Identification and Characterization of SppA, a Novel Light-inducible Chloroplast Protease Complex Associated with Thylakoid Membranes

Martin Lensch, Reinhold G. Herrmann, and Anna Sokolenko‡
From the Botanisches Institut der Ludwig-Maximilians-Universität, Menzingerstrasse 67, D-80638 München, Germany

A new component of the chloroplast proteolytic machinery from Arabidopsis thaliana was identified as a SppA-type protease. The sequence of the mature protein, deduced from a full-length cDNA, displays 22% identity to the serine-type protease IV (SppA) from Escherichia coli and 27% identity to Synechocystis SppA1 (sll1703) but lacks the putative transmembrane spanning segments predicted from the E. coli sequence. The N-terminal sequence exhibits typical features of a cleavable chloroplast stroma-targeting sequence. The chloroplast localization of SppA was confirmed by in organello import experiments using an in vitro expression system and by immunodetection with antigen-specific antisera. Subfractionation of intact chloroplasts demonstrated that SppA is associated exclusively with thylakoid membranes, predominantly stroma lamellae, and is a part of some high molecular mass complex of about 270 kDa that exhibits proteolytic activity. Treatments with chaotropic salts and proteases showed that SppA is largely exposed to the stroma but that it behaves as an intrinsic membrane protein that may have an unusual monotopic arrangement in the thylakoids. We demonstrate that SppA is a light-inducible protease and discuss its possible involvement in the light-dependent degradation of antenna and photosystem II complexes that both involve serine-type proteases.

Protein degradation is one of the major regulatory processes that allow the adaptation, repair, or removal of thylakoid proteins during environmental or developmental changes (reviewed in Refs. 1 and 2). However, our knowledge about chloroplast proteases remains limited. To date, the components of the degradation machinery that have been identified in the organelle include the stroma-located members of the Clp family, which are well conserved from prokaryotes to eukaryotes (3–7), the stromal (8, 9) and thylakoid (10) processing proteases that dissect imported protein precursors, the thylakoid-bound metalloprotease FtsH (11), and two luminal components, the heat shock protease HtrA (DegP; Ref. 12) and HtrA proteases (12, 23, 24). In chloroplasts, several light-dependent degradation processes point to the possible existence of light-regulated proteases. For instance, the accumulation of early light-inducible proteins, a class of proteins that are induced at early stages of illumination, has been suggested to be controlled by a light-repressed protease (25, 26). Conversely, degradation of LHCII, the major peripheral antenna protein of thylakoid membranes, is induced under high light and probably involves extrinsic (27) as well as thylakoid-intrinsic proteases (28). The best studied light-induced degradation process in chloroplasts is that of photosystem II. Photosystem II repair under light stress requires the specific removal and degradation of photodamaged D1 (29–31) and of the chlorophyll a core antenna protein CP 43 (32).

We describe here a new chloroplast-located protease encoded by a nuclear gene in Arabidopsis that fulfills the requirements for participating in light-induced degradation processes in the organelle. This protease displays significant homology to prokaryotic SppA proteases.

EXPERIMENTAL PROCEDURES

Plant Growth—Arabidopsis and spinach seedlings were grown for 3–4 weeks in a greenhouse at 25 °C under a 12-h light period with a moderate light regime (150 μE m⁻² s⁻¹). To monitor the effect of light on RNA or protein levels, the plants were transferred to darkness or high intensity light (700 μE m⁻² s⁻¹) for periods of 0–52 h. Plants exposed to different light regimes were harvested and used for purification of numerous chloroplast proteolytic activities in chloroplast subfractions (15) and from the genome analysis of one chloroplast progenic ancestor, the Synechocystis genome, which displays about 40 different protease sequences. Biochemical studies have provided information about substrate and inhibitor specificity for some proteolytic activities (6), but a detailed knowledge of proteolytic processes requires that the enzymes themselves and the corresponding genes are characterized at the molecular level.

Two kinds of proteases can be operationally distinguished from a physiological point of view: constitutive proteases that, for instance, are involved in the degradation of abnormal or damaged proteins and inducible proteases that are produced or activated only under conditions where specific degradation processes are required. The latter include enzymes that are induced by high light regimes or under heat shock. Well known representatives of that category from plants and bacteria are the heat shock protease Lon (16, 17), the Clp family (7, 15, 18–21), HslUV (22), and HtrA proteases (12, 23, 24). In chloroplasts, several light-dependent degradation processes point to the possible existence of light-regulated proteases. For instance, the accumulation of early light-inducible proteins, a class of proteins that are induced at early stages of illumination, has been suggested to be controlled by a light-repressed protease (25, 26). Conversely, degradation of LHCII, the major peripheral antenna protein of thylakoid membranes, is induced under high light and probably involves extrinsic (27) as well as thylakoid-intrinsic proteases (28). The best studied light-induced degradation process in chloroplasts is that of photosystem II. Photosystem II repair under light stress requires the specific removal and degradation of photodamaged D1 (29–31) and of the chlorophyll a core antenna protein CP 43 (32).

We describe here a new chloroplast-located protease encoded by a nuclear gene in Arabidopsis that fulfills the requirements for participating in light-induced degradation processes in the organelle. This protease displays significant homology to prokaryotic SppA proteases.

‡ To whom correspondence should be addressed. Tel.: 49-89-17861242; Fax: 49-89-1782274; E-mail: anna@botanik.biologie.uni-muenchen.d.

** This work was supported by Deutsche Forschungsgemeinschaft Grant DFG SO448/2-1 (to A. S.) and SFB 184 (to R. H.) and the Fonds der Chemischen Industrie. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received for publication, January 18, 2001, and July 5, 2001 
Published, JBC Papers in Press, July 6, 2001, DOI 10.1074/jbc.M100506200
SppA Protease from Higher Plants

cDNA Library Screening and Nucleotide Sequence Analysis—The Arabidopsis expressed sequence ESA107 (accession number AA042706), which displayed homology to prokaryotic SppA proteases, was obtained from the Arabidopsis Biological Resource Center (Ohio State University). A full-length SppA cDNA was isolated from an Arabidopsis (Arabidopsis thaliana var. Landsberg erecta) cDNA library using a probe comprised of the amino acid sequence of SppA (15) and expressed in the E. coli strain M15 and BL21 (Qiagen and Promega, Madison, WI). The insert comprised a full-length cDNA. It suggested that the insert comprised a full-length cDNA. It suggested that the insert comprised a full-length cDNA.

Characterization of the Corresponding cDNA

Identification of Hybridizing DNA Fragments in the A. thaliana cDNA Library—The library of BACs used was constructed at the Institut für Genbiologische Forschung-BAC Library—The library of BACs used was constructed at the Institut für Genbiologische Forschung-BAC Library—The library of BACs used was constructed at the Institut für Genbiologische Forschung-BAC Library. The library of BACs used was constructed at the Institut für Genbiologische Forschung-BAC Library used was constructed at the Institut für Genbiologische Forschung-BAC Library (Ref. 34). Individual recombinant BACs of this library are currently being ordered into contigs that at present represent more than 90% of the Arabidopsis genome. Additionally, representative DNA probes with known map positions were hybridized to BAC clones anchoring the available contigs on the genetic map of molecular markers. Taking advantage of these data (www.mpimp-golm.mpg.de/101/igf_bac_con-t.html), sppA was mapped by hybridization to library filters of 9216 BAC clones spotted in duplicate in 3 x 3 arrays. The filters were obtained from the Resorzentrum im Deutschen Humangenomprojekt, (Berlin, Germany; www.rzpd.de) and hybridized according to Legen et al. (45).

FIG. 1. Amino acid alignment of SppA proteases from A. thaliana (At), Synechocystis (Syn), and E. coli (Ec). Amino acid residues identical between Arabidopsis and one of the other sequences are marked by shaded grey boxes, and identical residues between all sequences are indicated by black boxes. The three purported transmembrane domains in the E. coli sequence are underlined. Predicted cleavage sites for the stroma-targeting transit peptide in the Arabidopsis sequence are marked by arrowheads. Possible "catalytic" amino acid residues are indicated by asterisks (see "Discussion"). The amino acid residues in the Arabidopsis and E. coli sequences, respectively, are marked above and below the alignments.

RESULTS

Identification of the SppA Protease from A. thaliana and Characterization of the Corresponding cDNA—A search of the Arabidopsis data base revealed a DNA sequence exhibiting homology to the prokaryotic protease SppA. An expressed sequence tag (EBA1077, accession number AA042706) of 835 base pairs was used as a probe for isolating the full-length DNA by screening of an appropriate A. thaliana cDNA library. Five clones were isolated. The longest clone (with the largest insert, 2196 base pairs (accession number AF114385), was sequenced. The presence of an in-frame stop codon at position −15, a typical translation initiation sequence

\[
\text{GGACAATGGGCA}^{-15} \text{ and a poly(A) tail at the 3'} \text{ end suggested that the insert comprised a full-length DNA. It contained a single open reading frame of 2068 nucleotides,}
\]
which corresponds to a polypeptide of 680 amino acid residues (Fig. 1) and a calculated molecular mass of 74.8 kDa. The coding region is preceded by a 28-base pair 5′-untranslated region and is trailed by an untranslated stretch of 119 base pairs. The N-terminal 68-residue domain of the protein displays typical features of a chloroplast stroma-targeting transit peptide (47); it initiates with MetAla characteristic of such proteins and contains positively charged (Arg) and hydrophobic short side chain residues (Ser, Ala) and a predicted amphiphilic β-sheet (residues 62–67). PSORT and ChloroP programs predicted two potential cleavage sites following Ala and Gln of the presequence (48, 49) and contains positively charged (Arg) and hydrophobic short side chain residues (Ser, Ala) and a predicted amphiphilic β-sheet (residues 62–67). PSORT and ChloroP programs predicted two potential cleavage sites following Ala and Gln of the presequence (48, 49)

SppA Is a Single Copy Gene in the Arabidopsis Genome—Southern analysis of total Arabidopsis genomic DNA as well as a search through the A. thaliana Data Base (Stanford, CA) provided no evidence for a second sppA gene in the Arabidopsis genome. High density filters of BAC contigs covering almost the entire Arabidopsis genome (44) were used to substantiate the gene copy number and to determine the chromosomal localization of sppA. A signal consistent with a single gene copy was found on a single BAC (number F2P9) originating in chromosome 1 (data not shown). The recent sequencing of the Arabidopsis genome (internet information at mips.gsf.de/proj/thal/) confirmed the presence of a single sppA gene on that chromosome (accession number AC016662).

SppA Is a Chloroplast Protein Located in Thylakoid Membranes—To evaluate the chloroplast location of SppA, we conducted in organello import experiments with radiolabeled precursor protein made in vitro from the SppA cDNA. The sample displayed various premature termination products together with the precursor form of SppA, labeled p in Fig. 2 (lane TP). The radiolabeled SppA precursor protein was successfully imported into intact chloroplasts from spinach and processed to the mature form (labeled m in Fig. 2). The imported mature SppA was resistant to proteolytic attack upon incubation of intact spinach chloroplasts with thermolysin or trypsin for 30 min (experiment not shown). Subfractionation of the organelles into thylakoid (Fig. 2, lane Th) and soluble stroma fractions (Fig. 2, lane S) showed that the radiolabeled SppA mature protein was recovered exclusively in the thylakoid fraction (Fig. 2, lane Th). The thylakoid localization of SppA within the organelle was confirmed by a serological approach with stromal, thylakoid membrane and lumenal proteins prepared from intact Arabidopsis chloroplasts using an antiserum elicited against the C-terminal part of the protein (amino acid residues 493–680; Fig. 3A). SppA could be detected as a 68-kDa polypeptide that was exclusively associated with thylakoid membranes (Fig. 3A). We further fragmented thylakoid membranes by digitonin treatment and subsequent differential centrifugation. This treatment allowed separate fractions enriched in grana membranes, grana margins, and stroma lamellae (for details see “Experimental Procedures”). As illustrated by Fig. 3B, the immunodetection of the 33-kDa subunit of the oxygen-evolving complex of photosystem II (control) were used for immunodetection in fractionated thylakoid membranes.

To estimate the nature of the association of SppA with the stroma lamellae, purified thylakoids were treated with solutions of chaotropic salts (NaBr and NaSCN) or with alkaline solutions (Na₂CO₃ and NaOH). The protein was partially released from the membranes only after treatment with the strong chaotropic reagent NaSCN (Fig. 4). That the membrane association of SppA resisted alkaline treatments as well as NaBr treatment suggests that SppA behaves rather as an integral than as a peripheral membrane protein.

The topology of SppA within thylakoid membranes was further investigated by protease protection assays. Freshly prepared intact thylakoids or thylakoids permeabilized by sonication were treated with thermolysin, trypsin, or proteinase K (Fig. 5). As a control experiment, we first assayed the thylakoid samples with an antibody against the 33-kDa protein of the oxygen-evolving system that is peripherally located at the luminal face of thylakoid membranes (51). In intact thylakoids (Fig. 5, left bottom panel), the 33-kDa protein remained fully protected against proteolytic attack, even using the harshest proteases.
Upon thylakoid sonication, the 33-kDa protein became mildly sensitive to thermolysin and produced a degradation product of low molecular mass seen at the gel bottom. It was fully degraded by trypsin and proteinase K under the same conditions. Thus, the externally added proteases got full access to the luminal membrane surface only upon sonication of the thylakoids.

In intact thylakoids, SppA was partially digested by thermolysin or trypsin, and it was extensively digested by proteinase K (Fig. 5, left upper panel). Thus, SppA was readily accessible from the stromal surface to externally added proteases. Sonicated thylakoids that were also accessible to proteases from the luminal surface displayed the same SppA proteolytic patterns as those observed with intact thylakoids. Thus, we conclude that SppA is exposed at the stromal face of thylakoids and that it has no notable protease-sensitive domains exposed to the thylakoid lumen.

SppA Is a Proteolytically Active Enzyme—To study the proteolytic activity of SppA we first attempted to overexpress...
precursor and mature forms of the protein in *E. coli*. Overexpression of the precursor form proved unsuccessful, whereas that of the mature form, starting from Phe<sup>60</sup>, resulted in low yield production of two truncated products (data not shown). These results suggest that the heterologous expression of chloroplast SppA was detrimental to the bacteria most likely by virtue of its homology to the bacterial SppA protease (59). Therefore, the proteolytic activity of SppA was further analyzed by gel exclusion chromatography after mild solubilization of the major protein complexes from the thylakoid membranes. SppA was part of a high molecular mass complex of ~270 kDa that eluted in fractions 15–17 marked in Fig. 6A, consistent with the homotetrameric structure of SppA from *E. coli* (59). The fractions collected from size exclusion chromatography were analyzed both for the presence of SppA by immunodetection (Fig. 6B) and for proteolytic activity (Fig. 6C). The SppA-containing fractions, when run on nondenaturing gels containing gelatin, showed high proteolytic activity as visualized by the white band on a dark background (Fig. 6C). The apparent molecular mass deduced from the electrophoretic migration of the proteolytically active band, ~270 kDa, corresponded well with that of the complex eluted by size exclusion chromatography. Similar data were obtained by sucrose gradient centrifugation of thylakoid lysates according to Ref. 41 (data not shown). The proteolytic activity of SppA was significantly inhibited by the specific antiserum (~90%); no suppression occurred with preimmune serum), by phenylmethylsulfonyl fluoride but not iodoacetamide consistent with a serine-type protease (data not shown).

**SppA Is a Light-inducible Protease in Arabidopsis**—RNA filter hybridization was employed to investigate the expression of SppA at the transcript level (Fig. 7). When *Arabidopsis* plants were grown under moderate light (150 μE m<sup>−2</sup> s<sup>−1</sup>), they displayed no detectable SppA transcript (Fig. 7, lane 0). However, the more sensitive combined reverse transcriptase-polymerase chain reaction technique on a Light Cycler machine (Roche Molecular Biochemicals) allowed to detect minute amounts of the transcripts (data not shown). SppA transcripts remained below detection using conventional Northern procedures in plants after heat shock treatment (data not shown). However, when Arabidopsis plants were transferred to higher light intensities (e.g. 700 μE m<sup>−2</sup> s<sup>−1</sup>), a 2.2-kilobase pair SppA transcript became detectable after 10 h of exposure. The stationary mRNA concentrations increased about five times during the next 40 h under high light (Fig. 7, lanes 10–52 h). This light-induced transcription was accompanied by a substantial increase in the steady-state levels of the corresponding protein (Fig. 8). In this experiment Arabidopsis plants grown under moderate light for 6 days were either kept under the same light regime or transferred to darkness or to higher light intensities for 52 h. A serological identification of SppA using radiolabeled <sup>125</sup>I-protein A (Fig. 8A) or the ECL method (Fig. 8B) revealed that the protein was already present in significant amounts in plants grown under moderate light conditions, although the corresponding transcript was hardly visible in Northern experiments (Fig. 7). Quantification using <sup>125</sup>I-protein A showed that the SppA protein increased approximately four times after transfer of *Arabidopsis* plants to higher light intensities. The amount of cytochrome f, used here as a control, remained virtually unchanged under these conditions (Fig. 8C). Using the nonquantitative but more sensitive ECL method (Fig. 8B), we observed a lower molecular mass product that accumulated in much higher amounts after transfer of the plants from moderate light to darkness but became less abundant upon transfer to high light. These observations indicate that mature SppA is slowly converted to a truncated product, especially in low light or darkness. On the other hand, the steady-state fraction of mature SppA increases drastically at higher light intensities in parallel with the light induction of SppA gene expression.

**DISCUSSION**

We provide evidence for a novel, light-regulated protease, designated SppA, that is located in chloroplast thylakoids. This protease appears to be ubiquitous because it possesses homologues in eubacteria, archaeabacteria, and DNA viruses (MEROPS, peptidase data base; www.merops.co.uk/). SppA was identified as a bona fide chloroplast protease in *Arabidopsis*, because (i) sequence analysis predicted a transit peptide typical of a stroma-targeting presequences, (ii) in *vitro* expressed SppA was successfully imported into intact spinach chloroplast, and (iii) SppA could be detected immunologically as well as functionally active among the proteins from isolated chloroplasts.

Organelle subfractionation demonstrated that SppA is a membrane-bound protease that is heterogeneously distributed along thylakoid membranes. On a total protein basis, it was highly enriched in stroma lamellae and grana margins rather than in grana. Purification of SppA by size exclusion chromatography after limited detergent solubilization of the thylakoid membranes showed that the protease is part of a high molecular mass complex. This complex possesses a similar molecular mass as SppA from *E. coli*, which was found to form a tetrameric structure (60). Thus, chloroplast SppA most likely undergoes a comparable oligomerization as in bacteria. The SppA complex showed proteolytic activity in gelatin-containing gels, implying that it constitutes a new chloroplast protease of the SppA family.

Interesting aspects emerge from comparisons of topology predictions for SppA in chloroplasts (thylakoid membranes) and *E. coli*, which may relate to different localizations and the...
relatively low homology between the corresponding enzymes. In thylakoids, SppA was largely exposed at the stromal surface of the thylakoid membrane because it was extensively digested from the stromal side of the membrane by proteinase K. However, its limited accessibility to trypsin and thermolysin suggests that the stroma-exposed domains of SppA are tightly folded. We found no evidence for the exposure of protein domains at the luminal face of the membrane, indicating that the protease is a monomeric component. This is intriguing because the association of SppA with the membranes resisted treatments with alkaline or NaBr solutions that release most of the peripheral membrane proteins. Thus, SppA behaved rather as an integral membrane protein. Monotopic integral membrane proteins with comparable properties have been described previously (for reviews see Refs. 52 and 53). The best characterized monotopic integral membrane protein is prostaglandin synthase, whose crystal structure has revealed an amphiphilic helix that is most likely lying at the junction between the polar head groups and the fatty acid chains of the membrane lipids (54).

To further understand the nature of the interaction of SppA with membranes, we analyzed the SppA sequences from three sources in greater detail, notably from Arabidopsis, Synechocystis, and E. coli (Fig. 1). The SppA protease from the Arabidopsis chloroplast displays 27% identity to Synechocystis SppA1 (sll1703) and 22% to E. coli SppA. Highest conservation was found in the central domain of the protein (Fig. 1; residues 418–485 of Arabidopsis sequence), with a homology exceeding 50% within two weakly hydrophobic stretches (underlined in the E. coli sequence included in Fig. 1). These stretches, together with an N-terminal hydrophobic segment (also underlined in Fig. 1), have been previously suggested to contribute to the transmembrane binding of the E. coli enzyme to the plasmamembrane (55, 56). However, the purported N-terminal transmembrane domain (Fig. 1), which is the most reasonable candidate for the membrane anchoring of the E. coli protease, is absent in the Arabidopsis sequence (Fig. 1). Therefore, the chloroplast-located SppA protease should interact with the lipid bilayer through one or both of the other hydrophobic stretches that are homologous to the corresponding E. coli sequence (in the domain defined by amino acid residues 398–441 in E. coli). As a serine-type protease (55), part of this SppA domain is likely to contribute to the catalytic site of the enzyme because it contains the four serine residues (Ser426, Ser453, Ser460, and Ser482) that are strictly conserved among all SppA proteases known so far. Studies of catalytic domains of the serine-type proteases (57, 58) have indicated that serine residues involved in catalysis should be found close to histidine or lysine residues to form the so-called catalytic dyad. Only the two first serine residues (Ser426 and Ser453) are located at an appropriate distance from highly conserved lysines (positions 418 and 448). Because the two subsequent serines (positions 460 and 482) are placed within a hydrophobic stretch without lysine or histidine residues in their vicinity, we infer that Ser426 and Ser453 are involved in the catalytic function of SppA, whereas the latter may be part of a membrane anchor region. The hydrophobic interaction of the protease with the membrane may then be based on the domain encompassing residues 454–492 in Arabidopsis. However, there are no hydrophobic stretches that are long enough to span the membrane in this region. Instead, a wheel presentation of this region from the three sources (Fig. 9A) shows the occurrence of an amphiphilic helix. Its more polar face (Fig. 9A, domains I–IV) could allow an interaction with the polar head groups of the lipids, whereas the more hydrophobic face (Fig. 9A, domains V–VII) could interact with the fatty acid chains. This proposal is fully consistent with the biochemical and topological analysis that we have discussed above. A schematic view of the monotopic positioning of a single SppA molecule with respect to the thylakoid membrane surface is given in Fig. 9B. The catalytic domains of the protease could thus be held close to the thylakoid surface, in an appropriate position for an interaction with membrane protein substrates (Fig. 9B).

A most remarkable finding of our study is that SppA is a light-inducible protease. Although the substrates of the chloroplast SppA enzyme are unknown at present, it is conceivable that this protease participates in the light-dependent turnover of thylakoid membrane proteins or of peptides released from such proteins under light stress conditions. If so, SppA is another example of a light-induced protease, besides the metalloprotease FtsH (11), which may be involved in the second step of D1, a photosystem II core protein, degradation (60). SppA appears as a reasonable candidate for participating in the light-induced degradation of photosystem II (61–63) and/or LHCI antenna proteins (27, 64) as well. Both degradation processes were reported to involve serine-type endoproteases (27, 65). Two candidate enzymes for LHC degradation have been described: a membrane-bound protease (28, 66) and an extrinsic component that can be reversibly removed from thylakoids (27). Our observation that SppA is firmly attached to the membranes and enriched in stroma lamellae strongly argues for its involvement in LHC degradation through the proteolytic activity described in Refs. 28 and 66. This suggestion is supported by the kinetics of the light-induced LHC degradation process. Acclimation of thylakoid membranes, leading to the disposal of part of the LHCI complexes, is a slow process and requires an induction phase of ~48 h after transferring the plants from low to high light intensities (27, 64). Maximal induction of SppA mRNA was reached between 26 and 52 h of illumination with high light intensities, a period that corresponds with the max-
imum degradation of LHC protein during light adaptation (28). LHClII was shown to become highly accessible to proteolytic digestion upon its dephosphorylation in nonapressed membranes. Thus, SppA should be considered as a potential candidate for participating in the LHC degradation process, because of its light induction, thylakoid localization in nonapressed membrane domains, and serine content in the purported catalytic domain. The physiological role of SppA in the light-induced degradation processes of core and antenna complexes of photosystem II is currently under study.

The outlined findings bear on an intriguing phylogenetic aspect. Whereas the light induction of SppA points to its possible role in the degradation of transmembrane proteins, knock-out mutants in *E. coli* have suggested that SppA functions in the degradation of signal peptides once they are released from the precursor proteins (67, 68). A similar role has been suggested for SppA from *Bacillus subtilis* (69). It will be interesting to see whether the functional differences between bacterial and chloroplast SppAs, if correct, reflect a phylogenetic change in substrate usage and correlate with the limited sequence conservation that is restricted to the predicted catalytic core domain of the protein, whereas other parts involved in substrate recognition may have diverged during evolution.

**REFERENCES**

1. Adam, Z. (1996) *Plant Mol. Biol.* 32, 773–783
2. Andersson, B., and Aro, E.-M. (1997) *Plant Physiol. Plantanum* 100, 780–793
3. Gottesman, S., Squires, C., Pichersky, E., Carrington, M., Hobb, M., Mattick, J. S., Dairymple, B., Kuramitsu, H., Shirizu, T., Foster, T., Clark, W. F., Ross, B., Squires, C. L., and Maurizi, M. R. (1996) *Biochemistry* 35, 3513–3517
4. Mauriz, M. R., Clark, W. P., Katayama, Y., Rudikoff, S., Pumphy, J., Bowers, B., and Gottesman, S. (1996) *J. Biol. Chem.* 271, 12536–12545
5. Shankin, J., DeWitt, N. D., and Flanagan, J. M. (1995) *Plant Cell* 7, 1713–1722
6. Søklenko, A., Altschmiel, L., and Herrmann, R. G. (1997) *Planta* 207, 285–290
7. Porankiewicz, J., Wang, J., and Clarke, A. K. (1999) *Mol. Microbiol.* 24, 449–458
8. VanderVerE, P. S., Bennett, T. M., Ohlhorn, J. E., and Lampka, G. K. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 92, 7177–7181
9. Richter, S., and Lampka, G. K. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 7463–7468
10. Chal, B. K., Mould, R. M., Barbrook, A. C., Gray, J. C., How, C. J. (1998) *J. Biol. Chem.* 273, 689–692
11. Lindahl, M., Tabash, S., Coke, L., Pichtersky, E., Andersson, B., and Adam, Z. (1996) *J. Biol. Chem.* 271, 19229–19234
12. Ithaki, H., Naveh, L., Lindahl, M., Cook, M., and Adam, Z. (1998) *J. Biol. Chem.* 273, 7094–7098
13. Inagaki, N., Fujita, S., Sato, K. (1996) *Plant Mol. Biol.* 30, 89–90
14. Deltmuller, R., Herrmann, R. G., and Pakrati, H. B. (1996) *J. Biol. Chem.* 271, 21848–21852
15. Søklenko, A., Altschmiel, L., Altschmiel, L., Herrmann, R. G. (1998) *Plant Physiol.* 115, 827–832
16. Charette, M., Henderson, G. W., and Morkovitz, S. (1981) *Proc. Natl. Acad. Sci. U. S. A.* 78, 4728–4732
17. Serrra, R., Lyazik, A., Vallesius, C. E., Mackenzie, S., A. (1998) *Plant Cell* 10, 1217–1228
18. Kreh, H. E., and Simon, L. D. (1990) *J. Biol. Chem.* 172, 6026–6034
19. Squires, C. L., Pedersen, S., Ross, B. M., and Squires, C. (1991) *J. Biol. Chem.* 173, 4254–4262
20. Shimrner, E. C., Glover, G. R., Singer, M. A., and Liddis, G. A. (1996) *Trends in Biochem. Sci.* 21, 289–290
21. Waszynow, A., Wójciak-Kowalczyk, D., Missalakie, J., Banek, B., Jensen, M., Graves, B., Georgopoulos, C., and Zylke, M. (1995) *EMBO J.* 14, 1867–1877
22. Chuaq, S.-E., Burland, V., Plunkett III G., Daniels, D. L., and Blattner, F. R. (1995) *Gene* 134, 1–6
23. Lipinska, B., Sharma, S., and Georgopoulos, C. (1988) *Nucleic Acids Res.* 16, 10553–10667
Identification and Characterization of SppA, a Novel Light-inducible Chloroplast Protease Complex Associated with Thylakoid Membranes
Martin Lensch, Reinhold G. Herrmann and Anna Sokolenko

J. Biol. Chem. 2001, 276:33645-33651.
doi: 10.1074/jbc.M100506200 originally published online July 6, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M100506200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 66 references, 27 of which can be accessed free at http://www.jbc.org/content/276/36/33645.full.html#ref-list-1