Plant Physiol. (1991) 95, 164–170
0032-0889/91/95/0164/07/$01.00/0

Received for publication July 23, 1990
Accepted October 2, 1990

Isolation of a cDNA Clone for Spinach Lipid Transfer Protein and Evidence that the Protein Is Synthesized by the Secretory Pathway[1]

Werner R. Bernhard, Sharon Thoma, Jose Botella, and Chris R. Somerville*

Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

A cDNA clone encoding a nonspecific lipid transfer protein from spinach (*Spinacia oleracea*) was isolated by probing a library with synthetic oligonucleotides based on the amino acid sequence of the protein. Determination of the DNA sequence indicated a 354-nucleotide open reading frame which encodes a 118-amino acid residue polypeptide. The first 26 amino acids of the open reading frame, which are not present in the mature protein, have all the characteristics of a signal sequence which is normally associated with the synthesis of membrane proteins or secreted proteins. *In vitro* transcription of the cDNA and translation in the presence of canine pancreatic microsomes or microsomes from cultured maize endosperm cells indicated that proteolytic processing of the preprotein to the mature form was associated with cotranslational insertion into the microsomal membranes. Because there is no known mechanism by which the polypeptide could be transferred from the microsomal membranes to the cytoplasm, the proposed role of this protein in catalyzing lipid transfer between intracellular membranes is in doubt. Although the lipid transfer protein is one of the most abundant proteins in leaf cells, the results of genomic Southern analysis were consistent with the presence of only one gene. Analysis of the level of mRNA by Northern blotting indicated that the transcript was several-fold more abundant than an actin transcript in leaf and petiole tissue, but was present in roots at less than 1% of the level in petioles.

Lipid transfer proteins, which have been isolated from animals, yeast, plants, and bacteria, are characterized by their ability to catalyze exchange of lipids between natural or artificial membranes *in vitro* (10, 12, 29). Under conditions in which the donor membrane is actively engaged in lipid synthesis, plant lipid transfer proteins have also been shown to catalyze net transfer from the donor to acceptor membrane (17). On this basis, it has been proposed that they participate in the transfer of lipids between membranes in intact cells (17). However, this family of proteins is relatively poorly characterized in several respects, and a role in intracellular lipid transport is not established (2).

[1] This work was supported in part by grants from the U.S. Department of Agriculture/National Science Foundation/Department of Energy Plant Science Center Program and the U.S. Department of Energy (No. DE-AC02-76ER01338).

In higher plants, both specific and nonspecific lipid transfer proteins have been characterized (12, 27). The basic lipid transfer proteins from maize seedlings (7), spinach leaves (13), and castor bean seedlings (27) are nonspecific. All nonspecific plant lipid transfer proteins are soluble proteins which may account for as much as 4% of the total soluble protein (12). The intracellular location of the lipid transfer proteins has not been established but they are located outside the chloroplast (20) and have been thought to be cytosolic. The most thoroughly characterized proteins have a pI of about 9 and a molecular mass of about 9000 D (13, 27). The amino acid sequences have been determined for the spinach leaf (3) and castor seedling proteins (22). In addition, on the basis of amino acid sequence identity to the known lipid transfer proteins, several polypeptides from barley and finger millet, which were originally described as probable amylase inhibitors (18), were identified as lipid transfer proteins (1, 4). Recently, a cDNA of the maize lipid transfer protein has been characterized (23). Comparison of the deduced amino acid sequences of cDNA clones encoding the maize and barley lipid transfer proteins with the directly determined amino terminal sequences of the mature proteins indicated that these proteins are synthesized as precursors containing 27 or 25 additional N-terminal amino acids, respectively.

In order to establish conditions for the analysis of the role of lipid transfer proteins by genetic methods, we have isolated and characterized a full length cDNA clone for the nonspecific lipid transfer protein from spinach leaves, characterized the level of mRNA abundance in various tissues and examined the number of closely related genes in the spinach genome. In addition, we described here the results of experiments indicating the synthesis of the mature protein involves processing of a preprotein by the secretory pathway.

MATERIALS AND METHODS

Materials

A λgt11 cDNA library derived from mRNA of spinach leaves (*Spinacia oleracea* L. American Hybrid 424), was obtained from W. L. Ogren (U.S. Department of Agriculture, Urbana, IL). Plasmid pSP65AB30, which carries a cDNA encoding the light harvesting Chl *a/b* binding protein from *Lemna gibba* under transcriptional control of the SP6 promoter (14) was obtained from E. Tobin (University of California, Los Angeles, CA). Plasmid pSPBP1 encoding a cDNA

for bovine prolactin under transcriptional control of the SP6 promoter was obtained from V. R. Lingappa (University of California, San Francisco, CA). Plasmid pSAc3 carrying an actin gene from soybean (21) was obtained from R. Meagher (University of Georgia, Athens, GA). The plasmid pBluescript (BS^+) was purchased from Stratagene. Oligonucleotides were synthesized by the phosphoramidite method on an Applied Biosystems 380A instrument.

A preparation of microsomal membranes suitable for *in vitro* translocation and processing of precursor proteins was generously provided by J. Miernyk (U.S. Department of Agriculture, Peoria, IL). The microsomes were prepared from maize endosperm cultures (16) by a modification (RG Shatters, Jr, JA Miernyk, unpublished data) of the method of Burr and Burr (6).

Plaque Screening

The cDNA library was plated on *Escherichia coli* Y1090 and nitrocellulose plaques lifts were screened with the oligonucleotide mixtures SLTP-1 (ATGTG(C/T)GGNGTNCA(C/T)AT) and SLTP-2 (AA(A/G)GGNAT(T/C)AA(T/C)TA(T/C)GG) which were labeled to an average specific activity of 10^9 cpm μg^{-1} with [γ-^{32}P]ATP (3000 Ci $mmol^{-1}$) and T4 polynucleotide kinase (15). The filters were prehybridized 3 to 5 h at 42°C for SLTP-1 and at 39°C for SLTP-2, in 6 × SSC[2], 50 mM $NaPO_4$ (pH 6.8), 5 × Denhardt's solution, and 0.5 mg mL^{-1} sonicated herring DNA (Sigma). The hybridization was carried out at the same temperatures for 36 h with the addition of 2 pmol of the appropriate oligonucleotide mixture. The filters were washed in 4 × SSC at the same temperature as the hybridization, with four changes of the washing buffer at 30 min intervals.

Nucleic Acid Manipulations

RNA and DNA was extracted from 6-week-old spinach by grinding 5 g of tissue in liquid N_2, then stirring for 15 min at 60°C in a mixture of 50 mL of 100 mM Tris-HCl(pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.5% (w/v) SDS, 2% (v/v) 2-mercaptoethanol, and 50 mL of phenol. The mixture was then extracted once with 100 mL of chloroform/isoamyl alcohol (24:1), once with phenol/chloroform/isoamyl alcohol (24:24:1) and the nucleic acids recovered by ethanol precipitation. The pellet was resuspended in 20 mL water, 1 volume of 4 M LiCl was added and after incubation for 16 h at −20°C the RNA was pelleted by centrifugation at 12,000*g* for 60 min. The RNA pellet was washed with 70% ethanol. DNA was isolated from the supernatant of the LiCl precipitation by adding two volumes of ethanol and centrifuging at 15,000*g* for 60 min, then purified by CsCl centrifugation.

Fragments to be sequenced were subcloned into the appropriate restriction site of pBluescript and sequenced by the chain termination method using Sequenase (US Biochemicals).

For Southern analysis DNA was restricted and resolved by electrophoresis on a 0.8% agarose gel (10 μg per lane). Following depurination in 0.25 M HCl and denaturation in 0.5 M NaOH, 1.5 M NaCl the DNA was transferred in 0.25 M NaOH, 1.5 M NaCl to nylon membrane (Hybond N, Amersham). The membrane was prehybridized 3 to 5 h at 50°C in 6 × SSC, 5 × Denhardt's solution, and 1 mg mL^{-1} sonicated herring DNA. The hybridization was carried out at 50°C for 10 h with the addition of 0.2 μg of the purified insert from the plasmid pWB2 labeled with [α-^{32}P]dCTP by random priming (8) to an average specific activity of 5×10^8 cpm μg^{-1}. The membranes were washed once with 1 × SSC, 0.2% SDS at 50°C and three times with 0.1 × SSC, 0.2% SDS at the same temperature.

For Northern analysis, 20 μg RNA was resolved by electrophoresis in an agarose gel containing formaldehyde (15). After the transfer (in 10 × SSC) to nitrocellulose, the blot was prehybridized at 42°C in 5 × SSC, 5 × Denhardt's solution, 50 mM $NaHPO_4$ (pH 7.0), 0.01% SDS, 50% deionized formamide, and 1 mg mL^{-1} sonicated herring DNA. The hybridization was carried out at the same temperatures for 10 h with the addition of 0.4 μg of the purified insert of the plasmid pWB2 labeled with [α-^{32}P]dCTP by random priming. The membranes were washed twice with 2 × SSC, 0.1% SDS at 23°C, twice with 2 × SSC, 0.1% SDS at 60°C, and once with 0.2 × SSC, 0.1% SDS at 60°C.

In Vitro Transcription and Translation

Transcripts were prepared from linearized plasmids with T3 RNA polymerase in the presence of 500 μM m^7 G(5′)ppp(5′)G (Pharmacia) and 2.5 mM of each ribonucleotide triphosphate at 38°C for 1 h. The reaction was terminated by addition of 1 unit of DNAse per μg of template DNA. After 10 min the reaction was extracted with chloroform/phenol (1:1), then with chloroform, adjusted to 0.3 M sodium acetate and RNA recovered by ethanol precipitation.

Translation assays containing 0.5 μg RNA, 15 μL of wheat germ extract (Promega), 15 μCi ^{35}S-methionine (1149 Ci/mmol; Amersham) in a final volume of 30 μL were incubated 1 h at 30°C. No additional KOAc or MgOAc was required for maximal rates of translation of transcripts from pWB2 or pSP65AB30. Maximal rates of translation of transcripts from pSPBP1 were obtained following addition of 33 mM KOAc. For some experiments 3 μL of canine pancreatic microsomes (Promega), or 8 μL (21.4 μg of protein) of microsomes from cultured cells of maize endosperm tissue, were added to the translation reactions. The translation products were resolved electrophoretically by loading equal amounts of incorporated radioactivity on 10 to 20% gradient SDS polyacrylamide gels. The resolved proteins were electrophoretically transferred to nitrocellulose then the filters were autoradiographed.

Protease Protection

Translation reactions were terminated by addition of 2 μL of a solution containing 3 mM cycloheximide and 0.12 M methionine, and divided into three identical samples. One sample was left untreated as a control. $CaCl_2$ (10 mM) and proteinase K (1 mg mL^{-1}) were added to the second sample. The third aliquot was adjusted to contain 1% Triton X-100

[2] Abbreviations: SSC, 150 mM NaCl, 10 mM sodium citrate (pH 7.0); bp, base pair.

```
TTGCATTATA TTT  ATG GCT AGC TCC GCT GTT ATC AAG TTA GCT TGT   46
                 M   A   S   S   A   V   I   K   L   A   C   -16

GCA GTC CTG TTG TGC ATC GTG GTC GCT GCA CCA TAC GCT GAA GCA   91
 A   V   L   L   C   I   V   V   A   A   P   Y   A   E   A   -1

GGT ATA ACT TGT GGG ATG GTT TCA AGC AAA CTT GCT CCT TGC ATT  136
 G   I   T   C   G   M   V   S   S   K   L   A   P   C   I   15

GGG TAC CTT AAA GGA GGC CCC TTG GGC GGT GGT TGC TGT GGT GGA  181
 G   Y   L   K   G   G   P   L   G   G   G   C   C   G   G   30

ATT AAG GCC CTG AAC GCG GCA GCT GCC ACC ACT CCT GAC AGG AAA  226
 I   K   A   L   N   A   A   A   A   T   T   P   D   R   K   45

ACT GCA TGC AAT TGC CTC AAA AGT GCT GCT AAT GCC ATT AAG GGA  271
 T   A   C   N   C   L   K   S   A   A   N   A   I   K   G   60

ATC AAC TAC GGA AAG GCT GCT GGT CTC CCT GGT ATG TGT GGC GTC  316
 I   N   Y   G   K   A   A   G   L   P   G   M   C   G   V   75

CAT ATT CCC TAC GCC ATT AGC CCC AGC ACC AAC TGC AAC GCC GTC  361
 H   I   P   Y   A   I   S   P   S   T   N   C   N   A   V   90

CAC TAA  ACCGCAAATGTTATAACAAAAATGGAAGATGGAGCTACATAGGAGTGGCC  417
 H   *

CAGTTACTAAGCTCGTAGAGTGTATGATAATAAAAGAAGAGAGATCATCTTTGCCAAGT  476

CGCTAGCTTGTATTTCTTGTTTCATGTATTATTGCAACTTTTCTATTACTTTTCGGGTT  535

ACAAATATCCTAATATTACCAAAAAAAAA                                564
```

Figure 1. Composite nucleotide sequences of the cDNA for spinach lipid transfer protein and the deduced amino acid sequence. The amino acids are numbered from the glycine residue found at the amino terminus of the mature protein (3). The regions of sequence homologous to the oligonucleotide probes used to isolate the gene are underlined.

before addition of the protease. The reactions were incubated at 24°C for 1 h and the treatments were terminated by addition of 0.1 volume of 100 mM phenylmethylsulfonyl fluoride in isopropanol.

RESULTS

Isolation and Sequence Analysis of a cDNA clone

On the basis of the amino acid sequence of the lipid transfer protein of spinach (3) two oligonucleotide mixtures composed of 64 (SLTP-1) and 96 (SLTP-2) different 17-mers were prepared, which contained all possible DNA sequences which could encode the corresponding regions of the protein sequence. Screening of a λgt11 cDNA library derived from spinach leaf poly(A^+) RNA resulted in the recovery of two positive plaques from among approximately 75,000 screened. Both were identical and contained two *Eco*RI fragments of 580 and 730 bp, respectively. These inserts were subcloned into the *Eco*RI site of pBluescript to produce the plasmids pWB2 and pWB3 and the DNA sequence of the inserts in both plasmids was determined. The insert in plasmid pWB2 was found to encode the spinach lipid transfer protein. The insert in pWB3, which also had a poly-(A^+) sequence at one end, appeared to be a fragment of an unrelated cDNA which could not be identified by sequence homology to any previously determined DNA or protein sequence and was not characterized further.

The composite nucleotide and deduced amino acid sequences of pWB2 are shown in Figure 1. The entire cDNA was 564 nucleotides long and contained one open reading frame of 354 bp which began 13 bp from the 5′ end and terminated 197 bp from the 3′ poly(A) tail. The open reading frame encoded a polypeptide of 118 amino acids. The carboxy terminal 92 residues of the open reading frame were almost identical to the directly determined amino acid sequence of the mature protein (3). The only differences were that the deduced amino acid sequence had cysteines rather than serines at residues 27 and 28. Cysteines are also found at these positions in all other homologous proteins (1). The deduced amino acid sequence also contained 26 amino terminal residues which were not found on the mature polypeptide. This observation is consistent with evidence from the analysis of the maize (23) and barley (1, 18) genes indicating that these polypeptides are produced by cleavage of a preprotein.

RNA Analysis

To assess the size of the transcript encoding the spinach lipid transfer protein, and to evaluate the tissue specificity of gene expression, the cDNA clone was used to probe a Northern blot containing spinach RNA from various tissues

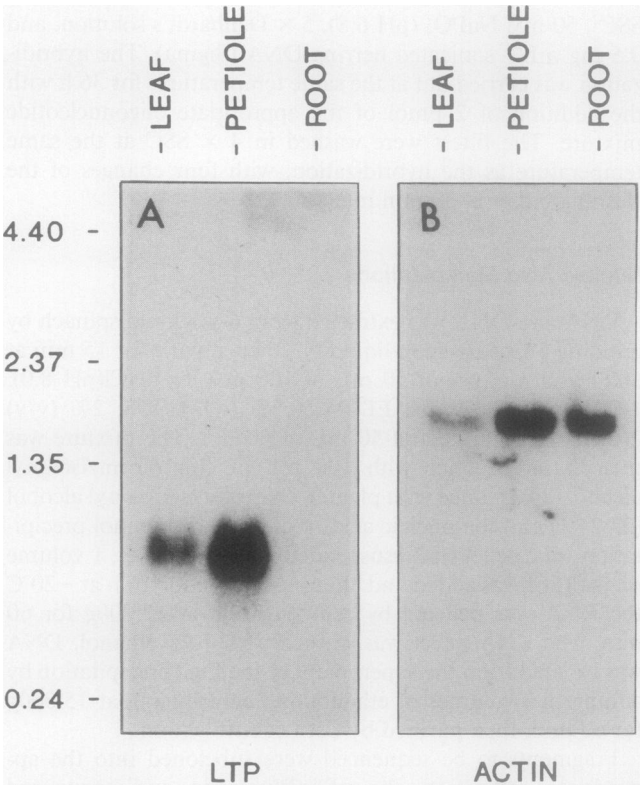

Figure 2. Northern blot of total RNA from spinach probed with the cDNA of spinach lipid transfer protein labeled to a specific activity of 2×10^9 dpm/μg DNA (A) then stripped and reprobed with an actin cDNA labeled to a specific activity of 3×10^9 dpm/μg DNA (B). Twenty μg of RNA was loaded in each lane. The filter in A was exposed 2 d; the filter in B was exposed 5 d. The size (in kb) and location of mol wt markers is indicated at the left of the figure.

(Fig. 2A). The results of this experiment indicated that the probe hybridized to an mRNA of approximately 700 bp in RNA preparations from leaves and petioles. Because this is similar to the size of the cDNA clone in pWB2, it appears that the insert in pWB2 represents a nearly full-length cDNA. There was a striking difference in expression of the mRNA in green tissues versus root tissue, where accumulation of the lipid transfer protein mRNA was not readily apparent. On prolonged exposure of the filter a faint signal could be observed in the lane of root RNA (results not presented). This signal was estimated to be approximately 100-fold less abundant than the leaf signal. To verify that the preparation of RNA from the various tissues were of comparable quality, the filter was stripped and reprobed with an actin gene which was previously shown to hybridize to a similar extent to RNA from roots, shoots and hypocotyls of soybean (11). The presence of hybridization signals of similar intensity in the root and petiole lanes when probed with the actin gene (Fig. 2B) verified that the two preparations of RNA were of similar quality and quantity.

Southern Analysis

Two protein fractions with lipid transfer activity have been reported for spinach (13), suggesting the presence of isoforms of lipid transfer protein. To investigate the number of homologous genes present in the spinach genome, Southern analysis was performed with genomic DNA cut with several restriction enzymes which do not have recognition sites in the cDNA sequence. For five of the six restriction enzymes used, the cDNA probe hybridized to only one fragment, and in four of these cases the fragment was less than 4 kb in size (Fig. 3). Thus, it appears likely that only one gene encoding the lipid transfer protein is present in the spinach genome. The presence of two bands in the lane containing DNA restricted with *Dra*I suggests the presence of at least one intron in the genomic sequence. In view of the very high abundance of the protein in leaf tissues, the mechanism by which a single gene appears to regulate accumulation of the protein may merit further investigation with respect to protein and mRNA turnover and rate of transcription.

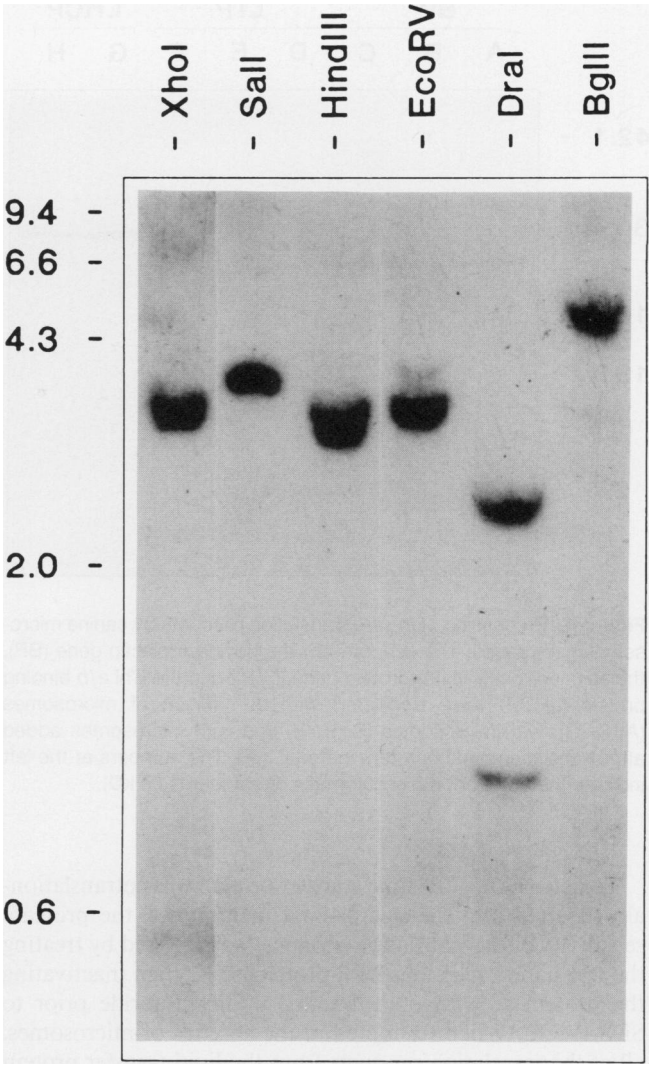

Figure 3. Southern blot of total spinach DNA probed with the cDNA of spinach lipid transfer protein. The size (in kb) and location of mol wt markers is indicated at the left of the figure.

In Vitro Translation and Processing

The sequence of the 26 amino-terminal residues of the deduced amino acid sequence of the spinach lipid transfer protein contained many of the features associated with signal sequences (25, 28). The general characteristics of signal peptides are: (a) they are 20 to 40 amino acids long, (b) there is a charged residue within the first five amino acids in the amino-terminal direction from the cleavage site and this is followed by a core of at least nine hydrophobic residues, (c) a helix-breaking residue (glycine or proline) frequently occurs four to eight residues before the cleavage site. The most stringent requirement is that an alanine residue occurs at the minus-1 and minus-3 positions. The prepeptide of the spinach lipid transfer protein satisfies all of these criteria.

The role of the amino-terminal sequence was tested by transcribing and translating the cDNA *in vitro* under conditions which lead to processing of signal peptides by the secretory pathway (26). When transcripts from the cDNA were translated without addition of microsomal membranes (Fig. 4D), or when the dog microsomal membranes were added after translation was complete (Fig. 4F), the translation product wasof the size expected from the open reading frame. By contrast, when dog microsomal membranes were present during translation, a translation product of the size expected for the mature polypeptide accumulated (Fig. 4E). Identical results were obtained with the positive control, prolactin (Fig. 4, A–C), whereas the presence of dog microsomes had no effect on the size of the Chl *a*/*b* binding protein (Fig. 4G, H) which is inserted into chloroplast membranes by a different mechanism. Replacement of dog microsomes with maize microsomal membranes resulted in processing of the primary translation product to a processed form of the same size as that obtained with the dog microsomes (Fig. 5). Thus, the amino terminus of the preprotein functions as a signal peptide in both plants and animals.

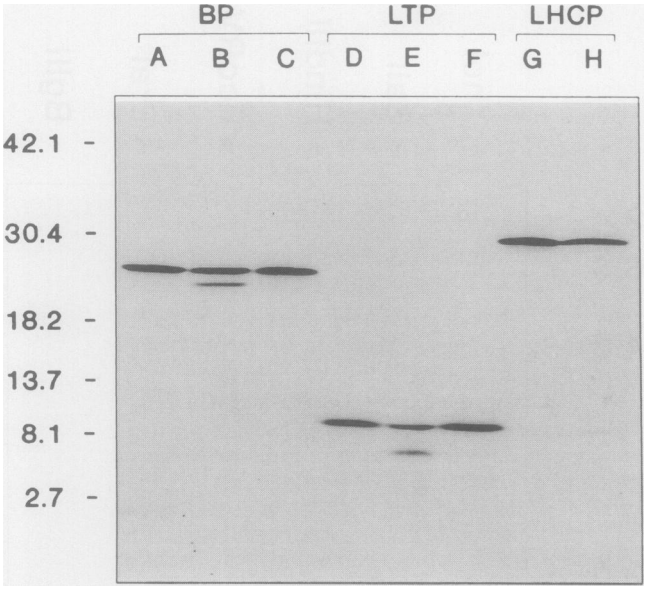

Figure 4. Processing of *in vitro* translation products by canine microsomal membranes. Transcripts from the bovine prolactin gene (BP), the spinach lipid transfer protein gene (LTP), and the Chl *a*/*b* binding protein (LHCP) were translated without addition of microsomes (A, D, G), with microsomes (B, E, H) and with microsomes added after translation had been terminated (C, F). The numbers at the left indicate the apparent molecular mass of standards (in kD).

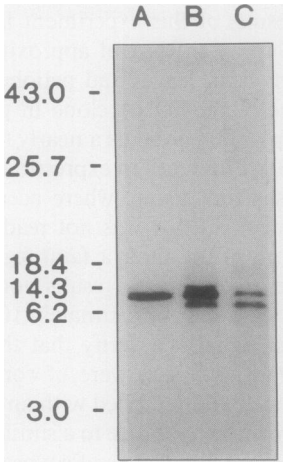

Figure 5. Processing of *in vitro* translation products by microsomal membranes from maize endosperm cell cultures. Transcripts of the lipid transfer protein were translated without the addition of microsomes (A), with maize microsomes (B), and with dog microsomes (C). The numbers at the left indicate the apparent molecular mass of standards (in kD).

To determine if the lipid transfer protein was cotranslationally inserted into the microsomal membranes, the protease sensitivity of the translation products was assessed by treating the translation reactions with proteinase K, then inactivating the proteinase with phenylmethylsulfonyl fluoride prior to SDS-PAGE. When translated in the absence of microsomes, all of the translation products from the lipid transfer protein gene and the control gene (prolactin) were protease sensitive (Fig. 6B, G). By contrast, when transcripts from the lipid transfer protein and prolactin genes were translated in the presence of dog microsomes, the low mol wt product was protected from proteolysis but the high mol wt product was not (Fig. 6D, I). The presence of microsomes also resulted in the appearance of a translation product from the lipid transfer gene of higher mol wt. This does not appear to be due to N-linked glycosylation since the polypeptide does not contain the glycosylation sites Asn-*X*-Ser or Asn-*X*-Thr. Addition of low amounts of Triton-X100 to these translation mixtures concomitantly with addition of the proteinase K rendered the low mol wt products protease sensitive (Fig. 6E, J). Thus, the evidence indicates that the mature lipid transfer protein is sequestered in the endoplasmic reticulum. This result is consistent with the results of recent experiments indicating that maize lipid transfer protein is synthesized on membrane-bound polysomes (24).

DISCUSSION

The mechanisms by which lipids are transferred between intracellular membranes are not established (2). However, the demonstrations that lipid transfer proteins could mediate rapid net transfer of lipid between a donor and acceptor membrane *in vitro* (17) suggests that this mechanism could be important *in vivo*. According to this hypothesis, the rate of transfer of lipid between membranes would be proportional to the rate of diffusion of the protein-lipid complex between donor and acceptor membranes. In this respect, the high abundance of the spinach leaf protein (*i.e.* up to 4% of total soluble protein) in rapidly expanding tissue is consistent with such a proposed role. By contrast, the very low abundance of the transcript in root tissue suggests that the protein is not involved in lipid transport in this tissue. This could, in prin-

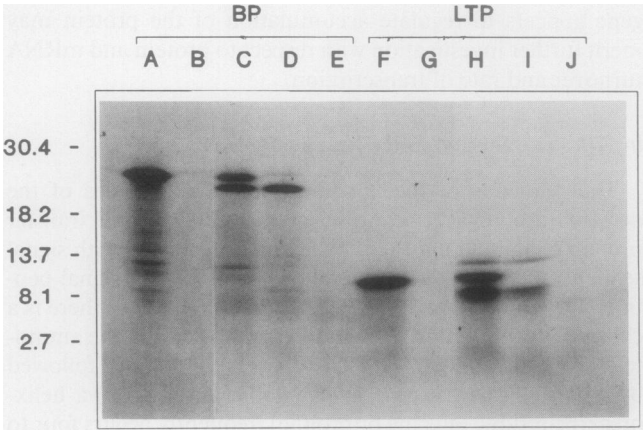

Figure 6. Protease sensitivity of translation products. Transcripts from the bovine prolactin gene (BP) and the spinach lipid transfer protein gene (LTP) were translated without addition of microsomes (A, B, F, G) or with dog microsomes (C, D, E, H, I, J). After translation, the reaction products were incubated with proteinase K for 1 h (B, D, E, G, I, J). In lanes E and J, Triton X-100 was added along with the proteinase K.

ciple, be due to the involvement of different isozymes in this tissue. Lipid transfer in leaves and other green tissues involves substantial flux between endoplasmic reticulum and chloroplast membranes (5). By contrast, in roots, relatively little flux of lipid is thought to take place between plastids and endoplasmic reticulum. Thus, lipid transfer proteins with different characteristics may be utilized in green and nongreen tissues. The possibility of additional isozymes of related proteins is not excluded by the Southern analysis reported here since the conditions used would not have revealed homology to any DNA sequence of less than about 80% sequence identity. Because of the relatively large size of the spinach genome, and the fact that it has not yet been possible to genetically transform spinach, a detailed analysis of a weakly homologous gene family in this organism is not considered worthwhile. However, we have used the spinach cDNA clone described here to isoalte the homologous cDNA and genomic clones from *Arabidopsis thaliana* (results not presented). The greatly reduced genome size of this organism, and the ease with which it can be genetically transformed, should facilitate a critical analysis of the question of tissue-specific isoforms.

The evidence, presented here, indicating that synthesis of the spinach lipid transfer protein involves cotranslational insertion into the endoplasmic reticulum, suggests that either this protein is synthesized by a novel mechanism, or it does not participate in intracellular lipid transfer. Since it lacks the carboxy-terminal KDEL sequence associated with proteins retained in the lumen of the endoplasmic reticulum (19) it is presumably transferred to another compartment of the cells by additional posttranslational processing. Because the mature protein is normally recovered as a soluble extrachloroplastic protein, it has been assumed to be located in the cytosol (12). However, if the mature protein is primarily located in the cytosol, it would appear to be the first instance in which a cytosolic protein is synthesized via the secretory pathway with cleavage of the signal peptide. The only reported instance in which synthesis of a cytosolic protein involves cleavage of a signal sequence by signal peptidase is in the case of the hepatitis B virus precore protein (9). However, in this case, translocation of the protein into the endoplasmic reticulum during synthesis is aborted at an early stage so that the protein is not inserted into the membrane. By contrast, the protease protection experiments indicate that the lipid transfer protein is taken up by the endoplasmic reticulum. There is no known mechanism by which it could subsequently be transferred to the cytoplasm.

If the spinach lipid transfer protein is not located in the cytosol, it cannot carry out the proposed role in transferring lipid between the endoplasmic reticulum and the other organelles. Unfortunately, the precise intracellular location of the protein has not been critically addressed by previous studies. Thus, a resolution of the alternate possibilities must await the results of an immunoelectron microscopic analysis of the cellular location of the protein.

ACKNOWLEDGMENT

We are grateful to Jean-Claude Kader for communicating unpublished results.

LITERATURE CITED

1. **Bernhard WR, Somerville CR** (1989) Coidentity of putative amylase inhibitors from barley and finger millet with phospholipid transfer proteins inferred from amino acid sequence homology. Arch Biochem Biophys **269:** 695–697
2. **Bishop WR, Bell RM** (1988) Assembly of phospholipids into cellular membranes: Biosynthesis, transmembrane movement and intracellular translocation. Annu Rev Cell Biol **4:** 579–610
3. **Bouillon P, Drischel C, Vergnolle C, Duranton H, Kader J-C** (1987) The primary structure of spinach-leaf phospholipid-transfer protein. Eur J Biochem **166:** 387–391
4. **Breu V, Guerbette F, Kader J-C, Kannangara C, Svensson B, Von Wettstein-Knowles P** (1989) A 10 kd barley basic protein transfers phosphatidylcholine from liposomes to mitochondria. Carlsberg Res Commun **54:** 81–84
5. **Browse J, Warwick N, Somerville CR, Slack CR** (1986) Fluxes through the prokaryotic and eukaryotic pathways of lipid synthesis in the 16:3 plant *Arabidopsis thaliana.* Biochem J **235:** 25–31
6. **Burr FA, Burr B** (1981) In vitro uptake and processing or prozein and other maize preproteins by maize membranes. J Cell Biol **90:** 427–434
7. **Douady D, Grosbois M, Guerbette F, Kader J-C** (1982) Purification of a basic phospholipid transfer protein from maize seedlings. Biochim Biophys Acta **710:** 143–153
8. **Feinberg AP, Vogelstein B** (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem **132:** 6–13
9. **Garcia PD, Ou JH, Rutter WJ, Walter WD** (1987) Targeting of the hepatitis B virus precore protein to the endoplasmic reticulum membrane: After signal peptide cleavage translocation can be aborted and the product released into the cytoplasm. J Cell Biol **104:** 705–712
10. **Helmkamp GM Jr** (1986) Phospholipid transfer proteins: Mechanisms of action. J Bioenerg Biomembr **18:** 71–93
11. **Hightower RC, Meagher RB** (1985) Divergence and differential expression of soybean actin genes. EMBO J **4:** 1–8
12. **Kader J-C** (1985) Lipid binding proteins in plants. Chem Physics Lipids **38:** 51–62
13. **Kader J-C, Julienne M, Vergnolle C** (1984) Purification and characterization of a spinach-leaf protein capable of transferring phospholipids from liposomes to mitochondria or chloroplasts. Eur J Biochem **139:** 411–416
14. **Kohorn BD, Harel E, Chitnis PR, Thornber JP, Tobin EM** (1986) Functional and mutational analysis of the light-harvesting chlorophyll a/b protein of thylakoid membranes. J Cell Biol **102:** 972–981
15. **Maniatis T, Fritsch EF, Sambrook J** (1982) Molecular Cloning. A Laboratory Manual. Cold Spring Harbor, New York
16. **Miernyk JA** (1987) Extracellular secretion of acid hydrolases by maize endosperm cells grown in liquid medium. J Plant Physiol **129:** 19–32
17. **Miquel M, Block MA, Joyard J, Dorne AJ, Dubac JP, Kader J-C, Douce R** (1987) Protein-mediated transfer of phosphatidylcholine from liposomes to spinach chloroplast envelope membranes. Biochim Biophys Acta **937:** 219–228
18. **Mundy J, Rogers JC** (1986) Selective expression of a probable amylase/protease inhibitor in barley aleurone cells: Comparison to the barley amylase/subtilisin inhibitor. Planta **196:** 51–63
19. **Munroe S, Pelham HRB** (1987) A C-terminal sequence prevents secretion of luminal ER proteins. Cell **48:** 899–907
20. **Schwitzguebel JP, Siegenthaler PA** (1985) Evidence for a lack of phospholipid transfer protein in the stroma of spinach chloroplasts. Plant Sci **40:** 167–171
21. **Shah DM, Hightower RC, Meagher RB** (1982) Complete nucleotide sequence of a soybean actin gene. Proc Natl Acad Sci USA **79:** 1022–1026
22. **Takishima K, Watanabe S, Yamada M, Mamiya G** (1986) The amino-acid sequence of nonspecific lipid transfer protein from germinated castor bean. Biochim Biophys Acta **870:** 248–255

23. **Tchang F, This P, Stiefel V, Arondel V, Morch MD, Pages M, Puigdomech P, Grellet F, Delseny M, Bouillon P, Huet JC, Guerbette F, Beauvais-Cante F, Duranton H, Pernollet JC, Kader J-C** (1988) Phospholipid transfer protein: Full-length cDNA and amino acid sequence in maize. J Biol Chem **263:** 16849–16855
24. **Vergnolle C, Arondel V, Tchang F, Grosbois M, Guerbette F, Jolliot A, Kader J-C** (1989) Synthesis of lipid transfer proteins from maize seedlings. Biochim Biophys Res Commun **157:** 37–41
25. **von Heijne G** (1985) A new method for predicting signal sequence cleavage sites. Nucleic Acids Res **14:** 4683–4690
26. **Walter P, Lingappa V** (1986) Mechanism of protein translocation across endoplasmic reticulum membranes. Annu Rev Cell Biol **2:** 499–516
27. **Watanabe S, Yamada M** (1986) Purification and characterization of a non-specific lipid transfer protein from germinated castor bean endosperms which transfers phospholipids and galactolipids. Biochim Biophys Acta **876:** 116–123
28. **Watson MEE** (1984) Compilation of published signal sequences. Nucleic Acids Res **12:** 5145–5159
29. **Wirtz KWA, Gadella Jr TWJ** (1990) Properties and modes of action of specific and nonspecific phospholipid transfer proteins. Experientia **46:** 592–599