Characterization and Expression of Photosystem II Genes (psbE, psbF, and psbL) from the Facultative Crassulacean Acid Metabolism Plant *Mesembryanthemum crystallinum*

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The PSII reaction center contains a number of polypeptides including the gene products encoded by the chloroplast genes psbE, psbF, and psbL. The psbE and psbF genes encode the 9.3-kD α subunit and the 4.5-kD β subunit, respectively, of Cyt b-559 (Andersson and Styring, 1991). The α and β subunits are integral thylakoid membrane proteins that form a heme-bridged heterodimer with the heme group bound by conserved His residues located on the transmembrane helices of each subunit (Cramer et al., 1993). Cyt b-559 is closely associated with the PSII reaction center; however, there is disagreement over whether there is one or more copies of the function, the exact role of Cyt b-559 (Andersson and Styring, 1991). The α and β subunits are conserved integral thylakoid membrane proteins, and the 4.5-kD hydrophobic protein is predicted to span the thylakoid membrane once. The psbL gene product may correspond to a low molecular mass phosphoprotein whose N terminus is located on the stromal side of the thylakoid membrane (Webber et al., 1989). The function of the psbL gene product remains unknown.

Here we report the isolation and expression of an ice plant (*Mesembryanthemum crystallinum*) cDNA clone containing a portion of a chloroplast operon that encodes three PSII integral thylakoid membrane proteins: psbE, psbF, and osbL. The psbL gene encodes a 4.5-kD hydrophobic protein whose amino acid sequence is also highly conserved and shares 97.4 to 100% amino acid identity with other higher plant psbL gene products (Cramer et al., 1993). The psbL predicted amino acid sequence presumably served as a priming site for cDNA synthesis using an oligo(dT)-containing primer-adaptor. Subsequent isolation and identification of the cDNA containing the PSII genes showed that it was fortuitously ligated in a head-to-head orientation onto the 5′ end of a second cDNA insert encoding a thiold protease gene (N. R. Forsthoefel and J. C. Cushman, unpublished results). The nucleotide sequences of both strands were determined using the dideoxy chain-termination method.

**Table I.** Characteristics of a cDNA clone encoding the psbE, psbF, and psbL chloroplast genes from the common ice plant

<table>
<thead>
<tr>
<th>Organism:</th>
<th><em>Mesembryanthemum crystallinum</em> L. (common ice plant).</th>
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<tbody>
<tr>
<td>Location:</td>
<td>Chloroplast genome.</td>
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<tr>
<td>Function:</td>
<td>psbE and psbF, α and β subunits of Cyt b-559, respectively, psbL, PSII integral thylakoid membrane protein of unknown function.</td>
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<tr>
<td>Cloning and Sequencing Techniques:</td>
<td>cDNA library in λ-UNIZAP XR (Stratagene) prepared from polyadenylated RNA isolated after 30 h of salt stress from leaf tissue of the ice plant (J. C. Cushman, unpublished results). A stretch of 19 A residues located at position 677 of the sequence presumably served as a priming site for cDNA synthesis using an oligo(dT)-containing primer-adaptor. Subsequent isolation and identification of the cDNA containing the PSII genes showed that it was fortuitously ligated in a head-to-head orientation onto the 5′ end of a second cDNA insert encoding a thiold protease gene (N. R. Forsthoefel and J. C. Cushman, unpublished results). The nucleotide sequences of both strands were determined using the dideoxy chain-termination method.</td>
</tr>
</tbody>
</table>

Sequencing Identities:
- Nucleotide and amino acid sequence comparisons with GenBank/EMBL data bases.
- Features of the Transcript:
  - Transcript of 1.1 kb detected by northern blotting.
- Features of the Deduced Amino Acid Sequences:
- Cell Type/Expression:
  - Expressed in the chloroplast in a constitutive manner. No change in transcript abundance detected during a 5-d period of stress with 0.5 M NaCl.
- Cellular Localization:
  - Chloroplast thylakoid.

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Willey and Gray, 1989). This four-gene transcription unit is transcribed and translated in the dark as well as in the light (Webber et al., 1989; Kawaguchi et al., 1992). We presume that the isolation of this chloroplast transcript from ice plant arose from hybridization of a stretch of 19 A residues (at positions 677–695 in the sequence) during chromatographic selection of mRNA using oligo(dT) cellulose. This poly(A) stretch corresponds to the spacer region between the psbL and psbJ genes, assuming that the gene order of this operon in ice plant chloroplasts is similar to that found in other higher plants.

The common ice plant is a facultative CAM plant that switches from C3 to CAM photosynthesis when subjected to water stress. During the induction of CAM by salt stress treatment, we observe no decline in the steady-state levels of the 1.1-kb transcript encoding these PSII genes. This expression pattern is similar to that observed for other thylakoid-localized gene products that have been characterized in the ice plant. For example, petH, which encodes Fe-NADP+-oxidoreductase, a component of the chloroplast electron transport chain (Michalowski et al., 1989b), shows no change in steady-state transcript levels during salt stress. These observations support the view that the photosynthetic apparatus remains relatively unaffected by salt stress (Koster and Anderson, 1989). In contrast, other chloroplast-localized genes involved in photosynthetic carbon metabolism, such as prkl (phosphoribulokinase) (Michalowski et al., 1992), and the rbcS (small subunit of Rubisco) gene family (Michalowski et al., 1989a; DeRocher and Bohnert, 1993), display decreases in mRNA levels and transcription rates during salt stress. These declines presumably do not limit carbon assimilation, because they concur with corresponding increases in the expression of genes involved in CAM (DeRocher and Bohnert, 1993).

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The GenBank accession number for the sequence reported in this article is U04314.

LITERATURE CITED


