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cDNA Sequence for the Ribulose 1,5 Bisphosphate Carboxylase/ Oxygenase Complex Protein A Protein that Accumulates in Soybean Leaves in Response to Fruit Removal

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Plant Gene Register

cDNA Sequence for the Ribulose 1,5 Bisphosphate Carboxylase/Oxygenase Complex Protein[1]

A Protein that Accumulates in Soybean Leaves in Response to Fruit Removal

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Soybean (*Glycine max* L.) transiently accumulates two abundant vegetative storage proteins, VSP A and VSP B, in vacuoles of above-ground vegetative tissues (Wittenbach, 1983; Staswick, 1990). As older leaves and stems become a source of exported metabolites for developing sinks, the VSPs are preferentially degraded and the resulting metabolites are presumably mobilized in the xylem and phloem stream. A striking feature of these proteins is that VSP gene expression is dramatically increased in mature leaves following the removal of reproductive sinks (seed pods) and the amount of these proteins increases accordingly (for review, see Staswick, 1994). Other changes in leaves associated with this shift from a source to a storage organ in response to fruit removal include the elevation of starch, retention of Chl, loss of Rubisco activity, and a delay of visible symptoms of senescence (Wittenbach, 1982).

A 30-kD RCP that is tightly associated with Rubisco following extraction from soybean leaves also accumulates in response to fruit removal (Table I) (Crafts-Brandner et al., 1991; Crafts-Brandner and Salvucci, 1994). The function of this protein is not known. To further investigate the regulation and the function of the RCP gene, we have isolated and sequenced the insert of a full-length cDNA clone called pRBP3.

An open reading frame of 283 amino acids encoding a protein with a predicted mol wt of 31,258 was identified. This size is close to that estimated for RCP (30 kD) by SDS-PAGE. The sequence was confirmed to encode RCP by the following results. First, an internal RCP tryptic peptide sequence (S. Crafts-Brandner and M. Salvucci, unpublished results) matched amino acids 49 to 57 predicted from the pRBP3 cDNA sequence. Second, polyclonal antisera obtained from a pRBP3 fusion protein expressed in *Escherichia coli* specifically recognized purified RCP (P. Staswick, unpublished results). Additionally, RNA blot hybridizations demonstrated that mRNA hybridizing to pRBP3 was elevated in leaves of defruited plants (P. Staswick, unpublished results), as is the protein (Crafts-Brandner and Salvucci, 1994). RCP mRNA also increased in response to treatment with methyl jasmonate and other factors that elevate VSP gene expression (Staswick, 1994).

The predicted sequence for RCP has no obvious homology with the previously characterized VSPs. The closest match obtained by a TFASTA search of GenBank was with narbonin, a 2S seed globulin of about 30 kD found in *Vicia pannonica* and *Vicia narbonensis* (Hennig et al., 1992). The amino acid sequence identity was only about 27% overall (47% similarity when conservative substitutions are considered). However, the sequence encompassing amino acids 47 to 149 of RCP was 40% identical (58% similar) to narbonin, although three gaps of four amino acids or less were introduced to maximize homology. No significant similarity with other sequences in GenBank was noted.

A typical signal peptide directing entry into the secretory pathway was not evident in the derived RCP amino acid sequence, which is consistent with the protein's immunolocalization in the cytoplasm (Crafts-Brandner and Salvucci, 1994). In contrast, narbonin genes encode a signal sequence. We consider that RCP is unlikely to be a storage protein, since reserve proteins, including soybean VSP A and VSP B, are sequestered in storage vacuoles. The predicted protein is devoid of sulfur-containing amino acids except for the initiating Met. Interestingly, these amino acids are also of low abundance in VSP A and VSP B (Staswick, 1988). Several hydrophobic domains are present, particularly in the carboxy-terminal half of the molecule.

The function of the soybean RCP protein remains unknown. Its marked increase in mature leaves in response to sink removal suggests that it may be involved in the transition from a source to a storage organ that occurs in these leaves. Whether this involves an interaction with Rubisco in vivo is unclear. Although narbonin has been considered a seed storage protein, its crystal structure (Hennig et al., 1992) indicates that it has a triose phosphate isomerase-like α/β barrel con-

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Abbreviations: RCP, Rubisco complex protein; VSP, vegetative storage protein.

Table I. *Characteristics of a cDNA encoding a RCP that is elevated in soybean leaves in response to fruit removal*

Organism:
Glycine max L. cv Hobbit.
Gene Function:
Unknown.
Gene Number:
One or two copies estimated from genomic Southern blot hybridization at high stringency.
Clone Type:
cDNA pRBP3 containing the entire coding sequence for RCP.
Source:
cDNA expression library containing 2×10^5 primary plaques constructed in λ ZAP (Stratagene). Poly(A^+) RNA was isolated from soybean leaves from plants that had been depodded twice weekly for 6 weeks after flowering. Polyclonal RCP mouse antisera (Crafts-Brandner and Salvucci, 1994) was used to select clones.
Sequencing Methods:
Sequence from both strands obtained from overlapping subcloned restriction fragments at the University of Nebraska-Lincoln DNA Sequence Analysis Facility. Specific primers were used where appropriate overlapping subclones were not obtained. Sequence was analyzed with an "Editbase" program (N. Nielsen, copyright USDA/ARS and Purdue Research Foundation) and with Genetics Computer Group programs.
Method of Identification:
Comparison of an internal tryptic peptide from purified RCP with amino acid sequence predicted from pRBP3. Generation of an RCP-specific antibody from the coding region of pRBP3 expressed in *E. coli*.
Features of cDNA:
1285 nucleotides plus a poly(A) tail of 24 nucleotides. Coding sequence for 283 amino acids. Untranslated 5′ and 3′ regions of 77 and 355 nucleotides, respectively.
Features of Predicted Protein:
Calculated mol wt of 31,258 and predicted isoelectric point of 6.1. Signal peptide not detected. A single (initiating) Met and no Cys residues. Carboxy-terminal half is notably hydrophobic.
Expression Characteristics:
mRNA levels elevated by reproductive sink removal, methyl jasmonate treatment, wounding, ammonium nitrate fertilization (P. Staswick, unpublished results). Protein is localized in cytoplasm (Crafts-Brandner and Salvucci, 1994).
Antibodies:
Polyclonal rabbit antisera raised against a pRBP3 fusion protein expressed in *E. coli*.

formation, typical of several other enzymes. Whether RCP has a similar structure that might be related to its function remains to be determined.

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The GenBank accession number for the sequence reported in this article is L28804.

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