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A chloroplast homologue of the signal recognition particle subunit SRP54 is involved in the posttranslational integration of a protein into thylakoid membranes

(translocation/signal peptide/protein transport/chaperone/endosymbiont)

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ABSTRACT **The mechanisms involved in the integration of proteins into the thylakoid membrane are largely unknown. However, many of the steps of this process for the light-harvesting chlorophyll a/b protein (LHCP) have been described and reconstituted *in vitro*. LHCP is synthesized as a precursor in the cytosol and posttranslationally imported into chloroplasts. Upon translocation across the envelope membranes, the N-terminal transit peptide is cleaved, and the apoprotein is assembled into a soluble "transit complex" and then integrated into the thylakoid membrane via three transmembrane helices. Here we show that 54CP, a chloroplast homologue of the 54-kDa subunit of the mammalian signal recognition particle (SRP54), is essential for transit complex formation, is present in the complex, and is required for LHCP integration into the thylakoid membrane. Our data indicate that 54CP functions posttranslationally as a molecular chaperone and potentially pilots LHCP to the thylakoids. These results demonstrate that one of several pathways for protein routing to the thylakoids is homologous to the SRP pathway and point to a common evolutionary origin for the protein transport systems of the endoplasmic reticulum and the thylakoid membrane.**

The chloroplast is highly compartmentalized and therefore must possess sophisticated sorting mechanisms that correctly route proteins to six different compartments. Most studies have focused on the targeting of thylakoid proteins—i.e., proteins that reside in the thylakoid lumen as well as a family of light-harvesting chlorophyll a/b binding proteins (LHCPs) that are integrated into the thylakoid membrane. These proteins are synthesized in the cytosol as precursors with cleavable N termini known as transit peptides (for review, see ref. 1). Transit peptides target preproteins to the chloroplast and are processed in the stroma (2). Transit peptides of lumenal proteins are bipartite (3); the N-terminal portion governs transport across the envelope, whereas the C-terminal portion directs translocation across the thylakoid membrane (4). In the case of LHCP, the mature apoprotein contains sufficient information for thylakoid targeting (5), although the specific signal has not yet been identified (6, 7).

Studies of the early events of LHCP trafficking using isolated chloroplasts showed that LHCP is present as a soluble form in the stroma prior to integration into thylakoids (8). When integration was inhibited, this intermediate LHCP accumulated in the stroma as a larger complex (9). Formation of this complex, which we have designated the "transit complex," maintains LHCP solubility and integration competence (9). Both transit complex formation and LHCP integration into thylakoids have been reconstituted *in vitro*; both require a proteinaceous component(s) of the stroma (9, 10).

The chaperone nature of this "stromal factor" led to the suggestions that heat shock proteins Cpn60 (11) and Hsp70 (12) were involved in LHCP integration. These molecular chaperones are abundant in the stroma (13, 14), are able to bind polypeptides and release them upon ATP hydrolysis, and have been shown to be involved in mitochondrial protein import (15). However, immunoprecipitation experiments with antibodies against the two proteins failed to reveal the presence of Cpn60 and Hsp70 in the transit complex (9, 14). Furthermore, stromal extract depleted of Hsp70 still supported transit complex formation and LHCP integration, indicating that neither process requires this chaperone (14).

The recent demonstration of a stromal GTP requirement (16) for LHCP integration now suggests the involvement of a guanine nucleotide-binding protein in this process. One likely candidate is 54CP, a chloroplast homologue of the 54-kDa protein of the mammalian signal recognition particle (SRP) (17). Protein transport across or integration into the endoplasmic reticulum (ER) is mediated by a GTP-dependent mechanism involving SRP (18). The 54-kDa polypeptide subunit of SRP (SRP54) plays a major role in SRP-dependent targeting. It binds guanine nucleotides as well as nascent polypeptides that are destined for ER transport or integration (19). Furthermore, SRP54 appears to be required for docking to the SRP receptor (20) and hydrolyzes the GTP required for dissociation of SRP from the receptor (21). In this report, we show that 54CP is bound to LHCP in the transit complex and is essential for LHCP integration into the thylakoid membrane.

MATERIALS AND METHODS

Materials. [^{35}S]Methionine and [^{3}H]leucine were from Amersham and DuPont/NEN, respectively. Reagents for transcription were from Promega. Protein A-Sepharose was from Sigma. Bis(sulfosuccinimidyl)suberate was from Pierce. All other chemicals were reagent grade. Chloroplasts, thylakoids, and stromal extracts were prepared from pea seedlings. Radiolabeled LHCP and 54CP precursors (p) were prepared by *in vitro* transcription and translation as described (7). Unlabeled pLHCP and pOE33 (33-kDa subunit of the oxygen-evolving complex) were expressed in *Escherichia coli* and purified from inclusion bodies as described (22).

Assays. Formation of transit complex was conducted essentially as described (9) by incubating radiolabeled pLHCP with

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Abbreviations: LHCP, light-harvesting chlorophyll a/b protein; p, precursor; SRP, signal recognition particle; ER, endoplasmic reticulum.

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stromal extract for 15 min at 25°C followed by analysis by nondenaturing PAGE. ATP or GTP at 1.5 mM was generally included in assays. Although not essential for complex formation, we have found these NTPs, as well as their nonhydrolyzable analogues, to be stimulatory. LHCP integration into thylakoids was conducted as described (10). Protein samples from integration assays were examined on SDS/12.5% polyacrylamide gels; cross-linking and immunoprecipitation samples were resolved on 15% gels. The radioactive proteins were visualized by fluorography or on a PhosphorImager (Molecular Dynamics).

Depletion of Hsp70 and 54CP from Stroma Extracts. Immunodepletion of stromal proteins was essentially as described by Yuan *et al.* (14) with minor modifications. Briefly, protein A-Sepharose 4B was hydrated in 10 mM Hepes·KOH, pH 8.0 (HK buffer), washed three times, and suspended in HK buffer. After addition of 0.4 ml of anti-Hsp70 or anti-54CP antibody, the beads were incubated at 4°C overnight followed by washing four times with HK buffer and then with HK containing 5 mM $MgCl_2$ (HKM). Stromal extract (0.6 ml at ≈1.5 mg of protein per ml) was adjusted to 1.0 mM ATP, applied to a column of the antibody-absorbed beads (0.42-ml packed volume), incubated with the beads for 15 min, and then allowed to pass through. The flow-through was reapplied to the column two more times, recovered from the column by centrifugation, and supplemented with dithiothreitol to 1 mM.

Immunoprecipitation of Transit Complex. Transit complex, formed by incubating pLHCP translation products with stromal extract (2.5 mg of protein per ml), was subjected to immunoprecipitation with protein A-Sepharose adsorbed with antibody (14). After an incubation for 2 hr at 4°C, the solution was clarified by centrifugation and the supernatant was analyzed for the presence of transit complex by nondenaturing PAGE (9).

Cross-Linking Analyses of Transit Complex. For cross-linking, stroma was desalted by centrifugation through a Sephadex G-25 column, equilibrated in HK, at 500 × *g* prior to formation of transit complex. Freshly prepared 12 mM bis(sulfosuccinimidyl)suberate was added to the reaction mixture to a final concentration of 0.2 mM. After incubation on ice for 2 hr, the reaction mixture was quenched by the addition of 1 M Tris·HCl (pH 7.5) to 50 mM. A portion of each reaction mixture was subjected directly to SDS/PAGE analyses. The remainder was divided into aliquots and incubated at 4°C for 2 hr with various antisera (150 μg of IgG) adsorbed onto protein A-Sepharose (6 mg) in a solution containing 50 mM Tris·HCl (pH 7.5), 150 mM NaCl, and 1% Tween 20. The matrix was washed three times in the same buffer and eluted with 40 μl of 0.3 M acetic acid. Twenty microliters of the eluate was removed, neutralized with 5 μl of 1 M Tris base, and subjected to SDS/PAGE analyses.

Transit Complex Formation Between Imported 54CP and LHCP. p54CP was translated *in vitro* and imported into chloroplasts (10) in the presence of 5 mM MgATP. Chloroplasts were repurified through 40% Percoll, washed in import buffer, and lysed at 2 mg of chlorophyll per ml in HKM. Stromal extract containing imported 54CP was obtained by centrifugation at 35,000 × *g* for 30 min to remove membranes. *E. coli*-produced pLHCP and pOE33 were solubilized in 10 M urea with 10 mM dithiothreitol for 4 hr and diluted 1:10 with HKM just prior to use. Assays were performed by mixing 20 μl of stromal extract, 5 μl of 10 mM GTP, and 5 μl of solubilized LHCP or OE33 followed by incubation at 25°C for 15 min. Complex formed was analyzed by native gel electrophoresis.

RESULTS

54CP Is Present in LHCP Transit Complex. Transit complex, formed by incubating radiolabeled pLHCP with a stromal extract, appears as a band on nondenaturing PAGE with an R_m (relative mobility) value of ≈0.4 (Fig. 1*A*). The presence of 54CP in the transit complex was demonstrated by immunoprecipitation with protein A-Sepharose adsorbed with monospecific antibodies. Transit complex was removed from solution with beads adsorbed with either anti-54CP or anti-LHCP

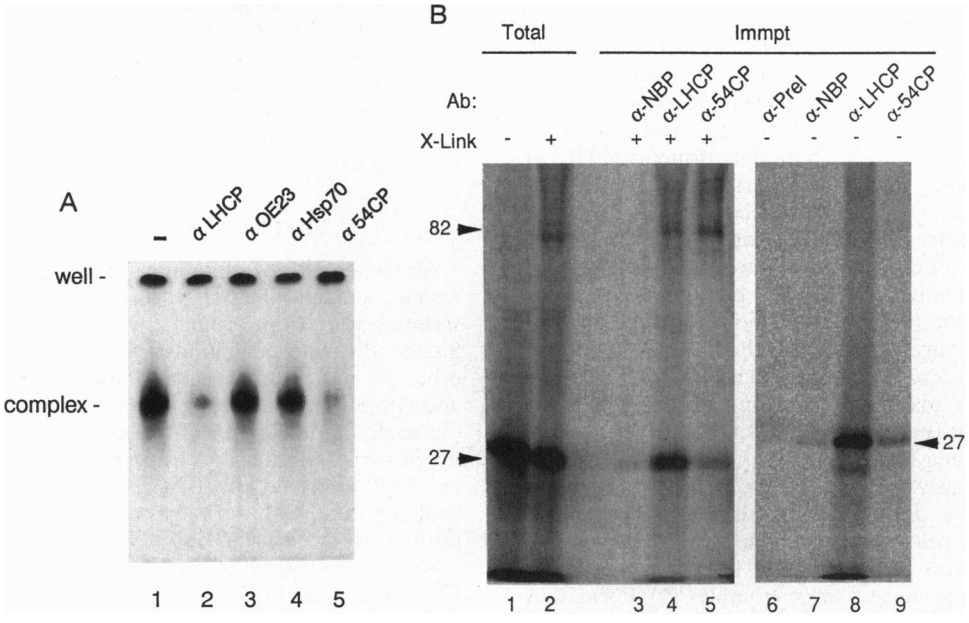

FIG. 1. Stromal 54CP associates with LHCP. (*A*) After the formation of transit complex, the reaction mixtures were subjected to immunoprecipitation without (lane−) or with antibody (α)-adsorbed beads as indicated. The samples were then analyzed by nondenaturing PAGE. The locations of transit complex and sample wells are marked. Uncomplexed LHCP aggregated and remained in the sample well (see Fig. 3*C*, no SE lane). (*B*) Transit complex was subjected to chemical cross-linking experiments by incubation in the absence (lanes−) or presence (lanes+) of cross-linker (X-Link) followed by SDS/PAGE directly (Total) or after immunoprecipitation (Immpt) with antibody-adsorbed beads as indicated. α-NBP, antiserum against a nonchloroplast protein; PreI, preimmune serum. Locations of cross-linking product (82 kDa) and free LHCP (27 kDa) are designated with arrowheads. Immunoprecipitated samples represent 2.5 times the amount loaded in the total sample gel lanes.

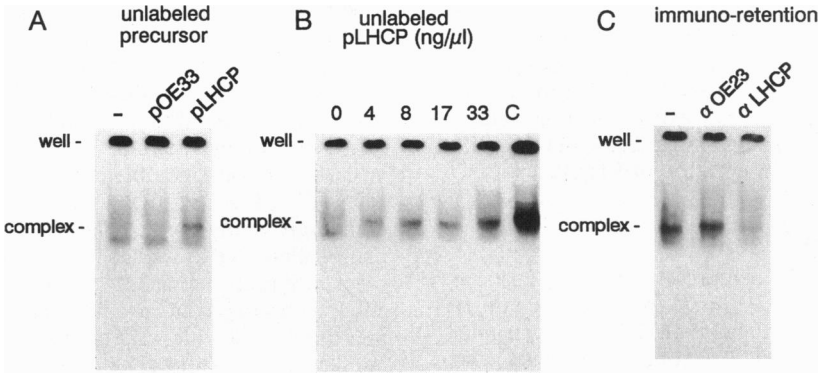

FIG. 2. Binding of imported 54CP and pLHCP. Stromal extract containing radiolabeled 54CP imported into isolated chloroplasts was incubated without (lane −) or with unlabeled pOE33 or pLHCP (33 ng/μl) (*A*) or with increasing amounts of unlabeled pLHCP as indicated (*B*) followed by nondenaturing PAGE. Transit complex formed using radiolabeled pLHCP is shown for comparison (*B*, lane c). (*C*) Reactions were conducted as in *A* using 33 ng of pLHCP per ml. Reaction mixtures were then treated without (lane −) or with 5 μl of antisera as indicated for 1 hr on ice prior to nondenaturing PAGE.

antibody (Fig. 1*A*). Beads adsorbed with other antibodies—i.e., anti-OE23 or anti-Hsp70 antibody, which served as controls for this experiment—did not diminish the amount of complex remaining in solution (Fig. 1*A*).

The association of 54CP with LHCP was further demonstrated by chemical cross-linking. After incubation of stromal extract with radiolabeled LHCP, an amine-reactive cross-linker, bis(sulfosuccinimidyl)suberate, was added to the reaction mixture. A single major labeled product, dependent on the presence of the cross-linker, was detected by SDS/PAGE (Fig. 1*B*, lane 1 vs. lane 2). The cross-linked product was precipitated by monospecific antiserum against either LHCP or 54CP (Fig. 1*B*, lanes 4 and 5). The estimated mass of the cross-linked product (82 kDa) (Fig. 1*B*) was consistent with a 1:1 cross-linking between 54CP (54 kDa) and LHCP (27 kDa). Anti-54CP antibody precipitated more free LHCP than the irrelevant antiserum or preimmune serum. This is consistent with the results shown in Fig. 1*A* that a stable interaction between LHCP and 54CP is not dependent on the cross-linker and that 30–35% of the LHCP added to stromal extract became incorporated into transit complex (9), whereas only 5–10% was cross-linked. Antiserum against a nonchloroplast protein did not precipitate the cross-linked product (Fig. 1*B*, lane 3). Together, these data demonstrate that 54CP directly binds LHCP in the transit complex.

Attempts to form transit complex by combining the purified proteins or translation products or by translating the two proteins simultaneously were unsuccessful. Furthermore, we were unable to form transit complex by incubating *in vitro* translated 54CP with stroma prior to incubation with purified LHCP. However, p54CP imported into intact chloroplasts was capable of forming transit complex. Fig. 2 illustrates the results from experiments in which stromal extract containing imported, radiolabeled 54CP was incubated with unlabeled pLHCP or pOE33 expressed in *E. coli*. pOE33, a precursor to a lumenal protein, was used as a negative control because it is targeted by a pathway distinct from that of LHCP (22, 23). For reasons not understood, most of the 54CP did not enter the nondenaturing gel. The addition of pLHCP but not pOE33 resulted in formation of a radiolabeled 54CP band (Fig. 2*A*) with the same mobility on nondenaturing PAGE as transit complex formed with labeled LHCP and unlabeled stroma (Fig. 2*B*; compare lane C to the other lanes). The 54CP in this band increased with increasing amounts of LHCP (Fig. 2*B*). When the samples containing the complex were incubated with antisera prior to running the nondenaturing gel, antibodies against LHCP but not OE23 were effective in preventing the complex from entering the gel (Fig. 2*C*), demonstrating that LHCP was present in the complex.

54CP Is Essential for LHCP Integration into Thylakoid Membranes. It has been our hypothesis that formation of the transit complex is a first and essential step of the integration process. Previous studies (9) suggested that the transit complex is an intermediate during the integration of LHCP. Moreover, LHCP in the transit complex is competent for integration, indicating that complex formation is a productive process (9). To address this hypothesis, we removed 54CP from stromal extract by immunodepletion. As a control, we removed Hsp70 from a stromal extract in a parallel immunodepletion reaction (14). These treatments were effective in removing the corresponding proteins, as demonstrated by immunoblotting (Fig. 3*A*), without otherwise altering the protein composition of the extract, as judged by Coomassie staining (Fig. 3*B*). As expected, depletion of 54CP from the stromal extract abolished its ability to form transit complex, whereas removal of Hsp70 had little effect (Fig. 3*C*). Moreover, removal of 54CP abol-

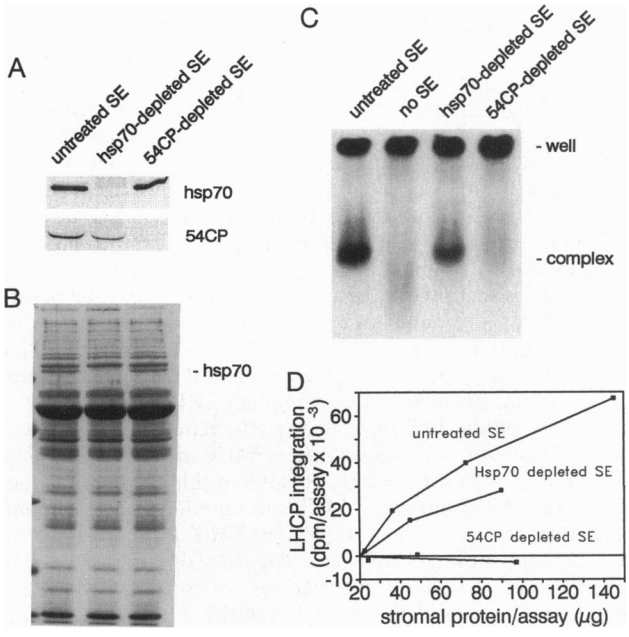

FIG. 3. Requirement of 54CP for transit complex formation and LHCP integration. The stroma was depleted of Hsp70 or 54CP, analyzed by immunoblot using Hsp70- or 54CP-specific antibodies (*A*), subjected to SDS/PAGE followed by Coomassie blue staining (*B*), used in assays to measure soluble complex formation (*C*), or used to assay LHCP integration into thylakoid membranes (*D*). SE, stromal extract.

ished the ability of the extract to support integration of LHCP into isolated thylakoids (Fig. 3*D*). The depletion of Hsp70 from stroma had only a slight effect on LHCP integration, which presumably reflects a small loss of integration activity that frequently occurs when stromal extract is subjected to chromatographic procedures. These data indicate the essential role of 54CP in the thylakoid integration of LHCP.

DISCUSSION

Several lines of evidence have been presented to demonstrate that 54CP interacts with LHCP. First, the transit complex generated by incubating radiolabeled LHCP with stromal extract can be immunoprecipitated by monospecific antiserum for 54CP (Fig. 1*A*). Second, LHCP was specifically crosslinked to 54CP, resulting in a product that was efficiently immunoprecipitated by 54CP-specific antiserum (Fig. 1*B*). Third, radiolabeled 54CP, when imported into chloroplasts, was able to form a complex with purified LHCP. This complex exhibited the same electrophoretic mobility as transit complex and was bound by antibodies against LHCP (Fig. 2). That both LHCP and 54CP can be incorporated into the same complex strongly argues for a specific interaction between the two proteins.

It is significant that 54CP did not stably interact with pOE33 (Fig. 2; X.L. and N.E.H., unpublished results) or pOE23 (R.H. and K.C., unpublished results). Recent studies have shown that thylakoid proteins are routed by at least three distinct pathways (22, 24–27). OE33 requires ATP and a stromal factor that is homologous to bacterial protein SecA (23, 28). OE23 uses a route that is independent of stroma and ATP but absolutely dependent on a pH gradient across the thylakoid membrane (24, 29). The third pathway—i.e., for LHCP integration—requires GTP (16), and as we have now shown requires 54CP. Whether transport or integration of other proteins is dependent on 54CP remains to be determined. However, genetic studies suggest the possibility that two other integral membrane proteins of the thylakoids, cytochrome *f* and D1, share at least one component of the pathway utilized by LHCP (30). These proteins, unlike LHCP, are encoded and synthesized within the chloroplast. Thus, it is possible that the 54CP pathway accommodates integral thylakoid membrane proteins that are synthesized in the plastids as well as in the cytosol.

It appears that 54CP does not function as a monomer. Complex-forming and integration activities fractionate as an ≈200-kDa form on gel filtration columns in the absence of LHCP (R.H. and K.C., unpublished results). One pool of 54CP is associated with this activity; the other pool of 54CP is associated with ribosomes (17). In addition, the fact that 54CP had to be imported into intact chloroplasts to be functional indicates that 54CP may require the chloroplast machinery for folding and assembly into a multisubunit complex. The mammalian SRP is a ribonucleoprotein that contains five polypeptides in addition to the 54-kDa polypeptide and an RNA scaffold. It is possible that 54CP is associated with components that are homologous to the mammalian SRP.

We have established that 54CP participates in LHCP integration. However, the precise role of 54CP in LHCP targeting still remains to be established. Results in this study as well as previous work (9) show that 54CP can function as a molecular chaperone. It is also conceivable that 54CP targets LHCP to the thylakoids in much the same way that SRP pilots nascent peptides to the ER. Two observations are consistent with this idea. First, previous work shows that purified LHCP, unfolded and then diluted out of denaturant, retains its competence for integration in the absence of any additional components for at least 30 min, the duration of the integration assay (14). At least in this *in vitro* assay, LHCP remains soluble in the absence of 54CP. As additional stroma is still absolutely required for LHCP integration, there is reason to believe that 54CP participates in the insertion step. Consistent with this idea is the observation that GTP hydrolysis is required for LHCP integration (16) but not for transit complex formation (9) and that 54CP is a GTPase (X.L. and N.E.H., unpublished results). Based on the SRP paradigm, the hydrolysis step is expected to occur at the membrane, implying that 54CP accompanies LHCP to the thylakoid.

There are some notable differences between the characteristics of 54CP and of SRP. First, 54CP is capable of interacting with preproteins posttranslationally. There is evidence that mammalian SRP as well as its homologous *E. coli* counterpart react with preproteins only cotranslationally (31, 32). Second, 54CP seems to recognize a different targeting signal than SRP. SRP binds to a wide variety of signal peptides, which display conserved and essential features of a charged N terminus and a hydrophobic core. SRP will even bind nonsignal peptides in the absence of a cytosolic factor termed NAC (33). Lumenal proteins such as OE33 and OE23 contain signal peptide motifs within their targeting elements. Yet, 54CP shows no ability to bind to these preproteins (see above). It should be interesting to determine which elements of LHCP are recognized by 54CP.

The data presented here demonstrate that 54CP is an essential component of one pathway for intraorganellar routing of thylakoid proteins. As such, it emphasizes the evolutionary relationship of the protein targeting pathways of thylakoid and ER membranes. Presumably both of these membranes resulted from invagination of the cytoplasmic membrane of ancestral prokaryotes. Prokaryotes also have an SRP-like pathway (34) that appears to participate in the export of a number of proteins, all of which are dependent on SecA for targeting (35–37). In contrast, trafficking of LHCP is independent of the chloroplast SecA (16, 23) and completely dependent on 54CP (this work). The results presented here suggest the possibility that proteins primarily dependent on a SRP-like targeting pathway in prokaryotes may be integral membrane proteins localized in the cytoplasmic membrane. Further analyses of this pathway in chloroplasts should reveal interesting insight into the ways in which the SRP system has evolved and functions in different organisms and organelles.

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1. Deboer, A. D. & Weisbeek, P. J. (1991) *Biochim. Biophys. Acta* **1071,** 221–253.
2. Robinson, C. & Ellis, R. J. (1984) *Eur. J. Biochem.* **142,** 337–342.
3. Smeekens, S., Bauerle, C., Hageman, J., Keegstra, K. & Weisbeek, P. (1986) *Cell* **46,** 365–375.
4. Ko, K. & Cashmore, A. R. (1989) *EMBO J.* **8,** 3187–3194.
5. Viitanen, P. V., Doran, E. R. & Dunsmuir, P. (1988) *J. Biol. Chem.* **263,** 15000–15007.
6. Auchincloss, A. H., Alexander, A. & Kohorn, B. D. (1992) *J. Biol. Chem.* **267,** 10439–10446.
7. Huang, L., Adam, Z. & Hoffman, N. E. (1992) *Plant Physiol.* **99,** 247–255.
8. Reed, J. E., Cline, K., Stephens, L. C., Bacot, K. O. & Viitanen, P. V. (1990) *Eur. J. Biochem.* **194,** 33–42.
9. Payan, L. A. & Cline, K. (1991) *J. Cell Biol.* **112,** 603–613.
10. Cline, K. (1986) *J. Biol. Chem.* **261,** 14804–14810.
11. Lubben, T. H., Donaldson, G. K., Viitanen, P. V. & Gatenby, A. A. (1989) *Plant Cell* **1,** 1223–1230.
12. Yalovsky, S., Paulsen, H., Michaeli, D., Chitnis, P. R. & Nechushtai, R. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5616–5619.
13. Ellis, R. J. (1990) *Science* **250,** 954–959.
14. Yuan, J. G., Henry, R. & Cline, K. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8552–8556.
15. Manningkrieg, U. C., Scherer, P. E. & Schatz, G. (1991) *EMBO J.* **10,** 3273–3280.
16. Hoffman, N. E. & Franklin, A. E. (1994) *Plant Physiol.* **105,** 295–304.

17. Franklin, A. E. & Hoffman, N. E. (1993) *J. Biol. Chem.* **268,** 22175–22180.
18. Rapoport, T. A. (1992) *Science* **258,** 931–936.
19. Lutcke, H., High, S., Romisch, K., Ashford, A. J. & Dobberstein, B. (1992) *EMBO J.* **11,** 1543–1551.
20. Zopf, D., Bernstein, H. D. & Walter, P. (1993) *J. Cell Biol.* **120,** 1113–1121.
21. Miller, J. D., Wilhelm, H., Gierasch, L., Gilmore, R. & Walter, P. (1993) *Nature (London)* **366,** 351–354.
22. Cline, K., Henry, R., Li, C. J. & Yuan, J. G. (1993) *EMBO J.* **12,** 4105–4114.
23. Yuan, J., Henry, R., McCaffery, M. & Cline, K. (1994) *Science* **266,** 796–798.
24. Cline, K., Ettinger, W. F. & Theg, S. M. (1992) *J. Biol. Chem.* **267,** 2688–2696.
25. Robinson, C., Cai, D., Hulford, A., Brock, I. W., Michl, D., Hazell, L., Schmidt, I., Herrmann, R. G. & Klosgen, R. B. (1994) *EMBO J.* **13,** 279–285.
26. Henry, R., Kapazoglou, A., McCaffery, M. & Cline, K. (1994) *J. Biol. Chem.* **269,** 10189–10192.
27. Michl, D., Robinson, C., Shackleton, J. B., Herrmann, R. G. & Klosgen, R. B. (1994) *EMBO J.* **13,** 1310–1317.
28. Nakai, M., Goto, A., Nohara, T., Sugita, D. & Endo, T. (1994) *J. Biol. Chem.* **269,** 31338–31341.
29. Klosgen, R. B., Brock, I. W., Herrmann, R. G. & Robinson, C. (1992) *Plant Mol. Biol.* **18,** 1031–1034.
30. Smith, T. A. & Kohorn, B. D. (1994) *J. Cell Biol.* **126,** 365–374.
31. Bernstein, H., Poritz, M. A., Strub, K., Hoben, P., Brenner, S. & Walter, P. (1989) *Nature (London)* **340,** 482–486.
32. Luirink, J., High, S., Wood, H., Giner, A., Tollervey, D. & Dobberstein, B. (1992) *Nature (London)* **359,** 741–743.
33. Wiedmann, B., Sakai, H., Davis, T. A. & Wiedmann, M. (1994) *Nature (London)* **370,** 434–440.
34. Wolin, S. L. (1994) *Cell* **77,** 787–790.
35. Phillips, G. J. & Silhavy, T. J. (1992) *Nature (London)* **359,** 744–746.
36. Luirink, J., Tenhagenjongman, C. M., Vanderweijden, C. C., Oudega, B., High, S., Dobberstein, B. & Kusters, R. (1994) *EMBO J.* **13,** 2289–2296.
37. Oliver, D. B., Cabelli, R. J. & Jarosik, G. P. (1990) *J. Bioenerg. Biomembr.* **22,** 311–336.