The stromal processing peptidase (SPP) catalyzes removal of transit peptides from a diversity of precursor proteins imported into chloroplasts. SPP contains an HXXEH zinc-binding motif characteristic of members of the metallopeptidase family M16. We previously found that the three steps of precursor processing by SPP (i.e. transit peptide binding, removal, and conversion to a degradable subfragment) are mediated by features that reside in the C-terminal 10–15 residues of the transit peptide. In this study, we performed a mutational analysis of SPP to identify structural elements that determine its function. SPP loses the ability to proteolytically remove the transit peptide when residues of the HXXEH motif, found in an N-terminal region, are mutated. Deletion of 240 amino acids from its C terminus also abolishes activity. Interestingly, however, SPP can still carry out the initial binding step, recognizing the C-terminal residues of the transit peptide. Hence, transit peptide binding and removal are two separable steps of the overall processing reaction. Transit peptide conversion to a subfragment also depends on the HXXEH motif. The precursor of SPP, containing an unusually long transit peptide itself, is not proteolytically active. Thus, the SPP precursor is synthesized as a latent form of the metallopeptidase.

Chloroplast biogenesis and function depends on the import of a large diversity of proteins from the cytoplasm. These proteins are synthesized as precursors containing an N-terminal targeting signal, called the transit peptide, that mediates multiple steps in the import pathway (for reviews, see Refs. 1–4). Whereas the chloroplast is unique to the plant cell, the mitochrondion and the endoplasmic reticulum (ER)1 are two other major eukaryotic organelles that depend on massive protein translocation mediated by N-terminal targeting signals. It has been predicted for Arabidopsis thaliana that up to 11,000 different precursor proteins may be targeted to these three organelles, ~3,500 to the chloroplast alone (5). Distinct properties of the targeting signals ensure organelle-specific sorting and translocation of the precursor proteins. Ultimately, highly specialized proteolytic enzymes that reside in each organelle remove the targeting signals (for a review, see Ref. 6).

We have identified a general stromal processing peptidase (SPP) that removes transit peptides from an array of precursors involved in different biosynthetic pathways and destined for different chloroplast compartments (7). The activity of SPP, which is a soluble, monomeric enzyme, depends on metal ions (8). Analysis of a full-length SPP cDNA from pea revealed that the enzyme contains an HXXEH zinc-binding motif characteristic of members of the metallopeptidase family M16, such as ptililysin, insulin-degrading enzyme, and the β-subunit of the mitochondrial processing peptidase (MPP) (9, 10). Conservation extends beyond the zinc-binding motif, where 25–30% identity is found in an N-terminal region of ~200 residues of SPP. The residues of the HXXEH motif are predicted to be essential for the catalytic function of these metallopeptidases (9, 11).

To verify the function of SPP in vivo, we demonstrated by the expression of an antisense gene for SPP in both transgenic tobacco and A. thaliana plants that not only is SPP essential in chloroplast biogenesis but also is required for normal plant development (12, 13). Down-regulation of SPP in A. thaliana, containing only one SPP gene, yielded many lines that were seedling-lethal. In surviving plants, import of a recombinant precursor of the green fluorescent protein was defective, indicating a critical role for SPP in the general protein import pathway.

To elucidate the mechanism of transit peptide removal, we used recombinant SPP in an affinity assay (14). We found that SPP initially recognizes a precursor by binding to its transit peptide and then removes the transit peptide in a single endoproteolytic step. The mature protein is immediately released, whereas the transit peptide remains bound to SPP. SPP unexpectedly terminates this interaction by a second cleavage that converts the transit peptide to a subfragment form. Thus, SPP is regenerated for multiple rounds of processing of precursors that enter the stroma. These experiments indicate that SPP contains a high affinity binding site for the transit peptide, and additional proteolysis of the transit peptide produces a subfragment that is no longer recognized by the enzyme. Interestingly, we discovered that a chloroplast extract not only converts the transit peptide to the subfragment form but also rapidly degrades the subfragment by a novel proteolytic activity that is ATP- and metal ion-dependent.

Previously, we investigated the functional organization of the transit peptide to identify determinants for the specific protein-protein interaction required for precursor recognition and cleavage by SPP (15). Competition experiments using synthetic oligopeptides of the transit peptide of the ferredoxin
precursor (preFD) showed that the C-terminal 12-mer of the transit peptide blocked binding, removal, and internal cleavage of the preFD transit peptide. Hence, this region alone is necessary to mediate each of the three steps catalyzed by SPP in a processing reaction that finally leads to the release of a degradable transit peptide subfragment (14).

In this study, we initiated a mutational analysis of SPP to identify structural determinants essential for its enzymatic function. We demonstrate that the HXXEH motif and the structural integrity of the entire SPP polypeptide are requirements for the proteolytic removal of the transit peptide, but they are not needed for the initial binding step. Moreover, these findings suggest that SPP are also determinants for the conversion of the transit peptide to the subfragment. Interestingly, the precursor of SPP (preSPP) exhibits a low affinity for the preFD transit peptide and does not carry out precursor cleavage, indicating that preSPP is a latent form of the enzyme.

**MATERIALS AND METHODS**

**Preparation of Radioactive-labeled Substrates**—The cDNA construct of the precursor of ribulose-1,5-bisphosphate carboxylase/oxygenase activase (preRBCA) from *Spinacia oleracea* (16) was used to synthesize radiolabeled [35S]Met preRBCA in vitro as described previously (15). The [35S]Met preFD transit peptide was prepared according to an earlier protocol (14).

**Synthetic Oligopeptides**—The oligopeptides T-[1–24] (MALTSTLVSASILPKPQPMVAS) and T-[25–48] (SLPTNMQALFLKKGSRGRVTAM), representing the N- and C-terminal halves of preFD transit peptide (17), respectively, were synthesized by the W. M. Keck Biotechnology Resource Center at Yale University (New Haven, CT). The oligopeptide representing the human tumor necrosis factor-a fragment 46–65 (NLVYPSEGLYLIYSQVLF) was from Sigma (18, 19).

**Preparation of SPP, SPP Mutants, and preSPP**—A cDNA fragment encoding SPP from pea (*Pisum sativum*) (GenBank accession no. U25111) (10) was cloned into the expression vector pHEX5BA (20) to express active SPP N-terminally fused to a peptide tag that contains a biotin residue, which is naturally attached in *Escherichia coli* cells (7). The SPP mutants E97Q and H94L were generated using the SPP expression vector construct as DNA template for a PCR-based mutagenesis method published previously (21). Fragments encoding preSPP and the deletion mutants SPP-[1–574], SPP-[564–1115], and SPP-[1–874] were amplified by PCR from the SPP cDNA and inserted into pHEX5BA as described above. The site-directed mutations and fragment deletions were verified by sequencing. All fusion proteins were expressed in *E. coli* for preparation of soluble cell extracts as outlined previously (7).

Extracts were dialyzed against 25 mM HEPES-KOH, pH 7.5, for 20 h at 4 °C. Relative amounts of biotinylated fusion proteins were compared using biotin blotting (see below) and adjusted by either diluting with 25 mM HEPES-KOH, pH 7.5, or concentrating using a centrifugal filter device (Centriplus; Millipore). Extracts were either directly tested for binding and conversion of [35S]Met preFD transit peptide or incubated with streptavidin-coated magnetic beads (Protein A magnetic beads; Sigma) and harvested using a magnetic manipulator (Protein A magnetic manipulator; Sigma). Incubation was for 6 h at 24 °C. Samples were analyzed by standard SDS-PAGE.

**Assays for Binding and Conversion of a Transit Peptide**—The assays for binding and conversion of [35S]Met preFD transit peptide were carried out as described previously (15). Radiolabeled peptide samples were separated by Tricine SDS-PAGE (24), and analyzed using the STORM 860 scanner coupled with ImageQuant software.

**RESULTS**

**Proteolytic Processing Requires Residues of the HXXEH Zinc-binding Motif**—In earlier experiments, we demonstrated that steps of the processing reaction include binding and cleavage of the precursor followed by conversion of the transit peptide to a subfragment form (see Introduction). To identify structural elements in SPP that determine these steps, we initiated a mutation and deletion study of the enzyme. Mature SPP from pea consists of 1115 amino acids with a calculated molecular weight of 123,752. An N-terminal region, extending from amino acid 53 to 288, contains the signature zinc-binding motif HXXEH that is found in all members of the metallopeptidase family M16; thus, we refer to it as the M16 peptidase region (25) (Fig. 1). To investigate the role of the HXXEH motif for the processing reaction, glutamic acid 97 and histidine-94 of SPP were replaced by glutamine (E97Q) and leucine (H94L), respectively (Fig. 1). Examination of the mutants sequences using the Network Protein Sequence Analysis program package predicts that each substitution will not alter local secondary structure (see web site: npsa-pbil.ibcp.fr) (26). The processing activity of the mutants was tested using equal amounts of wild-type SPP, E97Q, and H94L in a reaction with the radiolabeled substrate [35S]Met preRBCA. In earlier experiments using similar conditions, we have shown that SPP needs less than 20 min to complete processing of preRBCA (14). Interestingly, neither mutant generated detectable amounts of mature RBCA even after 6 h of incubation, which was in sharp contrast to wild-type SPP (Fig. 2B). Another precursor, preFD, was not processed by the SPP mutants either (data not shown). Thus, our findings verify that the residues of the HXXEH motif are essential for SPP activity, suggesting a critical role for the M16 peptidase region in precursor processing.

![Fig. 1. Schematic representation of the SPP mutants and preSPP.](image-url)
Mutation of the HXXEH Motif Does Not Block Binding to the Transit Peptide—Binding of the transit peptide to SPP initiates the processing reaction. To test whether the inactive mutants still interact with the substrate, preparations of wild-type SPP, E97Q, and H94L were immobilized onto magnetic bead matrix and incubated with [35S]Met preFD transit peptide (see “Materials and Methods”). The assay was stopped after 2 min, before the conversion to the subfragment by wild-type SPP would occur (see Introduction). Surprisingly, examination of the matrix fractions demonstrated that both mutants still bind the intact transit peptide (Fig. 3A, lanes 1–3). Quantification of an image of the binding experiment generated with a Phosphor-Imager (see Materials and Methods) indicated that wild-type SPP and E97Q bound similar amounts of transit peptide, and binding to H94L was reduced by only 10%. To test the specificity of the interaction, binding assays were carried out in presence of the synthesized oligopeptides T-(1–24) and T-(25–48), representing the N- and C-terminal halves of the preFD transit peptide, respectively (15). Analysis of the bound fractions revealed that the two oligopeptides have a different effect on binding by the SPP mutants (Fig. 3B). The presence of the N-terminal half T-(1–24) did not change the amount of bound transit peptide, whereas, in striking contrast, a concentration of 10 μM of the C-terminal half T-(25–48) reduced binding to E97Q and H94L by 68 and 61%, respectively. In a control experiment, an unrelated oligopeptide, tumor necrosis factor-α (TNF) (46–65), Lane 1, binding without oligopeptide; lanes 2–4, binding in presence of 0.1, 1, and 10 μM oligopeptide, respectively.

Conversion of the Transit Peptide to Its Subfragment Depends on the HXXEH Motif—The above results raised the question of whether an HXXEH-mutant could still convert the transit peptide to its subfragment form. Because the conversion reaction occurs after the removal of the transit peptide from the precursor, we investigated this question using a radiolabeled transit peptide as a substrate. A time course analysis with immobilized E97Q and the [35S]Met preFD transit peptide showed that the SPP mutant did not carry out the reaction (Fig. 4). Therefore, both functions of SPP, precursor cleavage and transit peptide conversion, depend on the HXXEH motif.

Separation of the N- and C-terminal Halves of SPP Abolishes Precursor Processing Whereas the N-terminal Half Alone Retains an Affinity for the Transit Peptide—Alignment of the SPP sequences from pea and A. thaliana revealed an identical stretch of mostly acidic residues and prolines, EPIPEPPELE, between the highly conserved N- and C-terminal halves of the proteins (7) (Fig. 1). The acidic, proline-rich stretch is predicted to be a flexible spacer often found to link functionally and structurally defined domains in both eukaryotic and prokaryotic proteins (27, 28). To investigate whether this region marks the transition between two functional domains within SPP, the N- and the C-terminal halves of SPP were expressed separately, yielding the constructs SPP-(1–574) and SPP-(564–1115), respectively (Fig. 1). Furthermore, a data base search of the genome of the cyanobacterium Anabaena sp. PCC 7120 identified the open reading frame ALL1021 (29) with significant similarities to SPP. ALL1021 is characterized by an N-terminal M16 peptidase region containing the HXXEH motif. Interestingly, in contrast to the other members of the M16 family with a sequence similarity restricted to the N-terminal region, SPP and ALL1021 also share 22% identity within 240 residues near their C termini. To examine whether this C-terminal region of SPP has a critical function in processing, a third construct, SPP-(1–874) (Fig. 1), was made by deleting the 240 residues from SPP.

Using the in vitro processing assay, we found that none of the deletion mutant constructs cleaved [35S]Met preRBCA (Fig. 5, A and B, lanes 2–4). We then tested whether the deletion of the C terminus in SPP-(1–574) and SPP-(1–874) could be complemented by the construct SPP-(564–1115), representing the C-terminal half of SPP. However, combining the constructs did not reconstitute processing activity (Fig. 5B, lane 5; not shown for SPP-(1–874) in combination with SPP-(564–1115)). Moreover, a test for conversion of the preFD transit peptide by these deletion constructs was negative as well (data not shown). We conclude that the intact SPP polypeptide is required to adopt an active conformation, which includes the C-terminal region with sequence similarity to the putative M16-type peptidase from the cyanobacterium Anabaena sp.

We next examined the affinity of the three deletion mutant...
Constructs for the \[^{35}S\]Met preFD transit peptide in a binding assay (Fig. 5C). The two constructs containing the M16 peptidase region, SPP-(1–574), and SPP-(1–874), bound 35 and 32% of \[^{35}S\]Met preFD transit peptide, respectively, compared with intact SPP. But binding by the C-terminal half, SPP-(564–1115), was reduced to ~5%. Thus, the N-terminal half of SPP represented by SPP-(1–574) contains structural elements necessary for interaction with the transit peptide. However, the lower amount of transit peptide bound to SPP-(1–574) or SPP-(1–874) indicates that the N-terminal half, either alone or extended downstream by 300 amino acids, is not sufficient to constitute the fully functional substrate binding site present in intact SPP.

PreSPP Is a Latent Form of the Metallopeptidase—The full-length SPP cDNA from pea encodes the precursor protein preSPP with an N-terminal region of 142 amino acids containing features characteristic of chloroplast transit peptides; i.e. it is enriched for the polar amino acids serine and threonine, the overall charge is basic, and 75% of the peptide is predicted to be devoid of regular secondary structure. We found previously that radiolabeled preSPP is imported into chloroplasts \textit{in vitro} and processed by recombinant SPP in \textit{trans} (7). When cleavage of preSPP was carried out in the presence of oligopeptides corresponding to regions of the preFD transit peptide, our results indicated that the determinants for preSPP transit peptide removal reside within the C-terminal 10–15 residues preceding the cleavage site, a feature shared with other transit peptides (15). However, the preSPP transit peptide is unusually long, and contains some additional features that might play a special role (see "Discussion"). Here we used a preSPP expression construct to test whether the transit peptide affects SPP activity (Fig. 1). Similar amounts of SPP and preSPP (Fig. 6A) were assayed for processing activity by incubation with \[^{35}S\]Met preRBCA as described above. The analysis shows that preSPP cannot produce mature RBCA (Fig. 6B). Furthermore, a binding assay using the \[^{35}S\]Met preFD transit peptide revealed that binding to preSPP is reduced to 14% compared with mature SPP (Fig. 6C). Hence, the transit peptide region of preSPP strongly reduces the ability of the enzyme to interact with the substrate and it completely blocks the cleavage reaction.

**Discussion**

In this study, we have analyzed SPP to identify determinants required for the post-translational removal of transit peptides from chloroplast precursors. Our previous work has shown that SPP interacts with a stretch of 10–15 amino acids at the C terminus of the transit peptide to cleave at a specific site and release the mature protein and produce a degradable transit peptide subfragment (see Introduction). In this study, mutant and deletion constructs have allowed us to gain insights into the role that certain features of SPP play during processing. Four important points emerge from our study. First, SPP depends on the HXXEH zinc-binding motif for catalytic activity, as we predicted based on the conservation of this motif in members of the M16 metallopeptidase family (see below). Moreover, adopting a proteolytic active conformation apparently requires the structural integrity of the SPP polypeptide. Second, binding to the transit peptide does not depend on catalytic activity, demonstrating that there are two separable steps in the overall processing reaction. Interestingly, an N-terminal domain of SPP contains structural determinants for binding to the transit peptide. Third, conversion of the transit peptide to the subfragment depends on the same catalytic site needed for precursor processing. Finally, the transit peptide region of preSPP blocks the processing activity. Hence, preSPP synthesized in the cytoplasm is prevented from prematurely processing other precursors that are post-translationally targeted to the chloroplast.

Interestingly, our experiments with the SPP mutants E97Q and H94L now demonstrate that the residues of the HXXEH zinc-binding motif play an essential role during processing of chloroplast precursors. In earlier works, mutant analyses of this motif were done with other M16 peptidases, each hydrolyzing a distinct range of substrates \textit{e.g.} pitrilysin, insulin-degrading enzyme, and MPP \(\beta\)-subunit (30–34). In all cases, nonconservative mutations of the defined residues of the motif abolished proteolytic activity, similar to our findings with the SPP mutants. Hence, the HXXEH motif is crucial for the activity of M16 peptidases, indicating a similar catalytic mechanism despite the diversity of the substrates cleaved by this family of peptidases. In fact, a crystal structure analysis of MPP localized the residues of the motif as part of an \(\alpha\)-helix at the catalytic center, with the two histidines separated by one helical turn and complexed to a metal ion (presumably \(\text{Zn}^{2+}\)). The internal glutamic acid was near a water molecule that was also bound to the metal ion (35). A model for catalysis based on the metallopeptidase thermolysin proposes that the internal...
Properties of the Chloroplast Stromal Processing Peptidase

39501

glutamic acid polarizes the zinc-bound water molecule for a nucleophile attack on the carbonyl carbon of the scissile peptide bond in the substrate (11).

Our earlier work has shown that SPP is a monomeric polypeptide (7, 8). Because of SPP’s relatively large size of 124 kDa, we examined the possibility that a “proteolytic” domain separable from other parts of the enzyme mediates its processing activity. However, we found that not even a construct of SPP’s M16 peptidase region extended downstream by nearly 600 residues (i.e. SPP-1–874) was sufficient to carry out precursor processing. The functional counterpart to SPP in mitochondria, MPP, is a heterodimeric complex of ~100 kDa cooperatively formed by α- and β-subunits of similar size (35). Sequence similarity to SPP, however, is restricted to the M16 peptidase region present in β-subunit (see above). We asked if the SPP polypeptide analogous to MPP is organized into two functional “subunit regions” that cooperate to produce an active enzyme. However, combining constructs representing the N- and C-terminal halves of SPP did not reconstitute processing activity. Therefore, we conclude that only the structurally intact SPP polypeptide is capable of adopting a proteolytic active conformation. The requirements for the binding to the transit peptide and its proteolytic removal depend on different features of SPP. The inactive mutants E97Q and H94L can still interact with the transit peptide, and specific determinants required for this interaction are present in the N-terminal half. However, the lower affinity for the transit peptide displayed by the N-terminal half alone, only 35% of that found for mature SPP, indicates that multiple subsites of the intact metallopeptidase participate for optimal substrate binding.

We have previously shown that SPP possesses a unique property. It catalyzes a second cleavage reaction, converting the transit peptide to a subfragment form (see Introduction) (14). Our analysis indicates that both conversion and precursor processing depend on the HXXEH motif. A reaction comparable with the transit peptide conversion has not been observed in mitochondria. The isolated targeting signal of a mitochondrial precursor remained stable in the presence of MPP (36). When a targeting signal was produced during processing in the mitochondrial matrix, however, its rapid hydrolysis by a novel peptidase occurred without detectable intermediates (37).

It can cleave such a diverse group of precursors possessing transit peptides that show little sequence, or apparent structural similarity. Our earlier analyses showed that SPP recognizes the C-terminal region of the transit peptide, which has a positive net charge but lacks defined secondary structures. That transit peptide binding can be separated from the proteolysis per se by the mutation of the HXXEH motif should facilitate the design of future experiments to establish the protein-protein interactions that occur and how SPP achieves its specificity.

Acknowledgment—We thank Ana Chee for preparing the preSPP expression construct.

REFERENCES

1. Keegstra, K., and Cline, K. (1999) Plant Cell 11, 557–570
2. Vothknecht, U. C., and Soll, J. (2000) Biol. Chem. 381, 887–897
3. Chen, K., Chen, X., and Schnell, D. J. (2000) Biochem. Soc. Trans. 28, 485–491
4. Bruce, R. D. (2001) Biochem. Biophys. Acta 1541, 2–21
5. Arabidopsis Genome Initiative (2000) Nature 408, 791–794
6. Pasteel, M., Karla, A., Strynadka, N. C., and Dalbey, R. E. (2002) Chem. Rev. 102, 4549–4580
7. Richter, S., and Lampka, G. K. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 7463–7468
8. Oblong, J. E., and Lampka, G. K. (1992) EMBO J. 11, 4401–4409
9. Rawlings, N. D., and Barrett, A. J. (1995) Methods Enzymol. 248, 183–228
10. VanderVeer, P. S., Bennett, T. M., Oblong, J. E., and Lampka, G. K. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7177–7181
11. Makarova, K. S., and Grishin, N. V. (1999) Protein Sci. 8, 2537–2540
12. Wan, J., Brand, R., and Lampka, G. K. (1998) Plant J. 15, 459–468
13. Zhang, H., Wan, J., Jin, R., and Lampka, G. K. (2000) Plant J. 34, 802–812
14. Richter, S., and Lampka, G. K. (1999) J. Cell Biol. 147, 33–44
15. Richter, S., and Lampka, G. K. (2002) J. Biol. Chem. 277, 43888–43894
16. Wernesk, J. M., Ziefinski, R. E., and Oregen, W. L. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 787–791
17. Smeekens, S., van Birgelen, J., and Weisbeek, P. (1985) Nucleic Acids Res. 13, 3179–3194
18. Petracca, D., Niedwia, G. E., Hayflick, J. S., Seeburg, P. H., Derynck, R., Palladino, M. A., Kehr, W. J., Aggarwal, B. B., and Goeddel, D. V. (1984) Nature 312, 724–729
19. Wang, A. M., Creasey, A. A., Ladner, M. B., Lin, S., Strickler, J., Van Arsdale, J. N., Yamamoto, R., and Mark, D. F. (1985) Science 229, 149–154
20. Richter, S., Hess, W. R., Krause, M., and Messer, W. (1998) Mol. Gen. Genet. 257, 534–541
21. Langner, U., Richter, S., Roth, A., Weigel, C., and Messer, W. (1996) Mol. Microbiol. 21, 301–311
22. Laemmli, U. K. (1970) Nature 227, 680–685
23. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
24. Schagger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379
25. Rawlings, N. D., O’Brien, E., and Barrett, A. J. (2000) Nucleic Acids Res. 30, 334–346
26. Combet, C., Blanchet, C., Geurjon, C., and Deleage, G. (2000) Trends Biochem. Sci. 25, 147–150
27. Chou, Y., and Klug, A. (1993) Nucleic Acids Res. 21, 3341–3346
28. de Chateau, M., and Bjoerk, L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8490–8495
29. Kanieko, T., Nakamura, Y., Wolk, P. C., Kuritz, T., Sasamoto, S., Watanabe, A., Iriuchi, M., Ishikawa, A., Kawashima, K., Kimura, T., Kishida, Y., Kohara, M., Matsutomo, M., Matsuno, A., Muraki, A., Nakazaki, N., Shimo, S., Sugimoto, M., Tkazawa, M., Yamada, Y., Yasuda, M., and Tabata, S. (2001) DNA Res. 8, 205–213
30. Becker, A. B., and Roth, R. A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3835–3839
31. Gehm, B. D., Kuo, W. L., Perlman, R. K., and Rosner, M. R. (1999) J. Biol. Chem. 268, 7943–7948
32. Kuo, B. D., Gehm, B. D., Kuo, W. L., and Rosner, M. R. (1999) J. Biol. Chem. 268, 21538–21544
33. Kitada, S., Shimokawa, K., Niidome, T., Ogishima, T., and Ito, A. (1995) J. Biochem. 117, 1148–1150
34. Striebl, H.-M., Rysavy, P., Adamec, J., Spizek, J., and Kalousek, F. (1996) Arch. Biochem. Biophys. 335, 211–218
35. Taylor, A. B., Smith, B. S., Kitada, S., Kojima, K., Miyaura, H., Owitowski, Z.,
Properties of the Chloroplast Stromal Processing Peptidase

36. Arretz, M., Schneider, H., Guiard, B., Brunner, M., and Neupert, W. (1994) J. Biol. Chem. 269, 4959–4967
37. Stahl, A., Meberg, P., Ytterberg, J., Panfilov, O., Brockenhaus von Lowenhielm, H., Nilsson, F., and Glaser, E. (2002) J. Biol. Chem. 277, 41931–41939
38. Novak, P., and Dev, I. K. (1988) J. Bacteriol. 170, 5067–5075
39. Weihofen, A., Binns, K., Lemberg, M. K., Ashman, K., and Martoglio, B. (2002) Science 296, 2215–2218
40. Springman, E. E., Angleton, E. L., Birgedal-Hansen, H., and Van Wart, H. E. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 5578–5582
41. Van Wart, H. E., and Birgedal-Hansen, H. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 5578–5582
42. Milla, M. E., Leesnitzer, M. A., Moss, M. L., Clay, W. C., Carter, H. L., Miller, A. B., Su, J. L., Lambert, M. H., Willard, D. H., Sheeley, D. M., Kost, T. A., Burkhart, W., Moyer, M., Blackburn, R. K., Pahel, G. L., Mitchell, J. L., Hoffman, C. R., and Becherer, J. D. (1999) J. Biol. Chem. 274, 30563–30570
43. Hawlitschek, G., Schneider, H., Schmidt, B., Tropschug, M., Hartl, F. U., and Neupert, W. (1988) Cell 53, 795–806
44. Witte, C., Jensen, R. E., Yaffe, M. P., and Schatz, G. (1988) EMBO J. 7, 1439–1447
45. Paces, V., Rosenberg, L. E., Fenton, W. A., and Kalousek, F. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 5355–5358
46. Geli, V. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 6247–6251
47. Kojima, K., Kitada, S., Shimokata, K., Ogishima, T., and Ito, A. (1988) J. Biol. Chem. 273, 32542–32546
48. Saavedra-Alanis, V. M., Rysavy, P., Rosenberg, L. E., and Kalousek, F. (1994) J. Biol. Chem. 269, 9284–9288