Supporting Information

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SI Quantitation of Imported Proteins

The quantities of proteins imported were determined on fluorographs or immunoblots by comparison with known amounts of precursor put into the import reaction. The film used for this purpose was preflashed to give a 15% fog absorbance to improve its sensitivity and extend its linear response range (1). For fluorographs, we also placed calibration strips derived from gels containing dilutions of radioactive protein on each piece of film along with experimental gel; this served to inform us of the film's linear response range. For immunoblots, all samples to be compared were loaded onto the same gels, thereby guaranteeing that each sample was treated equivalently, had the same background, etc. Multiple replicate import samples were loaded, typically with three lanes per time point or treatment. The standards used for comparison were also loaded in multiple lanes with dilutions, thereby allowing us to determine the film's linear range. All immunoblots were exposed multiple times to ensure that we obtained exposures with samples that were neither undetectable nor saturated. Films were scanned using a setting for Slides from a TMA (transparent materials adapter) fitting in a TMA template with an HP Scanjet G4050 scanner and accompanying software. Software ImageQuant 5.2 was used for quantification. The User Method of Volume Quantification was used, which calculates the volume under the surface created by a 3D plot of the pixel locations and pixel intensities. Local median was used to correct for the background. Bands on the scans were outlined with rectangles and analyzed as per instructions in the ImageQuant manual.

1. Laskey RA, Mills AD (1975) Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. *Eur J Biochem* 56(2):335–341.

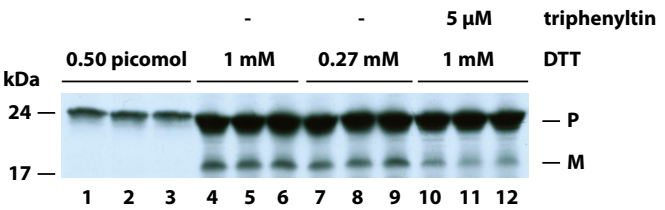

Fig. S1. Import of bacterially expressed [^{3}H]-precursor of the small subunit of RuBisCO into pea chloroplasts, representing the experiments of Table 1. The experiment was performed as described in the legend for Table 1. Lanes 4–6, 7–9, and 10–12 represent the experiments reported as experiments 2, 3, and 4, respectively, in that table. Chloroplasts equivalent to 6 μg chlorophyll were subjected to SDS/PAGE and fluorography. M, mature form of SSU; P, precursor of SSU.

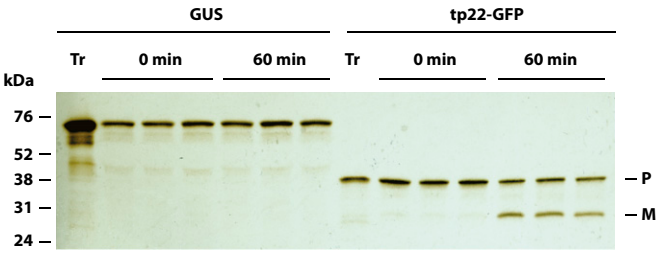

Fig. S2. Import of in vitro translated [^{3}H]-tp22-GFP into pea chloroplasts, representing the experiments of Table 2. The experiment was performed as described in the legend for Table 2; the data from this gel are reported as experiment 1 in that table. Chloroplasts equivalent to 4 μg chlorophyll were subjected to SDS/PAGE and fluorography. M, mature form of 22SSU-GFP; P, precursor tp22-GFP; SSU, small subunit; Tr, translation product representing 10% of the precursor added to the import reaction, equivalent to 0.72 picomoles of tp22-GFP.

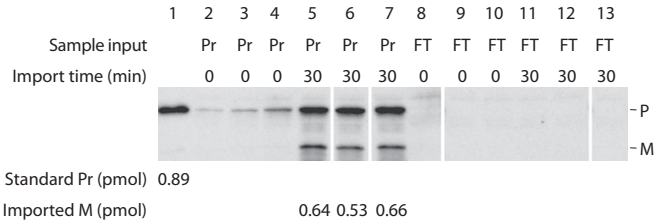

Fig. S3. Import of nonradioactive, bacterially expressed tp22-GFP into pea chloroplasts, representing the experiments of Table 3. The experiment was performed as described in the legend for Table 3; the data from this gel are reported as experiment 2 in that table. Precursor as a standard (lane 1) or chloroplast samples containing 4 μg Chl were separated by SDS/PAGE followed by immunoblotting against GFP antibody. The precursor band in lane 1 and mature bands in lanes 5–7 were quantified using ImageQuant. Bacterially expressed tp22-GFP representing 1.0% of the precursor added to the import reaction, equivalent to 0.89 picomoles (lane 1). The gel was originally run with the sample lanes containing the signals separated by flow-through lanes devoid of signal to facilitate accurate signal quantitation. The original (single) gel was rearranged using Photoshop to put the lanes into a more logical presentation order, with spaces indicating the moved-lane boundaries. FT, flow-through from the precursor concentration step added to the import reactions (lanes 8–13); M, mature form of 22SSU-GFP; P, precursor tp22-GFP; Pr, precursor added to the import reactions (lanes 2–7).

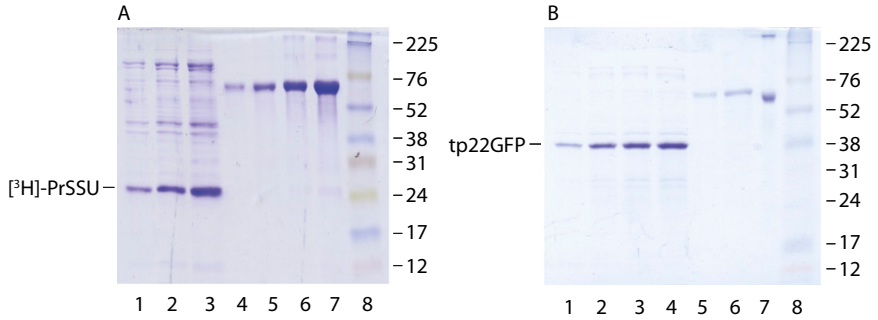

Fig. S4. Coomassie Blue R250-stained SDS gels showing the purified [^{3}H]-PrSSU (*A*, lanes 1–3) and tp22GFP (*B*, lanes 1–4) used in the experiments reported in Tables 1 and 3, respectively. BSA was loaded as a standard for quantification (*A*, lanes 4–7; *B*, lanes 5–7).

Table S1. Summary of effects of inhibitors, activators, and conditions on background chloroplast ATPase activity and protein import

Compound or condition	Known effect	Effect on background chloroplast ATPase activity	Effect on protein import	Concentration or temperature tested
DCMU	Inhibitor of electron transport	None	None	10 μM
Tentoxin	Inhibitor of CF1 ATPase	Decrease by 30–50%	None	0.5–10 μM
Mg^{2+}	ATPase cofactor	Increase	ND	1–100 mM
DTT	CF1 activator	Increase	Increase	1–2 mM
Vanadate	Inhibitor of ATPase	Increase	ND	0.05–50 μM
Molybdate	Inhibitor of ATPase	Increase	ND	200–400 μM
Phosphate	Product of ATPase	Slight increase	ND	5–100 mM
Triphenytin	Inhibitor of CF0	Decrease	Decrease	5 μM
Azide	Inhibitor of SecA	Increase	ND	10 mM
EDTA	Chelator	Decrease	Decrease	2.5–10 mM
Nigericin	Protonophore	None	ND	5 μM
Chloramphenicol	Inhibitor of protein synthesis	Slight decrease	ND	10 nM–100 μM
Spectinomycin	Inhibitor of protein synthesis	Slight decrease	ND	10 nM–100 μM
Bispyribac-sodium	Inhibitor of amino acid synthesis	Slight decrease	ND	10 nM–100 μM
Glyphosate	Inhibitor of amino acid synthesis	Slight decrease	ND	10 nM–100 μM
Halosulfuron-methyl	Inhibitor of amino acid synthesis	Slight decrease	ND	10 nM–100 μM
Penoxsulam	Inhibitor of amino acid synthesis	Slight decrease	ND	10 nM–100 μM
Imazethapyr	Inhibitor amino acid synthesis	Slight decrease	ND	10 nM–100 μM
Clomazone	Inhibitor of carotenoid synthesis	Slight decrease	ND	10 nM–100 μM
Malathion	Inhibitor of P450	Slight decrease	ND	10 nM–100 μM
Rifampicin	Inhibitor of RNA polymerase	Slight decrease	ND	10 nM–100 μM
Mild heat shock	Inhibitor of RuBisCO activase	None	ND	30 °C and 37 °C, 5–30 min
$NaNO_2$	Inhibitor of carbonic anhydrase	None	ND	10 mM
AMP	Inhibitor of phosphoribulokinase	Decrease	Decrease	10 mM
Glyceraldehyde	Inhibitor of phosphoribulokinase	None	ND	10 mM
3-Phosphoglyceric acid	Inhibitor of RuBisCO	None	ND	10 mM
P1, P5 – Di(adenosine-5′) pentaphosphate	Inhibitor of adenylate kinase	Slight decrease	ND	5–20 μM

ND, not determined.