Supporting Information

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SI Methods

Immunoelectron Microscopy. Twenty-four hours after bombardment, the transient transformed, cells were fixed for 2 h at 4 °C in 3% paraformaldehyde/0.5% glutaraldehyde/0.25 M sucrose in PHEM buffer (60 mM Pipes/25 mM Hepes/10 mM EGTA/2 mM $MgCl_2$, pH 7.4). An isolated transformant with GFP fluorescent was transferred onto a poly-L-lysine coated coverslip (18 × 18 mm) using micropipetting; it was then dehydrated for 5 min in each increment of the graded ethanol series (20, 40, 60, 80, and 100%), followed by infiltration with LR White:ethanol gradients of 1:2 for 1 h, 1:1 for 1 h, 2:1 for 1 h, and 100% for 12 h. All dehydration and infiltration steps were performed at 4 °C. The coverslip was placed on gelatin capsules filled with LR White, and it was polymerized at 58 °C for 24 h. The polymerized block was removed from the coverslip and sectioned on a Reichert Ultracut S ultramicrotome (Leica), using a diamond knife. Gold sections were collected onto Formvar-coated copper mesh or 1-slot grids. Before immunogold-labeling, the sections on grids were blocked with blocking solution (5% normal goat serum/2.5% skim milk/0.1% NaN_3 in PBS) for 1 h at room temperature. The grids were then incubated in 25 μL anti-GFP primary antibody (Clontech; Living Colors A.v. Monoclonal Antibody JL-8 no. 8371), diluted 1:25 with PBS, for 2 h at 30 °C. The grids were washed with PSB supplemented 0.05% Tween-20 for 5 min and 20 times on drops of PBS. The rinsed grids were then incubated on 30 μL anti-mouse IgG secondary antibody (Sigma) conjugated with 10 nm gold particles (diluted 1:20 with PBS) for 1 h at 30 °C. The labeled grids were rinsed with PBS and Milli-Q water, followed by staining with uranyl acetate for 10 min; the ultrathin sections were then observed under a JEM-1010 transmission electron microscope (JEOL) at 80 kV.

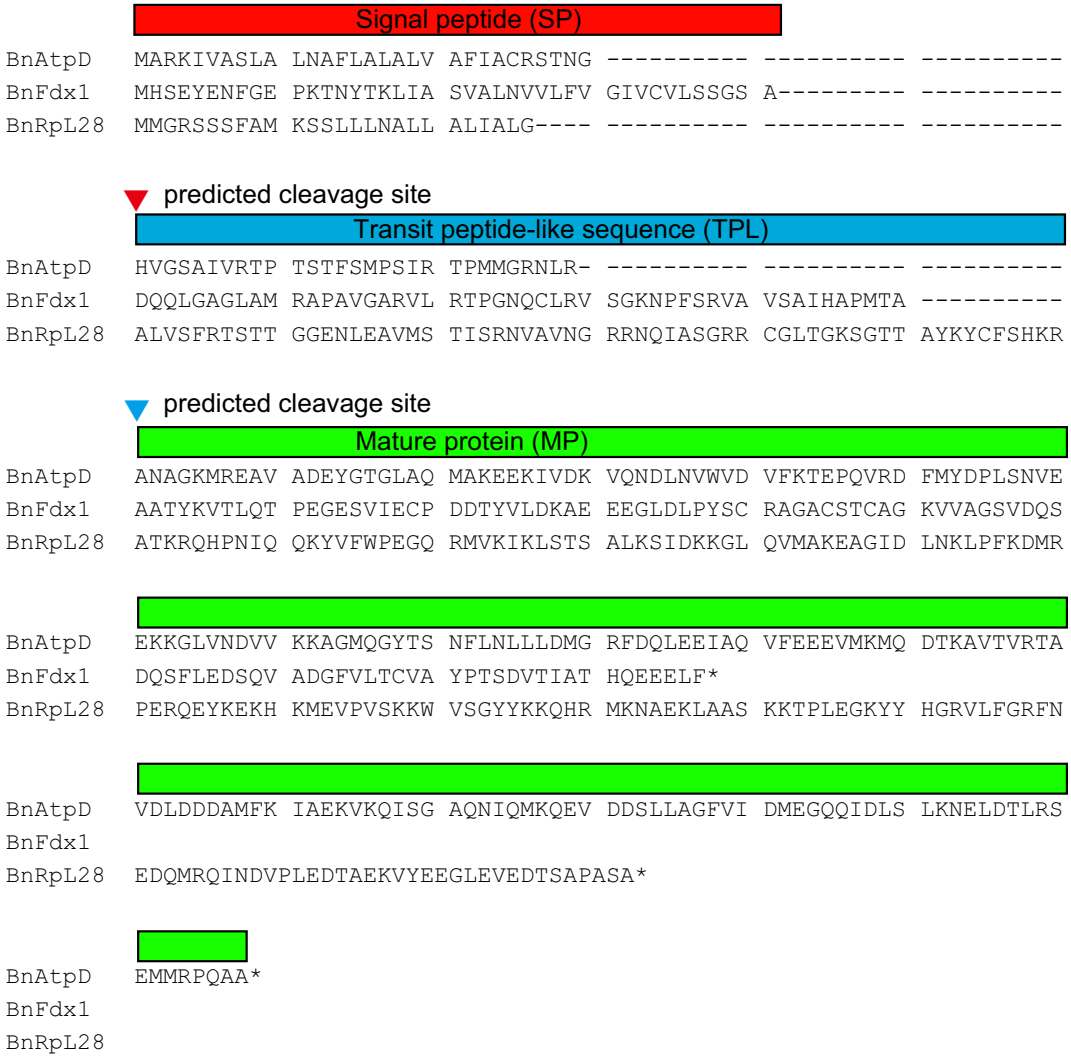

Fig. S1. An alignment of 3 plastid preprotein sequences (BnAtpD, BnFdx1, and BnRpL28) identified from a chlorarachniophyte *Bigelowiella natans*. These 3 preproteins have an N-terminal extension, which is predicted to be a bipartite targeting signal containing a signal peptide (SP) and a transit peptide-like sequence (TPL). The red arrowhead indicates the putative cleavage sites of the SPs, and the blue arrowhead indicates the putative TPL cleavage sites. The SP and TPL cleavage sites were predicted by the signal peptide prediction sever SignalP (www.cbs.dtu.dk/services/SignalP) and the chloroplast TP prediction server ChloroP (www.cbs.dtu.dk/services/ChloroP), respectively.

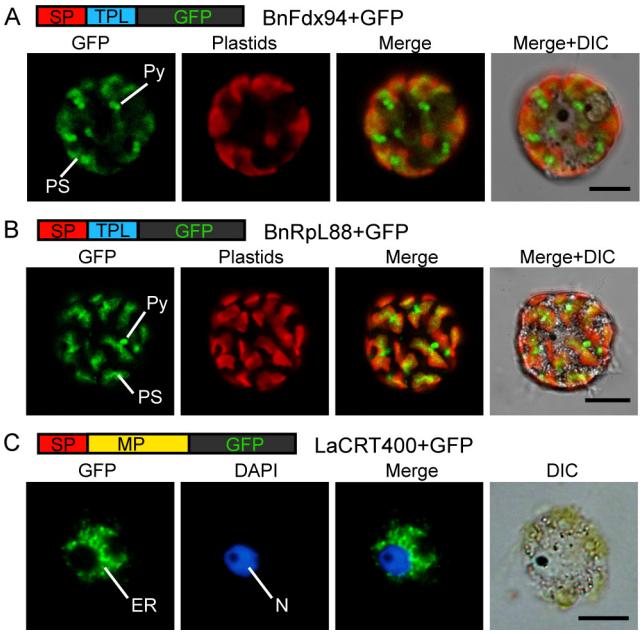

Fig. S2. Confocal images of transformed *Lotharella amoebiformis* cells with GFP fluorescence. The images labeled GFP show GFP localization (green); those labeled plastids show chlorophyll-autofluorescence (red). (*A*) A cell transformed with pBnFdx94+GFP showing the GFP localization in the stromas and pyrenoids in the plastids. (*B*) A cell transformed with pBnRpL88+GFP showing the GFP localization in the stromas and pyrenoids in the plastids. (*C*) Micrographs of a cell transformed with pLaCRT400+GFP and stained by DAPI. The cell showing fluorescent complex of DAPI and DNA in the nucleus, and showing GFP localization around the nucleus; nucleomorphs could not be detected by this staining. (Scale bar, 5 μm.) DIC, differential interference contrast; ER, endoplasmic reticulum; N, nucleus; PS, plastid stroma; Py, pyrenoid; MP, mature protein.

>ATP synthase delta subunit

HVGSAIVRTPTSTFSMPSIRTPMMGRNLR–ANAGK

>ATP synthase gamma subunit

LGVTRSAAFSPAGTTRDVSNIRANANLKEVRGRIESVSNTKKITSSMKLVAAAKVRKAQAAVLGGRPFAENLVKTLYGINQKVR–AEALT

>ferredoxin 1

DQQLGAGLAMRAPAVGARVLRTPGNQCLRVSGKNPFSRVAVSAIHAPMTA–AATYK

>photosystem II protein PsbO

AVYMSSGAQELSMVAPRQTRVFASKPNAMKNIAAGLA–AAGVS

>ribosomal protein rpL3

SIILSGLSPNLSSPM–AAAAR

>ribosomal protein rpL9

QQIGLPATKYNARVTSPVWRITPSRRCTSMSARRKKMVDVLLKEDVK–GSGKK

>Fe-S subunit of cytochrome c6f complex

VNRTQEGSLQLSAVRGKIAAPRTSFQNAVSRVSRNQLPSSSRKAVAQAFLSNPDMVPDMGKRKLMNNLVLAAVAPVV–ASAGG

>geranyl-geranyl reductase

ANPLGTAIVPSSASRSMYRAGPSTSSIPRGASGISSSSMGAMGAINNHNLKKMPKGTKFHCNWVAGERNLKGHAK–ADGSK

>phosphoglycerate kinase 1

NQQVAVEPLVGGGFSMAGVRPVSRMAVAPMHSVSPMNVGRFSKPSVTKFSAVSRPQQLKVN–VEKKM

>coproporphyrinogen III oxidase

TRGHFQVARSAPARSANTRITAAASRMQTAGKLFGGFSSATNKRDIGVRVIS–AESPT

>ribosomal protein rpS10

CFRGFSTPNNLERVLVQRISGNAPTGLTTINRMSRKLPTLVRSSNQPFSVGSTV–AEMAS

>photosystem II protein PsbW

SNLGATAIRSRVSPVSSVR–AGRMA

Fig. S3. TPL sequences of 12 plastid-targeted preproteins of a chlorarachniophyte, *B. natans*. These preproteins have consensus sequences that are similar to the C-terminal functional domain sequence, L-R-A-N-A, of BnAtpD TPL in chemical nature. The consensus sequences (I/L/M/V)-X-(A/G)-X-(A/G), indicated by red characters, are generally located near the putative TPL cleavage sites (−).