+) transformants. They were subsequently crossed to the two mt− nuclear mutants, *mcd1-F16* (see above) and *ccs1-ac206*, which is blocked at the level of apo- to holocytochrome *f* conversion (24) and overexpresses cyto-
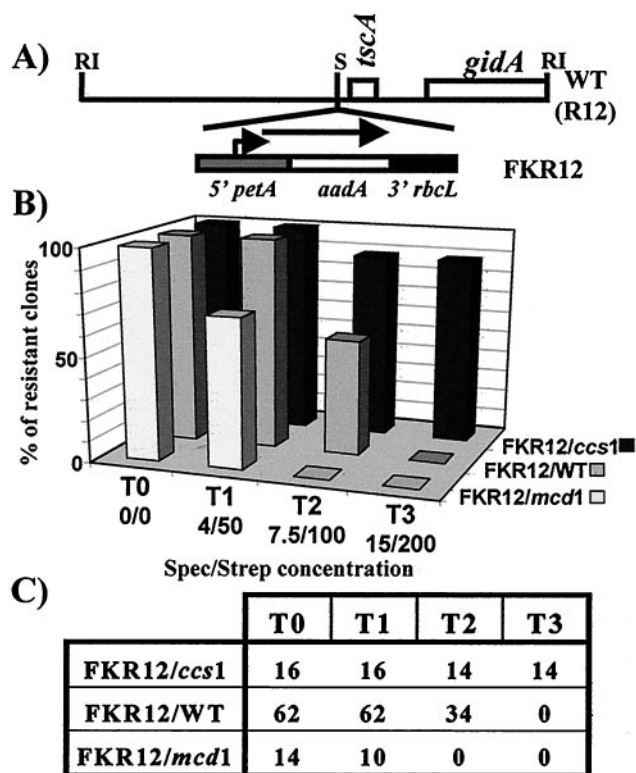
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FIG. 3.   *petA* 5′ UTR confers CES behavior to the *aadA* reporter gene product. (*A*) Map of the R12 fragment in wild-type and FKR12 strains. The positions of known genes and relevant restriction sites are indicated (S, *Stu*I; RI, *Eco*RI) (31). (*B*) Percentage of strains with FKR12/*mcd1*, FKR12/WT, and FKR12/*ccs1* genotypes, as indicated, growing on TAP (T0), T1, T2, or T3 medium, as indicated. Absolute numbers of growing clones (resistant clones) are indicated in *C*. The 62 FKR12/WT strains originated from three crosses—32 from FKR12 × WT, 14 from FKR12 × *mcd1-F16*, and 16 from FKR12 × *ccs1-ac206*—that yielded the same ratio of about 50% resistant clones on T2.

chrome *f* at the level of translation, as does the F52L-55V strain (data not shown). We identified the mutant progeny in the crosses by monitoring their fluorescence induction kinetics.

Because the *aadA* gene product cannot be detected in pulse-labeling experiments, the expression of the FKR cassette in the progeny of these crosses was monitored by testing their level of antibiotic resistance. To minimize *aadA*-independent background variations in their sensitivity to either spectinomycin or streptomycin, we used a combination of these two antibiotics. Growth of the progeny from the crosses was then assayed on solid TAP medium supplemented with increasing concentrations of antibiotics (streptomycin/spectinomycin, respectively): from 0 (as a growth control, T0), to 4/50 (T1), 7.5/100 (T2), or 15/200 (T3) μg/ml (Fig. 3*B*). FKR12 was also crossed to a mt⁻ wild-type strain to control for any unknown modifiers of antibiotic resistance.

The correlation between the genotype of the strains and their antibiotic resistance phenotype was drawn from analysis of at least seven tetrads for each cross. Growth data for all the clones that we tested are summarized in Fig. 3 *B* and *C*. All members of tetrads derived from the cross between FKR12 and wild-type, as well as the daughter cells from crosses with the *mcd1* and *ccs1* parental strains having the wild-type nuclear alleles (FKR12/WT), grew on T1 medium and died on T3 medium. Remarkably, about half of the FKR12/WT clones, whether analyzed in one batch or in three separate batches corresponding to each of the three crosses, grew on T2 medium whereas the other half did not, suggesting individual variation,

most likely of nuclear origin, in susceptibility to the combined antibiotics.

In contrast to these progeny with a wild-type nucleus, all but two *ccs1* mutant progeny were able to grow on T2 and T3 media (FKR12/*ccs1*). This demonstrates that the *aadA* reporter gene is, at least, 2-fold overexpressed in a *ccs1* relative to a wild-type nuclear context, in agreement with the 3-fold overexpression of cytochrome *f* in the *ccs1* strain.

In contrast, all *mcd1* mutant progeny died on either T2 or T3 medium. Moreover, 4 of these mutant products, of 14, were unable to grow on T1 medium (FKR12/*mcd1*). The *aadA* cassette, therefore, is underexpressed in a *mcd1* relative to a wild-type nuclear context. Indeed, the higher antibiotic susceptibility of the FKR12/*mcd1* progeny does not simply result from the loss of photosynthetic capability, because, in the FKR12 × *ccs1* cross, the more resistant progeny were the nonphotosynthetic ones. The decrease in antibiotic resistance was less than expected from the 10-fold decrease of cytochrome *f* synthesis in the *mcd1* strain. However, the physiology of antibiotic resistance in *Chlamydomonas* is poorly understood and it may not respond in a linear way to the accumulation of the AadA protein.

RNA filter hybridizations, using a probe specific for the *aadA* coding region, showed no correlation between the level of resistance to the antibiotics and the steady-state level of *aadA* mRNA. In fact, FKR12/WT strains contained less *aadA* mRNA than FKR12/*mcd1* strains but more than FKR12/*ccs1* strains (data not shown). This suggests that the chimeric transcript is degraded upon translation, a situation already observed for some *aadA*-containing cassettes (32).

DISCUSSION

How the CES Process Operates During Cytochrome b_6f Complex Biogenesis: Toward a Molecular Mechanism. Insight into the molecular basis for the regulation of cytochrome *f* synthesis can be drawn from the similar behavior of hemeless and C_{ter}-truncated cytochromes *f*, which are both expressed at high and SUIV-independent rates (ref. 20 and Fig. 1). The increased rates of synthesis cannot be explained by ribosome pausing during the translation of the C_{ter} domain, because this domain is still present in the hemeless mutant. One cannot invoke a rate-limiting step associated with the ligation of heme, because this would be bypassed in the strain expressing hemeless cytochrome *f* but not in the C_{ter}-truncated protein.

The up-regulation of cytochrome *f* synthesis has to be attributed to a common feature of both strains, the absence of unassembled C_{ter} domain, whose concentration is negligible in both cases. It is deleted in the C_{ter}-truncated mutant, which nevertheless accumulates the remainder of the protein in the lumen (20). It does not accumulate in the hemeless cytochrome *f* mutant, because the apocytochrome *f* is rapidly degraded (21). In contrast, unassembled C_{ter} domain readily accumulates in mutants expressing the wild-type version of cytochrome *f* but lacking SUIV or cytochrome b_6. These strains show very low rates of cytochrome *f* synthesis (6). We propose that, because most of the C_{ter} domain is shielded into assembled cytochrome b_6f complexes, wild-type cells display intermediate rates of cytochrome *f* synthesis controlled by the steady-state concentration of unassembled cytochrome *f*, which in turn depends both on the rates of assembly and dissociation constant of cytochrome b_6f complexes.

Translation initiation is, most likely, the key step in the regulation of cytochrome *f* synthesis. We can exclude other potential target steps, such as decreased rates of elongation during cytochrome *f* translation, decreased rates of cytochrome *f* translocation across the thylakoid membrane, or cotranslational degradation of the nascent polypeptide. In the strain AFFF, where cytochrome *f* synthesis is driven by the *atpA* promoter and 5′ UTR, we observed normal processing

and thylakoid insertion (Fig. 2); yet the CES process is not observed. Furthermore, experiments with the FKR cassette demonstrate that the *petA* 5′ UTR is able to confer CES behavior to the *aadA* reporter gene. Because the protein translated from this gene is completely different from that of cytochrome *f* (Fig. 3), the coding sequence *per se* cannot have a regulatory role. Thus, we conclude that translation initiation of cytochrome *f* is autoregulated via the *petA* 5′ UTR through a protein motif shielded upon assembly, most likely within the C_{ter} domain of unassembled cytochrome *f*, as illustrated in Fig. 4. The precise characterization of the regulatory residues is currently in progress.

Autoregulation of Cytochrome *f* Synthesis. How the C_{ter} domain of cytochrome *f* exerts a feedback control on translation initiation remains presently unknown. One possibility, illustrated in Fig. 4*A*, corresponds to the mechanism prevailing in most translational autoregulation processes described so far in both eukaryotes and prokaryotes. The proteins involved contain known or proposed RNA-binding domains. Typically, the RNA-binding motif of the protein under translational autoregulation interacts with its own mRNA to modify some posttranscriptional step—maturation, processing, or translation—that ultimately down-regulates protein expression (for review, see ref. 33). This is the case of certain *Escherichia coli* and yeast ribosomal proteins (34–36); the bacteriophage T4 gene 32 and 43 products (37, 38); the *E. coli* SecA, poly nucleotide phosphorlase, and poly(A) binding protein (39–41); the tumor suppressor p53 (42); and the yeast RNA helicase Dpb2p (43). Cytochrome *f*, however, has no reported
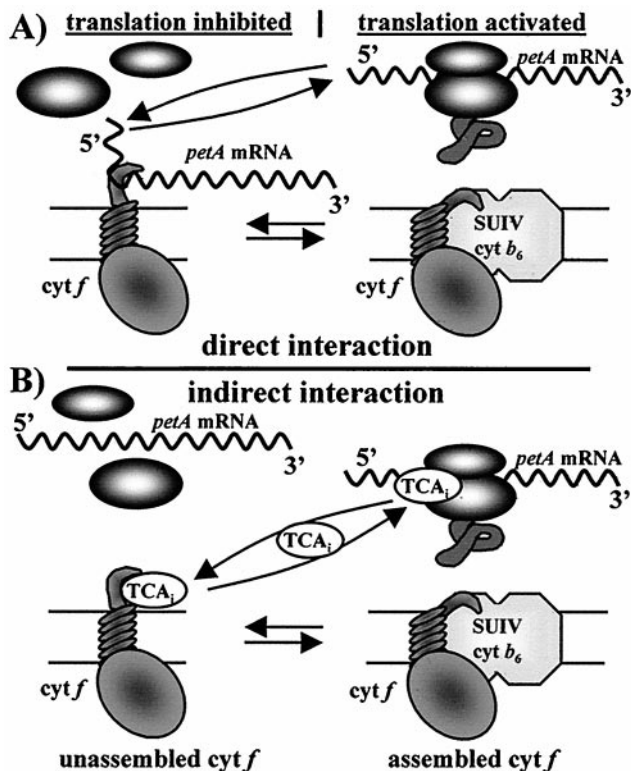

FIG. 4. Hypothetical mechanisms for the CES behavior of cytochrome f. (*A*) Direct interaction between the C_{ter} domain of cytochrome *f* and the *petA* 5′ UTR. (*B*) Indirect interaction which relies on a putative ternary effector, TCA_i, an activator of cytochrome *f* translation. (*Left*) Inhibition of cytochrome *f* synthesis caused by the accumulation of unassembled cytochrome *f*, as observed in strains lacking SUIV, but not in strains lacking accumulation of the C_{ter} domain of cytochrome *f*. (*Right*) Activation of cytochrome *f* synthesis, the *petA* messenger (*A*) or the TCA_i factor (*B*) being made available upon assembly. The intermediate rate of synthesis observed in wild-type cells results from an equilibrium between these situations.

RNA binding activity nor does its C_{ter} domain contain any typical RNA binding motif.

The regulation of cytochrome *f* expression is also distinct from that of β-tubulin, another non-RNA binding protein showing an assembly-dependent autoregulation of gene expression in animal cells. Unassembled β-tubulin prompts the degradation of polysome-bound tubulin mRNA (44), whereas the *petA* mRNA levels remain constant and show no correlation with cytochrome *f* translation rates.

We hypothesize therefore that there is an indirect interaction between the C_{ter} motif, firmly bound to the membrane, and the 5′ UTR of the *petA* mRNA. This autocontrol would implicate a ternary effector, a translational activator capable of competitive binding to the C_{ter} motif of cytochrome *f* and to the *petA* 5′ UTR. As illustrated Fig. 4*B*, the activator would be trapped by the C_{ter} domain of cytochrome *f* until this subunit assembles into a cytochrome b_6f complex. Upon assembly, the activator is released from the C_{ter} domain and becomes available for interaction with the 5′ UTR of *petA* mRNA, where it mediates translation initiation. Such an effector may be defective in several nuclear mutants we have isolated that are specifically defective in cytochrome *f* translation: these mutants define a single nuclear locus, *TCA1* (22). We are currently examining the possible participation of *TCA1* as an effector of the autocontrol.

Generality of the CES Process in Organelle Protein Synthesis. Apart from cytochrome *f*, CES subunits are found in all major thylakoid complexes in *C. reinhardtii* (refs. 6 and 13–16, see Table 1). An additional and recent example is that of the chloroplast-encoded large subunit (LS) of ribulose bisphosphate carboxylase, whose synthesis is down-regulated in the absence of the nuclear-encoded small subunit (17).

Most interestingly, several recent observations suggest that the same chloroplast CES subunits are conserved in higher plants. In the maize nuclear mutant *crp1*, which is primarily impaired in the processing of the *petB-petD* cotranscript and, therefore, lacks SUIV synthesis, the translation of cytochrome *f* is reduced (45). Failure to synthesize both apoCP47 and D1 was observed in the barley *vir-115* mutant, which has a primary defect in D1 expression (46, 47). Although one cannot exclude a dual effect of the *crp1* and *vir-115* mutations, these observations are easily understood if cytochrome *f* and apoCP47 were CES subunits in maize and barley as they are in *Chlamydomonas*. Also, the expression of large subunit of the ribulose bisphosphate carboxylase is decreased in tobacco antisense plants that underexpress the nuclear-encoded small subunit (48).

A CES process may also contribute to the biogenesis of mitochondrial complexes. In the yeast *Saccharomyces cerevisiae*, for example, the mitochondrion-encoded subunits 6 and 8 of the ATP synthase complex show reduced synthesis in a mutant affecting subunit 9 (2). Similarly, the rate of synthesis of cytochrome oxidase (COX) I, a protein centrally located in the cytochrome oxidase complex and encoded by a mitochondrial gene, is reduced in yeast strains deficient in other COX subunits (8, 49–52), including mitochondrion-encoded COXII and COXIII. Furthermore, COXI, although synthesized in reduced amount in a strain deficient in COX7, is stable, and the COXII and COXIII subunits are synthesized at wild-type levels but rapidly degraded (52), a hierarchical situation strikingly similar to the one we described for the cytochrome b_6f complex in *C. reinhardtii* (6).

Whether the CES process occurs through a unique molecular mechanism in organellar protein synthesis is not yet known. Stampacchia *et al.* (53) failed to observe an involvement of the *psaA* 5′ UTR in the regulation of synthesis of the CES subunit PsaA, one of the major photosystem I reaction center subunits in *C. reinhardtii*. In contrast, the CES process also relies on the regulation of translation initiation of the α subunit in the biogenesis of the chloroplast ATP synthase from

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C. reinhardtii (B. Rimbault, D. Drapier, J.G.-B., and F.-A.W., unpublished results). Similarly, the *rbcL* mRNA level is unaffected by the decrease accumulation of the small subunit of ribulose bisphosphate carboxylase, whereas its binding to polysomes is reduced, suggesting a specific decrease in the rate of translation initiation of large subunit (48).

The mechanism for the CES process in cytochrome b_6f biogenesis in *C. reinhardtii* chloroplasts could thus reflect a rather ubiquitous phenomenon in the biogenesis of organellar proteins. A number of nuclear factors acting specifically on the translation of a particular organellar gene, as does the TCA1 factor discussed above, have been identified in *C. reinhardtii* (for review, see ref. 54) and in yeast (for review, see ref. 55). These factors could turn out to be essential components of the regulatory circuitry resulting in the control of protein assembly through translational autoregulation in organelles.

The ubiquitous CES process in organelles is probably not an energy-saving mechanism aimed at limiting production of unneeded polypeptides. It concerns only a restricted subset of the organelle-encoded polypeptides that are protease-resistant but show limited accumulation when unassembled. Rather, the unique properties of the CES subunits offer a means to catalyze multiple-subunit assembly. The various protease-susceptible subunits arrive at their assembly site from the nucleo-cytosol and organelle compartments in a stochastic way. Instead of having a high probability of being recognized as substrates for degradation because they are transiently unassembled, they would be rapidly titrated by the protease-resistant CES subunits acting as anchors and shelters for assembly.

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