CHARACTERIZATION AND IN VITRO EXPRESSION OF THE CYTOCHROME *b*-559 GENES OF BARLEY I. LOCALIZATION AND SEQUENCE OF THE GENES

by

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The psbE and psbF genes encoding the 9.4 and 4.4 kD apoproteins of cytochrome *b*-559 have been located in the chloroplast genome of barley. As in other plant species they are found adjacent to each other in the large single copy region of the chloroplast DNA. Both the nucleotide sequence and the deduced amino acid sequence for the two polypeptides are identical to that of wheat and more than 95% similar to those of spinach, tobacco and *Oenothera*. The region between the two genes spans 10 nucleotides (excluding the stopcodon) and contains a typical procaryotic ribosomal binding site. A dicistronic transcript is identified, but the presence of a ribosomal binding site between the two genes may allow independent translation.

1. INTRODUCTION

Cytochrome *b*-559 is an ubiquitous component of photosystem II in the thylakoid membranes of higher plants and algae. To date the physiological role of this cytochrome and its multiple redox potential forms in photosynthesis remains unknown. While apparently not being a member in the main electron transport chain from water to NADP it can participate in

cyclic electron transport around photosystem II. These aspects and possible functions in the water-splitting activity of photosystem II are summarized and discussed by CRAMER et al. (7). Recent dissection of the composition of photosystem II reveals cytochrome *b*-559 to be closely associated with the D1 and D2 proteins in the photosystem II reaction center core (18).

Cytochrome *b*-559 can be isolated from thy-

Abbreviations: bp = basepairs; cp DNA = chloroplast DNA; kbp = kilobasepairs; kD = kilodalton; SDS = sodium dodecyl sulfate; SSC = 0.15 M-sodium chloride + 0.015 M-sodium citrate; Tris = tris-(hydroxymethyl)-aminomethane.

lakoid membranes as a lipoprotein complex with an apparent molecular weight of 110 kD (28). Dissociation of the complex under mild conditions yields subunits of about 30 kD that still contain heme (15). After complete denaturation of the complex a 9-10 kD polypeptide is obtained and this polypeptide can be synthesized in isolated chloroplasts (32).

The gene coding for the apoprotein was localized on the chloroplast genome of spinach (29) and its nucleotide sequence determined (12). The deduced amino acid sequence was shown to be consistent with the N-terminal amino acid sequence of the 9.4 kD polypeptide (30). The gene is named *psbE* and is bicistronically transcribed together with another gene located 3' downstream of *psbE*. This gene, *psbF*, is separated from *psbE* by only 8 nucleotides and codes potentially for a 4.4 kD polypeptide (12). Subsequently, it was shown that a polypeptide of this size indeed is an additional component of the cytochrome *b*-559 complex (31). Hitherto, only the well-known 9 kD protein has been detected in isolated photosystem II preparations (2, 18). Meanwhile the *psbE*- and *psbF* genes have been sequenced in *Oenothera hookeri*, *Nicotiana tabacum* (5) and wheat (13). More than 95% conservation of the nucleotide sequences is observed among the four species.

The molecular arrangement of the apoproteins and heme groups within the oligomeric complex is not yet known. Spectroscopic data indicate a biaxial ligation of heme within the complex (1). The 9.4 kD and the 4.4 kD polypeptides contain each one histidine residue which could bind coordinatively to heme. Models of homodimers or heterodimers as the smallest heme containing subunits of cytochrome *b*-559 have been proposed (12). However, these models hardly give an understanding of the molecular organization of the 110 kD complex containing only one heme molecule per protein moiety of 30-46 kD (15, 28).

The in vitro assembly of the complex by starting from the in vitro translation products of the cloned genes might provide an alternative approach to clarify whether both apoproteins are necessary to form a functional cytochrome *b*-559 and how these apoproteins are linked in the complex. Towards this end, the *psbE* and

psbF genes have been localized on the chloroplast DNA of barley and their nucleotide sequence has been determined. In a forthcoming paper (of this journal) the in vitro expression of the genes is analyzed by coupled transcription and translation.

2. MATERIALS AND METHODS

2.1. Cloned cp DNA and plasmids

Cloned chloroplast DNA (cp DNA) of barley was provided by C. POULSEN. *Pst*I fragments derived from barley cp DNA had been ligated into the *Pst*I site of the gene providing ampicillin resistance in pBR325, while *Hind*III fragments had been ligated into the gene of pBR322 giving tetracycline resistance (19). The plasmid pUC13 (27) was used for subcloning of cp DNA-fragments derived from the larger *Pst*I- and *Hind*III fragments. pUC13 contains the *bla*-gene for β -lactamase conferring ampicillin resistance to the bacterial host.

2.2. Strains and media

E. coli JM83 was used in all cloning experiments with pUC13. The recipient strain for the recombinant pBR322 and pBR325 clones was *E. coli* HB101 (19).

Bacteria containing recombinant pUC13 plasmids were grown on LB medium (1% (w/v) Difco Bactotryptone, 0.5% (w/v) Difco Bactoyeast, 1% (w/v) sodium chloride) supplied with ampicillin ($100 \mu\text{g} \times \text{ml}^{-1}$) and appropriate for screening with $50 \mu\text{g} \times \text{ml}^{-1}$ of 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal). For bacteria containing recombinant pBR322 or pBR325 plasmids minimal medium (17) supplied with 0.4% (w/v) casamino acids and containing tetracycline ($12.5 \mu\text{g} \times \text{ml}^{-1}$) or ampicillin ($100 \mu\text{g} \times \text{ml}^{-1}$), respectively, was found to be more appropriate than LB medium.

2.3. Chemicals and enzymes

All chemicals used were of analytical grade. Low melting agarose used in cloning experiments was from Litex, Copenhagen. Bal 31 exonuclease was purchased from IBI, Newhaven, Conn, USA. All other enzymes were from

Boehringer, Mannheim, FRG. Radioactive nucleotides were obtained from New England Nuclear, USA.

2.4. Transformation

E. coli cells were grown and prepared for transformation by standard methods (17). Competent cells were stored in 0.1 M-CaCl₂ including 14% (v/v) glycerol at -80 °C. Transformants were selected for ampicillin resistance.

2.5. Isolation and purification of DNA

For size screening of recombinant plasmids, cells of 1 ml overnight cultures were resuspended in 100 µl of a solution consisting of 0.3 M-sucrose, 25 mM-Tris/HCl, pH 8.0, 25 mM-EDTA and 0.02% (w/v) bromophenolblue. After addition of 50 µl of 0.3 M-NaOH and 2% (w/v) SDS the cells were lysed at 70 °C for 20 minutes. After a single extraction with 20 µl of a phenol/chloroform mixture (1:1) the DNA containing solution was directly analyzed on 1% (w/v) agarose gels.

More pure, digestable plasmid DNA was prepared on small scale according to HATTORI and SAKAKI (11). For preparing large amounts of plasmid DNA from 250 ml – 1 l cultures the method of BIRNBOIM and DOLY (4) followed by CsCl-equilibrium centrifugation (20) was used.

Individual DNA-fragments used in cloning experiments were isolated from low melting agarose gels by heating of the gel piece in an equal volume of 100 mM-Tris/HCl pH 8.0 buffer containing 10 mM-EDTA at 70 °C for 10 minutes, followed by a twofold extraction with phenol and a threefold extraction with ether before ethanol precipitation.

Large amounts of DNA-fragments were isolated from preparative agarose gels by electroelution into dialysis bags using a 60 mM-Tris-buffer containing 2 mM-EDTA and adjusted to pH 8.3 with acetic acid.

2.6. DNA endonuclease restriction site mapping

Restriction sites of endonucleases were mapped on DNA-fragments according to SMITH

and BIRNSTIEL (24). Following their procedure the fragments were labelled with ³²P at both ends by filling in nucleotides with Klenow enzyme, and then cleaved with an appropriate restriction enzyme into two asymmetrical subfragments. The two resulting fragments were separated on agarose gels and after electroelution partially digested with the relevant enzyme. The lengths of the products with a common labelled terminus correspond directly to the positions of restriction sites along the DNA-fragments.

2.7. DNA-sequencing

Chloroplast DNA-fragments were sequenced after subcloning into pUC13 with the dideoxy-chain-termination method of SANGER et al. (22) using direct and reverse primers. Plasmid DNA was denatured in 0.2 M-NaOH before annealing with the primer (6). The radioactive label was α-³⁵S-dATP (18.5×10¹² Bq×mmol⁻¹). The dried gels were exposed to Kodak XRP1 films for 2-3 days.

2.8. Analysis of DNA by hybridization to radioactive DNA-probes

After digestion of large cpDNA-fragments cloned into pBR322 or pBR325 the subfragments were electrophoretically separated on 0.8-1.0% (w/v) agarose gels and transferred to nitrocellulose according to the method of Southern (25). Synthetic oligonucleotides complementary to sequences in the non-transcribed strand of the *psbE* and *psbF* genes of spinach (12) were prepared on an Applied Biosystems Oligonucleotide Synthesizer and used as probes. One probe covers amino acid residues 17-22 of the 9.4 kD apoprotein including the histidine residue. The second probe covers amino acid residues 14-19 of the 4.4 kD apoprotein including the histidine residue of this polypeptide. The oligonucleotides were labelled with ³²P by polynucleotide kinase.

For comparison, hybridizations were carried out with heterologous DNA from spinach (clones pCYb 559/1 and pCYb 559/2, kindly provided by Professor R.G. HERRMANN). The isolated DNA-fragments were labelled with ³²P by nick-translation.

Figure 1. Localization of the *psbE* gene encoding cytochrome *b*-559 apoprotein I in the barley chloroplast genome. A *psbE* specific oligonucleotide hybridized to the barley chloroplast subclone pHvC 186. After digestion with PstI, HindIII, or PstI and HindIII the DNA fragments were separated electrophoretically on a 0.8% (w/v) agarose gel and stained with ethidium bromide (A). After transfer to nitrocellulose DNA fragments were hybridized with the ^{32}P -labelled oligonucleotide.

The autoradiogram is shown in panel B. Standard sizes are shown in kbp on the left and arrows indicate the hybridizing bands.

Figure 2. Localization of the barley *psbF* gene encoding cytochrome *b*-559 apoprotein II. ^{32}P -labelled oligonucleotides specific for the *psbE* gene (B) and *psbF* gene (C) hybridize to the same DNA-fragment obtained by digesting the 8.3 kbp *Hind*III insert of pHvC 46 with *Bam*HI but to different fragments after digestion with *Bam*HI and *Eco*RI.

The fragments were separated electrophoretically on a 0.8% (w/v) agarose gel and were stained with ethidium bromide (A). After Southern blotting of the gel the fragments were hybridized with ^{32}P -labelled *psbE*- and *psbF*-oligonucleotides. The autoradiograms are shown in panels B and C, respectively.

Hybridizations were performed in a 6×SSC and 0.1% (w/v) SDS containing 0.25% (w/v) solution of non-fat dry milk (Blotto = bovine lacto transfer technique optimizer) (14). The filters were washed twice with 2×SSC including 0.1% (w/v) SDS and once with 0.2×SSC including 0.1% (w/v) SDS. Autoradiography was carried out using Kodak X-Omat X-ray films with Kodak X-Omatic regular intensifying screens at -80 °C.

2.9. Analysis of RNA

Chloroplast RNA was isolated from 12 hours illuminated barley seedlings as described earlier (3). The RNA was denatured and size fractionated by electrophoresis in a 1.5% (w/v) agarose gel containing 20% (v/v) formaldehyde with a buffer consisting of 40 mM-MOPS, 10 mM-sodium acetate and 1 mM-EDTA, pH 7.2. The RNA was blotted onto nitrocellulose as described by MANIATIS et al. (17). For hybridization, the gene

Figure 3. Physical map of the 13.5 kbp *Pst*I insert of pHvC 186. Only cleavage sites of enzymes relevant to localize the *psbE* and *psbF* genes are shown. The positions of endonuclease restriction sites were mapped according to (24) by labelling the DNA-fragments with ^{32}P at both ends and subsequently cleaving them into two asymmetrical subfragments. This was achieved for the 8.3 kbp *Hind*III fragments with *Xho*I and for the 4.2 kbp *Bam*HI fragment with *Kpn*I. Arrows indicate the cleavage sites of these enzymes. The subfragments were partially digested with *Bam*HI and *Eco*RI, respectively. The lengths of the fragments with a common labelled end reflect the positions of endonuclease restriction sites on the DNA. The positions of *Hind*III cleavage sites on the pHvC 186 insert had been determined by POULSEN (19).

specific 1.5 kbp EcoRI and 350 bp EcoRI DNA-fragments (see 3.1) were labelled with ^{32}P according to the protocol of FEINBERG and VOGELSTEIN (8) using a mixture of hexadeoxyribonucleotides (Pharmacia) as primers.

Hybridization was carried out at 45 °C in 6×SSC including 0.25% (w/v) Blotto, 40% (v/v) deionized formamide and 200 mM-phosphate-buffer, pH 6.5. Filters were subsequently washed with 2×SSC and 0.2×SSC at 50 °C, and exposed to Kodak X-Omat X-ray films at -80 °C.

3. RESULTS

3.1. Localization of the *psbE*- and *psbF* genes on the chloroplast DNA of barley

PstI- and HindIII fragments of barley chloroplast DNA which had been cloned into the plasmids pBR325 and 322, respectively, (19) were separated on agarose gels, blotted onto nitrocellulose filters and probed with synthetic 18-mer oligonucleotides corresponding to sequences in the transcribed strand of the *psbE* and *psbF* genes of spinach (12). These sequences are indicated in Figure 6 by thin horizontal brackets. Both probes hybridized to the 13.5 kbp PstI insert of pHvC 186 and to the 8.3 kbp HindIII fragment (Figure 1). The HindIII fragment was obtained by digestion of the larger PstI fragment with HindIII and is also available as subclone pHvC 46 (19). The probes hybridized only to the 8.3 kbp HindIII fragment but not to any adjacent HindIII fragment (Figure 1).

Digestion of the 8.3 kbp HindIII fragment with BamHI released four subfragments of 4.2 kbp, 2.1 kbp, 1.2 kbp and 0.8 kbp lengths (Figure 2A). Only the large 4.2 kbp BamHI fragment hybridized with the probes specific for the *psbE* and *psbF* genes (Figure 2B, C) and hence contains both genes. EcoRI digestion of this fragment yielded five fragments of 1.5 kbp, 1.2 kbp, 350 bp, 2×300 bp and 150 bp lengths (cf. Figure 3). The probe specific for the *psbE* gene hybridized only to the 1.5 kbp fragment, while the probe specific for the *psbF* gene hybridized to the 350 bp EcoRI fragment (Figure 2). Hybridizations with heterologous DNA from spinach (clones pCYb 559/1 and pCYb 559/2, kindly provided by R.G. HERRMANN) yielded analogous results (data not shown).

The positions of BamHI restriction sites within the 8.3 kbp HindIII insert of pHvC 46 and the positions of EcoRI restriction sites within the 4.2 kbp BamHI fragment were mapped as described under 2.6 using XhoI and KpnI, respectively, to produce the asymmetric fragments. The results are summarized in Figure 3 and revealed that both genes are entirely included in the 4.2 kbp BamHI fragment deriving from pHvC 46.

3.2. Transcript analysis

Chloroplast RNA from illuminated barley seedlings was size fractionated on formamide containing agarose gels and blotted onto nitrocellulose filters. As probes the 1.5 kbp EcoRI fragment which hybridizes to the *psbE* specific oligonucleotide, and the 350 bp EcoRI fragment which hybridizes to the *psbF* specific oligonucleotide (Figures 2, 3) were used. As shown in Figure 4 both probes hybridized to the same RNA-species of 1.5 kbp length.

3.3. Characterization of the *psbE*- and *psbF* genes of barley

For the nucleotide sequence analysis of the genes, the 1.5 kbp EcoRI fragment and the 350 bp EcoRI fragment were subcloned into pUC13. A 330 bp fragment isolated by AccI digestion of the 1.5 kbp EcoRI fragment as well as a 490 bp fragment including both genes and obtained by partial digestion of a 1.2 kbp AccI-BamHI fragment with HinfI were also subcloned into pUC13 and used for sequencing. The relevant restriction sites and the sequencing strategy are shown in Figure 5.

The nucleotide sequence (Figure 6) reveals that the two adjacent genes are separated by an intergenic region of only 10 nucleotides (excluding the stopcodon) which includes a putative ribosomal binding site, GGAGG (23) for the *psbF* gene at position -11. Another possible ribosomal binding site, GGAG, is located 10 bp 5' upstream of the *psbE*-start codon. 110 bp 5' upstream of the *psbE* start codon a hexamer sequence identical with the procaryotic "-35" promoter consensus sequence, TTGACA (21) can be identified. It is preceded and followed by other possible regulatory sequences. They are

Figure 4. Autoradiogram of the "Northern" blot showing the common transcript of the *psbE* and *psbF* gene. Chloroplast RNA (cp RNA) from plants grown in the light was denatured, electrophoresed through a 1.5% (w/v) agarose gel containing 1% (w/v) MOPS, and blotted onto nitrocellulose. For comparison, a preparation enriched in the partly purified 350 bp *EcoRI* fragment and a DNA preparation enriched in the 1.5 kbp *EcoRI* fragment deriving from pHvC 46 were electrophoresed through the same gel. After labelling with ^{32}P the 1.5 kbp fragment was used as a probe specific for the *psbE* gene, and the 350 bp *EcoRI* fragment was used as a probe preferentially hybridizing to the *psbF* gene. Hybridization was carried out in the presence of 40% (v/v) formamide, 0.25% (w/v) Blotto (14), 6×SSC and 50 mM-sodium phosphate buffer (pH 6.5) at 45 °C. The arrow on the right indicates the hybridizing RNA band. *E. coli* rRNA size standards of 23 S rRNA (2904 nucleotides), 16 S rRNA (1541 nucleotides) and 5 S rRNA (120 nucleotides) (Boehringer, Mannheim) are shown on the left.

underlined in Figure 6 and compared with similar sequences of other plant species in Figure 7.

4. DISCUSSION

The cytochrome *b-559* encoding genes *psbE* and *psbF* of barley are located at equivalent positions on the chloroplast DNA as in spinach and other dicotyledones (5) as well as in wheat (13). The positions of the genes in the large single copy region adjacent to the gene for cytochrome *f* are indicated on the physical map of barley chloroplast DNA illustrated by SØGAARD and v. WETTSTEIN-KNOWLES (26).

In all cases an *EcoRI* restriction site in the terminal part of *psbE* facilitates sequencing of the two genes. *PsbE* and *psbF* of barley are separated by 10 nucleotides. While the length of this intercistronic region is the same in the case of wheat, it spans only 8 nucleotides in spinach (12) and 9 nucleotides in *Oenothera* and tobacco (5). The TAG termination codon of *psbE* overlaps the putative ribosomal binding site, GGAGG (23) of *psbF*, in all plant species investigated so far. Also the *psbE* gene is consistently preceded by a similar sequence, GAGG which might function as a ribosomal binding site for this gene.

The nucleotide sequences of the *psbE* and *psbF* genes of barley are identical with those of wheat (13) and are more than 95% similar with those of spinach, tobacco and *Oenothera* (5). Likewise, the non-coding regions of the sequence 5' upstream of *psbE* and 3' downstream of *psbF* in barley are almost identical to those available in wheat. Only one "C" in position 451 (Figure 6) is exchanged by a "G" in wheat (13).

A comparison of the non-coding region 5' upstream of the barley *psbE* gene (Figure 6) with those of spinach and *Oenothera* (5) reveals quite high similarity until about position -60. Except for possible regulatory sequences, no homology is detectable further upstream. In the 3' downstream region of *psbF* the barley sequence shows no homology with the corresponding sequence of spinach (12).

In the 5' upstream region of the barley *psbE* gene are located possible regulatory sequences (Figures 6, 7) resembling the procaryotic "-35" and "-10" promoter consensus sequences (21). Interestingly, in barley two promoter-like "-35" sequences are arranged close to each other. The first heptamer sequence starting at position -121, TTGGTAC, appears also in the sequence 5' upstream of the spinach *psbE* gene (12). The second promoter-like "-35" sequence in barley,

Figure 5. Sequencing strategy of the 350 bp *EcoRI* fragment and part of the neighbouring 1.5 kbp *EcoRI* fragment. All fragments indicated were subcloned into the double-stranded vector pUC13 and sequenced by the dideoxy chain termination method of SANGER et al. (22) using direct and reverse primers.

Figure 6. Nucleotide sequence of the 350 bp EcoRI fragment and the neighbouring 330 bp AccI-EcoRI fragment derived from the larger 1.5 kbp EcoRI fragment. The nucleotides of the non-transcribed strand of the genes are arranged in codons corresponding to the derived amino acid sequence of the proteins. Numbering starts at the first base of the psbE start codon. Putative ribosomal binding sites are boxed and relevant endonuclease restriction sites indicated by horizontal brackets or at the start and end of the sequence by arrows. Double-lined arrows indicate the positions up to which non-coding sequences adjacent to the genes were degraded by Bal 31 exonuclease treatment in preparation for in vitro expression studies. Sequences underlined by thin horizontal brackets are complementary to the synthetic oligonucleotides used as probes for the psbE and psbF genes. Potential promoter-like sequences are underlined.

TTGACA, starting at position -110, is identical with the canonical “-35” consensus sequence of procaryotic promoters (21). This hexamer is followed at a distance of 17 bp by another hexanucleotide, TATCAG, and at a distance of 23 bp by the hexanucleotide TATTTT (Figures 6, 7). Although one cannot exclude a regulatory function of the first promoter-like “-35” sequence we assume that the TTGACA hexamer together with the 17 bp downstream TATCAG

sequence constitutes a promoter. Since the distance between the two promoter-like sequences in chloroplasts can vary from 11 to 24 bases (16) it is also feasible that the TATTTT hexamer which is separated by 23 bp from the “-35” sequence constitutes the second part of a putative promoter. While in the first case the spacing between the relevant hexanucleotides is optimal (17 bp), in the second case the “-10” region contains the canonical “T” in the last position

	-120	-100	-80
Barley	TTTGGTACAAAATTGACAATCTCACAAGGATGAAATATCAGTAATTT		
Spinach	TTTGGTACTATATTGGCAATCCTACAAGTATTTTACGTTTTTACGTG		
Oenothera	GTTATACTCATTAGCTCTATACTGATTCGGTATACTCGAAAAACACC		

Figure 7. Possible regulatory sequences resembling the procaryotic “-10” and “-35” promoter consensus sequences (21) in the 5' upstream region of the *psbF* genes of barley, spinach and *Oenothera*. The corresponding heptamer and hexamer sequences are underlined. A second possible regulatory sequence preceding the procaryotic like “-35” hexamer TTGACA in barley is indicated by a broken line. Numbering is only valid for the barley sequence.

(21). However, promoter-like structures of a few other chloroplast genes lack a “T” in this position (16). Expression of chloroplast genes in a homologous *in vitro* system has been found to be more efficient with the TTGACA hexamer than with similar sequences containing altered bases (9).

The possible regulatory sequences 5' upstream of the barley *psbE* gene are lined up with corresponding sequences of spinach and *Oenothera* (5) in Figure 7. In spinach the TTGGTAC heptamer is followed at a distance of 14 bp by a TACAAG hexamer, and in *Oenothera* a TTATAC starting at position -159 is separated by 23 bp from the hexamer sequence TATACT. The possibility to deduce two different promoter-like structures from the nucleotide sequence seems to be unique to barley.

Whether these sequences resembling procaryotic promoter structures are really used by the homologous RNA-polymerase is still unclear (10). Nevertheless, they probably do serve as promoters for *E. coli* RNA polymerase which is often used in studies on the expression of chloroplast genes.

The start of transcription has not yet been determined for the *psbE* and *psbF* genes of barley. In spinach and *Oenothera* the probable transcription initiation sites are located at appropriate positions behind the promoter-like sequences (5). “Nothorn” blot analysis revealed that in barley both genes are transcribed into a common single RNA species of 1.5 kbp length (Figure 4). Initially, a single RNA of 1.1 kbp length was described for the two genes in spinach

(29). Later studies with spinach, *Oenothera* and tobacco (5) revealed a minor 1.7 kbp long transcript in addition to the prominent 1.1 kbp transcript. The results obtained by “Nothorn” blot analysis clearly show that *psbE* and *psbF* are co-transcribed, which may facilitate the 1:1 stoichiometry of the 9.4 and 4.4 kD apoproteins as observed in spinach (31) and support the heterodimer model for cytochrome *b*-559 (12).

However, the existence of two separate putative ribosomal binding sites preceding the *psbE*- and *psbF* genes prompts the question whether both proteins might be synthesized independently, and how such an independent expression on the translational level might be regulated. Since no protein precursor containing both polypeptides has been detected until now, an independent translation of the two apoproteins is conceivable. One can speculate that the stoichiometry of the two apoproteins might influence the function of cytochrome *b*-559. Results obtained with a coordinate and an independent *in vitro* expression of the barley *psbE*- and *psbF* genes are described in a forthcoming paper in this journal.

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