Leucine aminopeptidase (LAP) is induced by wounding and bacterial pathogen infection in tomato. DNA blot analysis of XbaI-digested Map genomic clones demonstrated that LapA1 and LapA2 cDNAs were encoded by two different LapA genes in the tomato genome. The coding and untranslated regions of LapA1 and LapA2 mRNAs shared more than 93% identity. The deduced amino acid sequences of LapA cDNA clones and in vitro translation of LapA1 mRNA indicated that LAP-A was synthesized as a 60-kDa precursor protein. The processing of a 60-kDa preLAP-A into the mature 55-kDa LAP-A was demonstrated in vivo by expression of the full-length LapA1 cDNA in insect cells. Sequencing of a single LAP-A form isolated from a two-dimensional polyacrylamide gel indicated that LAP-A proteins had two different N termini that were separated by two residues. The LAP-A presequence had features similar to chloroplast transit peptides. Comparison of LAP-A levels in chloroplast and total protein extracts from methyl jasmonate-treated leaves indicated that a small proportion of LAP-A proteins was detected in the plastids. Inspection of the LAP-A presequence indicated the presence of a dibasic protease (Kex2/furin) processing site motif 6–8 residues upstream from the LAP-A N termini. Its potential role in LAP-A precursor biogenesis is discussed.

Aminopeptidases catalyze the hydrolysis of amino acids from the N terminus of peptides and proteins and were first identified in 1929 (1). Since then, a wealth of aminopeptidase activities have been defined in animals, plants, and prokaryotes (2–5). While monomeric aminopeptidases have been extensively used as a tool to monitor genetic diversity in plant breeding schemes (6), far less is known about the role of plant aminopeptidases in plant growth and development and in response to biotic and abiotic stress (for reviews, see Refs. 3 and 4). The findings that the plant peptide hormone, systemin, is a wound signal (7), N- and C-terminal processing is essential for the maturation of prosystemin to a bioactive peptide (8, 9), aminopeptidases are induced during the plant wound and defense responses (10–12), and aminopeptidase activity may be important for the regulation of the plant-defense response (13) have given the field of plant protein processing and turnover a new vitality. The aminopeptidases induced during the plant-defense response are analogs of the mammalian leucine aminopeptidase (LAP; EC 3.4.11.1) (14–16).

In mammals, the hexameric leucine aminopeptidase is a ubiquitous enzyme that is well characterized at the biochemical and biophysical levels (15). While the exact role for LAPs in mammalian cells has yet to be proven, changes in LAP activity levels have been correlated with lens aging (17) and hepatic diseases (18). Since LAP activities and mRNAs are detected in all organs examined (16, 19, 20), it has been proposed that the mammalian LAPs are involved in the turnover of normal and/or damaged proteins and peptides, which are essential for cell maintenance. More recently, a possible role for LAP in the human defense response was proposed (21). Since interferon-γ induces the accumulation of LAP in human cells cultured in vitro, LAP may be involved in antigen presentation or the antiviral effects of interferon.

Similar to the animal LAPs, plant LAPs may serve several functions (3). Aminopeptidases with biochemical characteristics similar to the hexameric LAP of animals are detected in coryledons of barley (22) and kidney bean (23). Although LAP activity levels do not rise after seed germination, it is possible that the levels of LAPs stored in these seeds are sufficient to aid in the turnover of storage proteins. Studies in Arabidopsis and tomato indicate that some LAPs are constitutively expressed in plants (12, 24, 25). For example, the Arabidopsis LAP is expressed in all organs examined and its accumulation is not responsive to changes in development or exogenous hormones (25). It is probable that these constitutive LAPs have an important role in protein and peptide catabolism in plants.

A second form of LAP is detected in the Solanaceous plants, tomato and potato. While sharing a high peptide sequence identity with the constitutive LAP of Arabidopsis and high degree of epitope conservation with the constitutive LAPs of tomato (10, 12), this second class of LAP proteins and genes is regulated in a distinct manner. The potato LapA mRNA is induced in leaves by two wound signals, abscisic acid and jasmonic acid, but its mRNA is not induced by bacterial and fungal pathogens (11, 26). In healthy potato plants, LapA mRNAs are found at high levels in developing floral buds and lower levels in tubers; they are not present in any other plant organs examined (16, 19).

**References**

1 The abbreviations used are: LAP, leucine aminopeptidase; AcMNPV, A. californica nuclear polyhedrosis virus; hp, base pair(s); LIHC, light-harvesting complex protein; MeJA, methyl jasmonate; OEC, oxygen-evolving complex; PCR, polymerase chain reaction; PGAMI, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase; UTR, untranslated region; PAGE, polyacrylamide gel electrophoresis; CAPS, 3-(cyclohexylamino)propanesulfonic acid.

2 C. J. Tu and L. L. Walling, unpublished results.

3 W. C. Chao, Y.-Q. Gu, V. Pautot, F. M. Holzer, and L. L. Walling, manuscript in preparation.
organ examined (11). Similar to potato, the tomato LapA mRNAs, proteins, and activity are induced after mechanical wounding (10, 12) and in developing floral buds (27). Unlike potato, the tomato LapA mRNAs and proteins are systemically induced after wounding and are induced after Pseudomonas syringae pv. tomato (F.s. tomato) infection and insect infestation of tomato leaves (10, 12). Recent data indicate that during F.s. tomato infection, the LapA mRNAs, proteins, and activities are induced primarily in response to the pathogen toxin coronatine. Using polyclonal antibodies to the tomato LAP-A, four classes of LAP and LAP-like proteins are detected in wounded tomato leaves (12). While the LAP-like polypeptides and the LAP proteins are systemically induced after wounding, the class of LAP and LAP-like proteins are detected in wounded potato, the tomato LapA gene (12) and are induced after mechanical wounding and are induced after P.s. tomato (P.s. tomato) infection and insect infestation of tomato leaves (10, 12). Recent data indicate that during P.s. tomato infection, the LapA mRNAs, proteins, and activities are induced primarily in response to the pathogen toxin coronatine.

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**EXPERIMENTAL PROCEDURES**

**Plant Material and RNA Isolation—Lycopersicon esculentum** Peto238R plants were grown to the 4-leaf to 6-leaf stage in a growth chamber. Details on plant growth conditions, methods for wounding, tissue harvest, and RNA isolation and quantitation have been described previously (28).

**Constriction and Screening of a Wound-induced cDNA Library—**Poly(A+) mRNA was isolated from tomato leaves 24 h after mechanical wounding. cDNAs were synthesized and packaged into Agt11 SfiI-NotI cDNA arms according to the manufacturer’s instructions (Promega, Madison,WI). The primary library contained 1.5×10⁶ recombinants. Approximately 10⁷ phage from the unamplified cDNA library were screened using a partial LapA1 cDNA clone (pDR57) (10). pDR57 was labeled with [α-³²P]dCTP (3000 Ci/mmol; Amersham) using the random primer method (29). The prehybridization, hybridization, and wash conditions have been described (30). LapA-positive clones were plaque-purified by secondary and tertiary screenings.

**Characterization of LapA cDNA Clones—**Phage were eluted from individual plaques in 500 μl of SM buffer (0.01% gelatin, 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 8 mM MgSO₄). Using the left (5'-TGGCGGACGTGTGGGAGCG-3') and right (5'-TGAACACAGGACACTTGGAATAAGG-3') agt11 primers and 10 μl of phage eluate, LapA cDNA inserts were amplified using the polymerase chain reaction (PCR). The agt11 primers were synthesized by the Biotechnology Instrumentation Facility (University of California, Riverside). The temperature cycle for PCR amplification was 1 min at 94°C, 1 min at 60°C, and 3 min at 72°C. Only clones with near full-length inserts (approximately 2 kilo-base pairs) were further analyzed. PCR products were digested with XhoI to identify the two classes of LapA cDNAs. λ DNAs were purified (31), and SfiI/NotI-digested LapA cDNA inserts were cloned into pGem11 (Promega). For DNA sequencing, LapA1 and LapA2 cDNA inserts in pGem11 were cloned into Smal-digested pBlapA1-SK (pBlapA1 and pBlapA2 respectively) DNA sequencing was facilitated by generating a series of deleted using exonuclease III (32). The DNA sequence of both strands was determined by the dideoxy chain-termination method using Sequenase (U.S. Biochemical Corp., Cleveland, OH) and ³²P-labeled dATP (>1000 Ci/mmol; Amersham). The degree of peptide similarity for the deduced LapA polypeptides and the alignments of the animal, prokaryotic, and plant LAP proteins were determined using the GAP or PILEUP programs of the University of Wisconsin Genetics Computer Group.

**DNA Blot Hybridization—**The isolation and characterization of the LapA genomic clones, lap1-1, lap1-2, lap2-2, and lap3-4, will be described elsewhere. DNA blots with XhoI-digested λap clones were hybridized with a ³²P-labeled pBlapA1-3UTR probe (29). pBlapA1-3UTR contains a SpeI/EcoRI subclone of pBlapA1 and contains only the 3'-UTR of the LapA1 cDNA. Hybridization conditions and washes were performed as described by Walling et al. (30).

In Vitro Translation and Immunoprecipitation—pBlapA1 (2 μg) was transfected into 25881 cells (2×10⁶ cells) were cotransfected with 0.5 μg of recombinant plasmid and 0.5 μg of pBlapA1-3UTR. The translation mixture was directly fractionated by 12% SDS-PAGE (33) or immunoprecipitated with the tomato LapA polyclonal antiserum (12). Immunoprecipitation of translated proteins was performed as described by Kessler (34) with the following modifications. The translation mixture (20 μl) was diluted to 1 ml with 1×TPBS (170 mM NaCl, 6.2 mM KCl, 12.6 mM Na₂HPO₄, 2.2 mM KH₂PO₄, pH 7.4, 0.5% Triton X-100). LAP polyclonal antibodies (50 μl) were added and incubated on ice for 1 h with gentle shaking. The antibody-antigen complexes were precipitated by adding 50 μl of Immunoprecipitin (Life Technologies, Inc.), incubating on ice for 1 h, and centrifugation at 12,000×g for 2 min. The pellet was resuspended and washed three times with 1×TPBS. The protein pellet was boiled for 2 min in 250 μl of 2×SDS and 6 M urea. After removal of Immunoprecipitin by centrifugation at 10,000×g for 2 min, SDS sample buffer was added to give a final concentration of 2.5% SDS, 5% β-mercaptoethanol, 10% glycerol, 0.05% bromphenol blue, and 62.3 μM Tris-HCl (pH 6.8). The immunoprecipitated proteins were fractionated by 12% SDS-PAGE (33).

**Expression of the LapA1 cDNA in Insect Cells—**A LapA1 cDNA lacking the 5'-UTR was ligated into the baculovirus polyhedrin expression vector, pV1393 (InVitrogen, San Diego, CA), which was digested with BamHI and Smal1. The resulting plasmid, pV1apATG, fuses the baculovirus polyhedrin promoter and its 5'-UTR with the complete coding region and 3'-UTR of LapA1. Site-directed mutagenesis of the LapA1 insert in pBlapA1 was necessary to create a BamHI site and to minimize the number of nucleotides added to the polyhedrin promoter-directer transcript. These gene manipulations are detailed in (35).

**Triochoplusian TNS5 cells were propagated in monolayer cultures and Autographa californiae (AcMNPV) infections were performed as described by Summers and Smith (36). TNS5 cells (2×10⁶ cells) were cotransfected with 0.5 μg of linearized AcMNPV virus (PharMingen, San Diego, CA), 3 μg of pV1apLATG, and 5 μg of liposomes (Life Technologies, Inc.). Five days later, the media from the transfected cells were screened for recombinant virus (AcMNPV-lapA1) by infecting TNS5 cells in a 96-well plate with serial dilutions of the transfection media. Media from recombinant-positive wells were used for several rounds of infection of TNS5 cells to produce a high titer recombinant virus. For LAP expression studies, TNS5 cells were plated at 2×10⁶ cells/60-mm culture dish and were infected with 3×10⁶ plaque-forming units of either wild-type AcMNPV or AcMNPV-lapA1. Cells were harvested every day for 6 days after infection.

**Methyl Jasmonate Treatment and Isolation of Chloroplasts—**A 500 mM methyl jasmonate (MeJa), 90% ethanol stock was used directly or diluted in water prior to use. One-month-old tomato plants were excised at the base of the stem. Excised plants were placed in a flask with 250 ml of 10 mM MeJa, 0.002% ethanol in an air-tight glass desiccator. The desiccator contained a cotton-tipped applicator that was wetted with 1 ml of 500 mM MeJa, 90% ethanol. Excised plants incubated in 250 ml of 0.002% ethanol in a desiccator with a cotton-tipped applicator wetted with 1 ml of 100% ethanol served as controls. MeJa-treated and control plants were incubated for 24 h prior to tissue harvest. Chloroplasts were isolated using the method described by Chabot et al. (37), and total proteins were extracted. Integrity of plastids was confirmed by light microscopy.

**Extraction, Fractionation, and Immunoblot Analysis of Proteins—**Extraction of soluble and insoluble proteins in TNS5 cells was according to MacDonald et al. (38); total proteins from insect cells, wounded and MeJa-treated tomato leaves, and chloroplasts from MeJa-treated

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4 V. Pautot, F. M. Holzer, J. Chaufaux, and L. L. Walling, submitted for publication.
leaves were extracted as described by Wang et al. (33). Proteins were dissolved in the solubilization buffer for two-dimensional PAGE.

For extraction of soluble leaf proteins, 15 g of wounded tomato leaves were harvested and homogenized with a blender in 30 ml of extraction buffer (50 mM Tris-HCl, pH 6.8, 1% β-mercaptoethanol, and 5% insoluble polyvinylpyrrolidone). The homogenate was filtered through three layers of Miracloth and centrifuged at 100,000 × g for 60 min at 4 °C to remove organelles and membranes. Soluble proteins were precipitated by adding 5 volumes of cold acetone and stored at −20 °C for 16 h. After centrifugation, the protein pellet was washed twice with cold acetone (−20 °C). The final pellet was dried under vacuum for 1 min and resuspended in the solubilization buffer for two-dimensional PAGE.

SDS-PAGE and two-dimensional PAGE were performed as described by Wang et al. (33). After electrophoresis, the gels were stained with Coomassie Blue R-250 or electrophoretically transferred to nitrocellulose filters (0.48 μm, Schleicher & Schuell BA85). The pI values of polypeptides were determined as described previously (12). The immunoblot analyses were carried out according to Gu et al. (12) using a 1:500 dilution of the polyclonal antibodies made against the LAP-A1 protein of tomato. The antisera against the chlorophyll a/b-binding proteins of the light-harvesting complex (LHCP) of photosystem II and the precursor of the 23-kDa oxygen-evolving complex protein (OEC23) have been described (42). The PGAMi antiserum was produced in rabbits as described by Cline et al. (41). The OEC23 and LHCP antisera were diluted 1:1500 prior to use. The antisera against the Lilium longiflorum plastocyanin (2,3-bisphosphoglycerate-independent phosphoglycerate mutase (PGAMi) has been described (41). The PGAMi antiserum was diluted 1:750 prior to use.

**RESULTS**

Isolation of Full-length cDNAs Encoding the Tomato Wound-induced LAPs—To isolate full-length LapA cDNA clones, a Agt11 SfuNotI cDNA library was constructed from mRNA isolated from wounded leaves of tomato and screened using a partial LapA1 cDNA clone, pDR57. Two classes of lap cDNA clones were distinguished by the presence or absence of an XbaI site. The LapA1 and LapA2 cDNA clones chosen for study had the longest cDNA inserts identified. The complete lapA1 cDNA clone (pBlapA1) lacked an XbaI site and had a 1930-bp insert. Based on results from RNase protection assays and the size of the LapA mRNA (2.0 kilobases) (10), the LapA1 sequence presented here is full-length. The cDNA had a short 5′-UTR (18 bp) that was followed by a large open reading frame encoding a 571-amino acid residue protein (Fig. 1). This protein has a molecular mass of 60 kDa, which is 5 kDa larger than the mature LAP-A protein (12). The context of the translation start site (UAACAUUGGC) fits the translational consensus sequence (AACAAUUGGC) of plant genes (43). Four putative polyadenylation signals (AAUAAA) were noted within the 170-bp 3′-UTR of LapA1 (Figs. 1 and 2).

**LapA2 cDNA clones with XbaI sites were also characterized. Only six nucleotide substitutions (at positions 1090, 1558, 1561, 1562, 1617, and 1647) were noted in the LapA1 and LapA2 coding regions. Two changes resulted in amino acid substitutions. One in LapA1 and two in LapA2 cDNAs were a Gly in LapA2 and the LapA1 Thr-515 was changed to Leu in LapA2. The 3′-UTR of LapA1 and LapA2 were more divergent, although still highly conserved in sequence (93% identity). Nine nucleotide substitu-
cleotide 19) identified a large open reading frame encoding a 60-kDa LAP-A protein with a pI of 6.3. A second potential initiation codon was located 291 nucleotides downstream, and if this ATG was utilized, a protein of 50 kDa would be synthesized. While this AUG was not in a nucleotide context (UGA-CAGG) that favored efficient translation in plants (43) and the deduced polypeptide was 5 kDa smaller than the observed size of the mature LAP-A from wounded leaves as determined by SDS-PAGE (12), the pI (5.5) of this protein was close to that observed for the mature LAP-A from wounded leaves (12). Therefore, it was essential to demonstrate which translational start codon was utilized.

To discriminate between the alternative initiation sites at nucleotide positions 19 and 301, the LAP-A1 protein was synthesized in vitro using the pBlapA1 plasmid and an in vitro coupled transcription-translation system. 35S-Labeled proteins were fractionated by SDS-PAGE (Fig. 3); a prominent 60-kDa protein revealed that it was a plant preprotein. To ensure that the 55-kDa LAP-A protein that accumulated in TN5 cells was indeed the mature protein, molecular size, 35S-labeled LAP-A are indicated by arrows. The protein sizes were determined by Bio-Rad prestained protein markers shown at left.

Processing of the LAP-A Precursor Protein in Insect Cells—To test whether the processing of the 60-kDa LAP-A precursor into the 55-kDa mature LAP-A occurred in vivo, expression of the full-length LAP-A was evaluated in E. coli and in insect cells. The LAP-A preprotein was not processed to its mature form in E. coli and accumulated to high levels in inclusion bodies (data not shown). However, processing of the LAP-A preprotein was observed in TN5 cells. TN5 cells were infected with AcMNPV-lapA1 or AcMNPV; cells were harvested at 1, 2, 3, 4, 5, and 6 days after infection. Soluble proteins and insoluble proteins were isolated and subjected to SDS-PAGE and immunoblot analyses with the plant LAP-A antiserum (Fig. 5). The TN5 cells accumulated multiple forms of the 60-kDa LAP-A proteins with pIs ranging from 6.2 to 6.4. This correlated well with the pI of the precursor protein deduced from the LapA1 cDNA clone (pI 6.4). In addition, multiple forms of the mature 55-kDa protein were detected and their pIs ranged from 5.6 to 5.8. These results were consistent with our previous findings that the 55-kDa LAP-A protein was detected in wounded tomato leaves had five forms in a pI range from 5.6 to 5.9 (12).

Determination of the N-terminal Residues of the Mature Acidic LAP Protein—The acidic LAPs were significantly enriched in soluble protein extracts from wounded tomato leaves. The two-dimensional PAGE profiles of total and soluble leaf protein profiles were distinct when visualized by Coomassie Blue staining (Fig. 6, A and B). The amount of the abundant ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (55 kDa, pI 7.3) in total protein extracts was dramatically reduced in soluble protein extracts, since intact nuclei, plas-
tides, and membrane structures were removed by high speed centrifugation (see “Experimental Procedures”). The two-dimensional immunoblot pattern of soluble proteins from wounded leaves was similar to that of phenol-extracted total leaf proteins (Fig. 6C; Ref. 12). All four classes of LAP and LAP-like proteins were detected. In the acidic region of these two-dimensional gels, three abundant, soluble, 55-kDa polypeptides were visualized by Coomassie Blue staining (Fig. 6B). To determine which of these polypeptides corresponded to the acidic LAP-A, the three polypeptides were excised from a two-dimensional gel, refractionated by SDS-PAGE, blotted, and incubated with the LAP polyclonal antiserum. The results indicated that protein 3 corresponded to the mature acidic LAP-A protein (data not shown).

The mature LAP-A was fractionated by preparative two-dimensional PAGE, the major LAP-A protein was identified and subjected to N-terminal analysis (see “Experimental Procedures”). Equimolar amounts of two amino acids were detected at the first five N-terminal positions, and a single amino acid was detected at the sixth position (Table I). Inspection of the deduced amino acid sequence of the LapA1 and LapA2 cDNA clones revealed that these data were consistent with the presence of two N termini for the LAP protein that were present in equivalent amounts. One polypeptide began at residue 54 and had the sequence Ile-Ala-Gly-Asp-Thr-Leu; the second polypeptide began with residue 56 and had the sequence Gly-Asp-Thr-Leu-Gly-Leu (Figs. 1 and 7). This predicts a 53- and 55-residue presequence was processed in vivo.

Localization of the LAP-A Proteins—While tomato and potato LAP presequences had features that were very similar to transit peptides that target proteins to the plastid (44, 45), cell fractionation studies suggested that the tomato LAP-A proteins were soluble proteins (12). To determine if LAP proteins were plastid-localized, chloroplast proteins and total proteins were isolated from leaves from control plants and plants that were treated with MeJA. Jasmonic acid and MeJA are potent inducers of some wound and defense response genes, including LapA genes (13). Fig. 7 displays a Coomassie Blue-stained SDS gel and immunoblot data using antisera to the wound-induced LAP-A and antisera to proteins with either a cytosolic or plastid localization. The immunoblots incubated with the antisera recognizing the chlorophyll a/b-binding proteins of the light-harvesting complex (LHCP) of photosystem II or the 23-kDa oxygen-evolving protein (OEC23) are shown (Fig. 7, D and E). These proteins are known to be localized to the thylakoid lumen and membrane, respectively. The chloroplast proteins were detected in the total protein extracts of healthy and MeJA-treated leaves and were more abundant in protein extracts from isolated chloroplasts (Fig. 7, D and E). Recent immunocytochemistry studies have shown that the 2,3-bisphosphoglycerate-independent phosphoglycerate mutase, PGAMi, is localized in the cytoplasm and nucleus in cells of higher plants (42). This protein was detected at equivalent levels in total protein extracts from control and MeJA-treated leaves, while it was not detected in the chloroplast proteins isolated from MeJA-treated plants (Fig. 7C). The tomato LAP antiserum immunoblots indicated that LAP proteins could be detected in chloroplasts from leaves of MeJA-treated plants. However, a comparison of the chloroplast and total protein extract immunoblots indicated that the majority of the tomato LAP proteins were not plastid-localized (Fig. 7B).
The wound-induced LAP-A proteins of tomato were encoded by two highly conserved genes, LapA1 and LapA2. The coding regions of the LapA genes were over 99% identical, and only two of the six nucleotide substitutions caused changes in the deduced amino acid sequence of the LapA1 and LapA2 proteins. The high degree of nucleotide identity observed in the coding regions of the LapA1 and LapA2 genes is not uncommon in tightly linked genes from multi-gene families in tomato (46, 47). The LAP-A polypeptide sequences deduced from the LapA1 and LapA2 cDNA clones predicted that LAP-As were synthesized as 60-kDa preproteins. This was supported by in vitro translation of the full-length LapA1 mRNA and by expression of the LapA1 cDNA in TN5 insect cells. These data unequivocally demonstrated that the LapA1 gene encoded the acidic LAP-As and suggested that the proteolytic machinery important for preLAP-A processing was conserved in animal cells.

While five acidic LAP-A forms were detected in wounded tomato leaves (12), only two LAP-A forms were detected in insect cells. It is likely that the other three forms were the result of the expression of the LapA2 gene and post-translational modifications of the LAP-A1 and LAP-A2 proteins in plant cells. The nature of these modifications is unknown.

N-terminal sequence analysis of the most abundant, mature LAP-A form resolved by two-dimensional PAGE indicated that the mature LAP-A protein had two N termini separated by 2 residues (Ile-54 and Gly-56). These data indicated that 53 or 55 residues of the LAP-A presequence were proteolytically removed in vivo. Since the LAP-A1 and LAP-A2 proteins were identical for the first 357 amino acid residues, it is impossible to determine if the two ends represented alternate termini for one of the acidic LAP-A proteins or if the two termini could represent LAP-A1 and LAP-A2 polypeptides that were differentially processed but co-migrate as a single isoelectric form during two-dimensional PAGE.

The deduced amino acid sequence from a bovine kidney Lap cDNA suggests that the animal LAP is also synthesized as a preprotein (20). It has a 26-amino acid residue N-terminal extension that is removed to yield the mature LAP form (48). In animals, LAP is a cytosolic enzyme (49). Comparison of the bovine LAP presequence and that tomato LAP-A presequences showed that there was no sequence similarity (data not shown). Neither the bovine or tomato LAP-A presequences was sufficient to be a likely candidate for targeting to the endoplasmic reticulum. While the bovine presequence was proline-rich (27% of the residues), the 53- and 55-residue LAP-A presequences had a high percentage of hydroxylation amino acids (>30% Ser and Thr). A second distinguishing feature was that the tomato presequence had nine basic residues and no acidic residues in this region. The features noted for LAP-A are consistent with the presequence serving as a transit peptide for transport in to the chloroplast.

A similar presequence was also observed in the deduced peptide sequence of the wound-induced potato LAP (26) and was found to be lacking in the Arabidopsis LAP cDNA (25). This implies that the Arabidopsis LAP was processed differently and may have a different subcellular localization than the wound-induced LAP-As or the Arabidopsis cDNA was not full-length. While the N termini of the tomato LAP-A proteins that accumulated in vivo were determined here, the location of the actual processing site for the LAP-A proteins is harder to determine. It is not clear if the two N termini of the mature LAP-As were the result of the concerted action of endo- and exo-proteases or the result of one or two distinct endonucleolytic events.

Despite the fact that the LAP-A presequences had features similar to transit peptides important for targeting proteins to plastosids, two lines of evidence suggested that this was not the primary location of LAP-A. Cell fractionation studies on wounded leaves (12) and the analysis of total and chloroplast proteins from MeJA-treated leaves indicated that LAP-A was a soluble protein and only a small fraction of the LAP-A protein was plastid-localized. These data suggest that LAP-A proteins reside in two locations in the tomato cell: the cytosol and the plastid. There are several examples of single-copy genes in higher plants that encode polypeptides that are localized to more than more one cell compartment. In Arabidopsis, a bifunctional gene encodes both the cytosolic and mitochondrial forms of alanyl-tRNA synthetase (50), and in pea, a single gene.

| TABLE I |
| --- |
| N-terminal residue determination for the mature acidic LAP-A polypeptide |
| Residue no. | Amino acid residues |
| 1 | Ile, Gly |
| 2 | Ala, Asp |
| 3 | Gly, Thr |
| 4 | Asp, Leu |
| 5 | Thr, Gly |
| 6 | Leu |

"The residues are numbered from the N terminus of the LAP-A polypeptide."
Fig. 8. N termini of the mature LAP-A and location of the dibasic protease processing motif. The deduced peptide sequence of the LAP-A leader and mature protein (residues 44–61) are presented. The dibasic protease motif A is boxed (48). The two N termini for the major LAP-A form were identified by Edman degradation (Table I) and are indicated by arrows.

encodes the cytosolic, chloroplastic, and mitochondrial glutathione reductase (51).

Inspection of the LAP-A presequence revealed the presence of two motifs that could potentially be involved in the maturation of the LAP-A preproteins. The small amount of LAP-protein that is localized to the chloroplasts may utilize the transit peptide consensus motif of (Val/Ile)-(A/ala/Cys) (ala) that was described by Gavel and von Heijne (45). Residues 51 to 55 of LAP-A were a close match to this consensus: Val-His-Cys-Ile.

The dibasic protease motif A is the LAP-A leader and mature protein (residues 44–61) are presented. The subcellular localization of LAP-A will in part determine the nature of its potential substrates and its exact role in the plant cell and the integrity of that cell compartment in response to pathogen invasion, pest attack, or mechanical wounding will ultimately control the accessibility of the wound-induced leucine aminopeptidase to plant- or pathogen-encoded peptides or proteins.

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