CpSufE Activates the Cysteine Desulfurase CpNifS for Chloroplastic Fe-S Cluster Formation*

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CpNifS, a cysteine desulfurase required to supply sulfur for iron-sulfur cluster biogenesis in Arabidopsis thaliana chloroplasts, belongs to a class of NifS-like enzymes with low endogenous cysteine desulfurase activity. Its bacterial homologue SufS is stimulated by SufE. Here we characterize the Arabidopsis chloroplast protein CpSufE, which has an N-terminal SufE-like domain and a C-terminal BolA-like domain unique to higher plants. CpSufE is targeted to the chloroplast stroma, indicated by green fluorescent protein localization and immunoblot experiments. Like CpNifS, CpSufE is expressed in all major tissues, with higher expression in green parts. Its expression is light-dependent and regulated at the mRNA level. The addition of purified recombinant CpSufE increased the V_{max} for the cysteine desulfurase activity of CpNifS over 40-fold and decreased the K_m toward cysteine from 0.1 to 0.043 mM. In contrast, CpSufE addition decreased the affinity of CpNifS for selenocysteine, as indicated by an increase in the K_m from 2.9 to 4.17 mM, and decreased the V_{max} for selenocysteine lyase activity by 30%. CpSufE forms dynamic complexes with CpNifS, indicated by gel filtration, native PAGE, and affinity chromatography experiments. A mutant of CpSufE in which the single cysteine was changed to serine was not active in stimulating CpNifS, although it did compete with WT CpSufE. The iron-sulfur cluster reconstitution activity of the CpNifS-CpSufE complex toward apoferredoxin was 20-fold higher than that of CpNifS alone. We conclude that CpNifS and CpSufE together form a cysteine desulfurase required for iron-sulfur cluster formation in chloroplasts.

Iron-sulfur (Fe-S) cluster proteins perform a variety of biological roles in electron transfer, catalysis, gene regulation, and sensing of iron and oxygen (1). Iron-sulfur cluster proteins are particularly important to photosynthesis. Measurements of metal ions in Arabidopsis thaliana have indicated that about 70% of the iron in green tissue is present in chloroplasts, and 40% is found in the thylakoids (2). Estimates in other plants indicate that up to 90% of the iron in leaves may be present in the chloroplasts (3). Within the thylakoids, the majority of the iron is found in Fe-S cluster proteins that function in photosynthetic electron transport (4). Next to photosynthetic carbon fixation, other pivotal plastid functions that require Fe-S clusters include nitrogen assimilation, sulfur assimilation, and pigment synthesis (for a review, see Ref. 5). Whereas all plastid types contain a number of important Fe-S cluster proteins, especially the green chloroplasts need to synthesize and maintain a variety of Fe-S proteins with at least five different cluster types (5).

In bacteria, three separate Fe-S formation machineries have been characterized (for a review, see Ref. 6). All systems include an NifS-like Cys desulfurase protein, which catalyzes the conversion of cysteine to alanine and elemental sulfur or the conversion of selenocysteine (SeCys)2 to alanine and elemental selenium. Every Fe-S machinery also has scaffold proteins thought to function in the preassembly of clusters before transfer to target proteins. The first discovered Fe-S assembly machinery was the Nif system of Azotobacter vinelandii, which is responsible for the formation of Fe-S clusters for nitrogenase (7). The second machinery was the Isc system first discovered in A. vinelandii and later in Escherichia coli, which has a housekeeping function in the formation of other cellular Fe-S proteins (8). Mitochondrial Fe-S assembly systems in eukaryotes are remarkably similar to this Isc system (6, 9). The third machinery was the suf system of E. coli and Erwinia chloranthi, which appears to be responsible for the formation of Fe-S clusters under oxidative stress and iron limitation (10–12). Based on sequence similarities, several of the plastid Fe-S biosynthesis components tentatively identified to date are most related to the bacterial suf cluster genes; other components are unique, however (5).

Chloroplasts have their own Fe-S assembly systems (13, 14). Fe-S cluster assembly into radiolabeled freshly imported ferredoxin precursor was demonstrated using isolated intact chloroplasts (15). The reaction proceeds in the absence of cytosol (16). The presence of supersaturated amounts of oxygen in green tissues provides a challenge for the synthesis and maintenance of plastid Fe-S cluster proteins because of the sensitivity of these clusters to oxygen (1). Therefore, it can be anticipated that next to the synthesis of new clusters, chloroplasts must have unique mechanisms to replace or repair oxidatively damaged clusters.

Characterization of the chloroplastic Fe-S formation machinery started with the identification of a Cys desulfurase CpNifS (17, 18) and of scaffold proteins CpNfu2 (19, 20, 21) and CplscA (22). CpNifS is the Cys desulfurase that converts cysteine into alanine and elemental sulfur for Fe-S formation. CpNfu2 can hold a transient Fe-S cluster. Insertion mutants in the CpNfu2 gene have a dwarf phenotype and are deficient in 2Fe-2S and 4Fe-4S proteins (20, 21). CplscA is a putative alternative scaffold that can accept a 2Fe-2S cluster from CpNifS, which can be transferred to apoferredoxin in vitro (22). In addition, other Suf-type system components (23–25) and HCF101 (26) may assist the Fe-S formation in plastids. The CpSuBCD complex is an ATPase and may be involved in providing ferrous iron or in transferring the Fe-S cluster from the scaffold protein to the target protein (23–25). HCF101 (high chlorophyll fluorescence 101) encodes a NifH-related P-loop ATPase that seems to be required for 4Fe-4S but not 2Fe-2S assembly in chloroplasts (26).

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2 The abbreviations used are: SeCys, selenocysteine; WT, wild type; GFP, green fluorescent protein; IDA, iminodiacetic acid; HPLC, high pressure liquid chromatography; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.
Since cysteine was identified as the sulfur source for Fe-S formation in chloroplasts (13, 14), the Cys desulfurase activity of CpNifS is probably essential for Fe-S formation in chloroplasts. Indeed, the depletion of CpNifS led to the loss of Fe-S reconstitution activity of chloroplast stroma (27). This Cys desulfurase of chloroplasts is distinct from the Cys desulfurases NiifS and IscS of the Nif and Isc type assembly systems and is more similar in sequence to SufS (18).

### MATERIALS AND METHODS

**Cloning and Plasmid Construction**—The *A. thaliana* CpSufE coding sequence was amplified by PCR using cdNA as a template. cdNA was prepared from Dnase-treated total RNA prepared from 2-week old seedlings as described (18). Primers used for CpSufE amplification were **SufE-precursor** and **SufE-Bam** (Table 1). The PCR product was digested with Ncol and BamHI and then ligated into vector [pET11d](https://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=nuccore&cmd=Retrieve&dopt=SearchAsTable&list_uids=4000350) resulting in [pMNFSC388S](https://www.ncbi.nlm.nih.gov/nuccore/198753198). All constructs were verified by DNA sequencing. The plasmids used for protoplast transformation were prepared using the Plasmid Midi Kit (Qiagen, Valencia, CA).

**Preparation of Proteins**—For overexpression of CpSufE, *E. coli* BL21 (DE3) codon + (Stratagene, La Jolla, CA) was transformed with plasmid pMSufE or with pMSufEC65S for the mutant protein. Two liters of LB medium containing 50 µg ml−1 kanamycin was inoculated with one-hundredth volume of overnight culture. Cells were grown at 37 °C to an *A*<sub>600</sub> of 0.5, and expression was induced by the addition of 0.4 mM isopropyl-β-d-thiogalactopyranoside followed by incubation for 3 h at 37 °C. The culture was chilled on ice, and the cells were collected by centrifugation for 5 min at 5,000 × g at 4 °C. From here on, all procedures were performed at 4 °C except where mentioned. The bacterial pellet was washed with 150 mM NaCl and resuspended in 50 mM Tris–HCl, pH 7.5, and then passed twice through a French press (8,000 p.s.i.) to disrupt the cells. The lysate was centrifuged for 20 min at 12,500 × g, and the cleared supernatant was loaded at a flow rate of 3 ml min−1 onto His–Bind iminodiacetic acid (IDA)–agarose (Novagen, Madison, WI) in a 1.6 × 20-cm column, which was saturated with NiSO₄, washed, and equilibrated in 50 mM Tris–HCl, pH 7.5. The column was washed with 4 volumes of 50 mM Tris–HCl, pH 7.5, followed by 4 volumes of 1 M NaCl, 50 mM Tris–HCl, pH 7.5, 6 volumes of 50 mM Tris–HCl, pH 7.5, again, and 2 volumes of 0.1 M imidazole in 50 mM Tris–HCl, pH 7.5, respectively. His–tagged CpSufE was eluted with 4 volumes of 1 M imidazole in 50 mM Tris–HCl, pH 7.5. Fractions of 6 ml were collected. Peak fractions

**Sequence Alignment**—Sequence analysis was performed using the Mac Vector sequence analysis software (International Biotechnologies, New Haven, CT). Searches for sequence similarity were performed using the BLAST network service provided by the National Center for Biotechnology Information (available on the World Wide Web at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). Sequence alignment was performed using ClustalW at European Bioinformatics Institute, ExpASY Proteomics tools (available on the World Wide Web at [www.ebi.ac.uk/clustalw](http://www.ebi.ac.uk/clustalw)).

### Table 1

| Oligonucleotide | 5′−3′ sequences | Restriction site underlined |
|----------------|-----------------|-----------------------------|
| SufE-precursor | CATGGCATGGCAAGCATAGTCTTCTTTC | Ncol |
| SufE-mature | GGAATTCCATATGGCTTCATCATCTGGTCAG | Ncol |
| SufE-Bam | CCGGATCCTAGAATCACTCAAGAAGTCT | Ncol |
| SufE-GFP-F | AATGATGGCCATCTGAGGGTCG | SalI |
| SufE-GFP-R | T7 terminator | T7 |
| SufEC65S-F | CATGCCATGGCAGCAGCGATGTCTTCTTC | Ncol |
| SufEC65S-R | T7 terminator | T7 |
| SufE-mature | TGGATGATTTATTTGCTCGGCGTCT | Ncol |
| SufE-GFP-RT | T7 terminator | T7 |
| SufE-mature | TGGATGATTTATTTGCTCGGCGTCT | Ncol |
| SufE-Bam | T7 terminator | T7 |
| SufE-Bam | TGGATGATTTATTTGCTCGGCGTCT | Ncol |
| SufE-Bam | T7 terminator | T7 |
| SufE-Bam | TGGATGATTTATTTGCTCGGCGTCT | Ncol |
| SufE-Bam | T7 terminator | T7 |
| SufE-Bam | TGGATGATTTATTTGCTCGGCGTCT | Ncol |
| SufE-Bam | T7 terminator | T7 |

**Sequence of oligonucleotides used for cloning and plasmid constructions**
Chloroplast Cysteine Desulfurase Activation

were pooled and dialyzed overnight against 25 mM Tris-HCl, pH 7.5. Pure His-tagged CpSufE ran as a single band on SDS-PAGE after staining with Coomassie Brilliant Blue.

To produce cleaved CpSufE, the pooled peak fractions were dialyzed overnight against 20 mM Tris-HCl, pH 8.4, followed by incubation with thrombin in a 1:1,000 (w/w) ratio (thrombin/target protein) at 4 °C for 8 h in 20 mM Tris-HCl, pH 8.4, 150 mM NaCl, 2.5 mM CaCl₂, as suggested by the manufacturer (Novagen, Madison, WI). The cleavage mixture was subsequently applied to a 10 × 1-cm Resource-Q column (Amersham Biosciences), equilibrated in 25 mM Tris-HCl, pH 8.0, at room temperature and connected to a Summit HPLC system (Dionex, Sunnyvale, CA). The column was eluted with a linear gradient from 0 to 1 M NaCl in 25 mM Tris-HCl, pH 8.0, and fractions of 2 ml were collected. Elution was monitored by detection of the OD at 280 and 220 nm. The purified cleaved CpSufE was dialyzed overnight against 25 mM Tris-HCl, pH 7.5, and stored frozen at −80 °C before use in activity assays. Typical yields were 5–10 mg/liter of culture. The purified protein migrated as a single band on SDS-PAGE (12.5% gel) and ran as a single peak in analytical HPLC runs on a 1-ml Resource Q column (Amersham Biosciences).

The His₆-tagged and cleaved CpSufEC388S were purified essentially as the WT CpSufE protein. WT CpNifS protein was prepared as described before (18). For overexpression of CpNifSC388S, E. coli BL21 (DE3) codon + (Stratagene, La Jolla, CA) was transformed with plasmid pMNFS₂₅⁸₃. The recombinant CpNifSC₃₈₈₅ was prepared essentially as WT CpNifS (18). Purified CpNifSC₃₈₈₅ was eluted from a calibrated 1 × 30-cm Superdex-200 gel filtration column (Amersham Biosciences) at the same retention time as the WT protein, and a native molecular mass of 83 kDa was calculated, suggesting that CpNifSC₃₈₈₅ is a dimer like the wild type protein. Holo- and apoferrodoxin were prepared as described (27).

**Enzyme Assays—**Cys desulfurase activity was assayed at 25 °C essentially as described (29). Briefly, 160 μl of reaction mixture contained 25 mM Tris-HCl, pH 7.4, 100 mM NaCl, 2.5 mM enzyme (0.11 mg/ml CpNifS, 0.09 mg/ml CpSufE), 10 μM pyridoxal 5'-phosphate (Acros Organics, Morris Plains, NJ), 1 mM dithiothreitol (Roche Applied Science), and 500 μM cysteine (Sigma). The reaction was stopped by the addition of 20 μl of 20 mM N,N-dimethyl-p-phenylenediamine in 7.2 M HCl. Methylene blue was formed by the addition of 20 μl of 30 mM FeCl₃ in 1.2 M HCl and was assayed by measuring the absorbance at 670 nm. The Scys lyase activity was measured as described (18). One unit of enzyme activity corresponds to 1 μmol of substrate converted/min. To estimate kinetic constants for both the Cys desulfurase and Scys lyase activities of CpNifS, the reaction velocities were measured over a wide range of substrate concentrations (0.01–20 mM). The data in Michaelis-Menten plots were fitted by an iterative method to estimate Km and Vmax values, using the software program Enzfitter (Biosoft, Cambridge, UK). The Fe-S reconstitution assay was performed as described before (27). For all statistical analyses, the JMP-IN software (SAS institute, Cary, NC) was used.

**Gel Filtration—**Sizes of protein complexes were estimated by gel filtration experiments, as described before (27). The column used was a 1 × 30-cm Superdex-200 column (Amersham Biosciences), which was connected to a Summit HPLC system with a UVD170 detector and controlled by Chromeleeeon software (Dionex, Sunnyvale, CA). The loop size was 0.2 ml. The column was equilibrated in 25 mM Tricine/KOH, pH 7.9, 50 mM KCl. The flow rate was 0.75 ml/min, and fractions were collected every 0.5 min. Elution was monitored by absorbance at both 280 and 220 nm and by immunoblotting of collected fractions. The void volume was determined with blue dextran. Standards used for calibration were IgY, bovine serum albumin, ovalbumin, chymotrypsinogen, and RNase.

**Protein Coelution Experiment—**100 μg of His-tagged CpSufE (WT or mutant) and possible partner proteins were mixed at room temperature in 500 μl of buffer (50 mM Tris-HCl, pH 7.5) and loaded on a 0.5-ml His-Bind IDA-agarose column (Novagen, Madison, WI). The column was washed with 2 ml of buffer and subsequently with 2 ml of 1 M NaCl, 50 mM Tris-HCl, pH 7.5, followed by 2 ml of 50 mM Tris-HCl, pH 7.5, again, and 1 ml of 50 mM Tris-HCl, pH 7.5, 0.1 M imidazole. Finally, the column was eluted with 2 ml of 1 M imidazole, 50 mM Tris-HCl, pH 7.5, and 1-ml fractions were collected. Samples incubated with cysteine were eluted with all solutions containing 1 M cysteine, whereas samples incubated without cysteine were eluted with solutions free of cysteine. Proteins eluted with 1 M imidazole were analyzed by SDS-PAGE and staining with Coomassie Brilliant Blue.

**Separation of CpNifS-CpSufE Complexes on Native Gel—**5 μg of CpSufE (WT or mutant) was mixed with 5 μg of CpNifS (WT or mutant) in a final volume of 15 μl, with or without 1 mM cysteine. After incubation at room temperature for 10 min, proteins were mixed with an equal volume of 20% (w/v) glycerol, 250 mM Tris-HCl, pH 6.8, and separated by native PAGE, using a 4% stacking gel, a 10% separating gel, and the Laemmli buffer system with the omission of SDS. 5 μg of each single protein was loaded as control. The gel was stained with Coomassie Brilliant Blue.

**Plant Sampling—**A. thaliana (Ecotype Columbia-0) plants were grown on soil with supplementary light on a 15-h light/9-h dark cycle for 4 weeks. Total leaf homogenate, chloroplast stroma, and RNA from different tissues were prepared as described (18). For light regulation analysis of CpSufE, Arabidopsis plants were grown on half-strength Murashige and Skoog agar medium (30) for 2 weeks, either on a 15-h light/9-h dark cycle or in complete darkness. Protein and RNA preparations from total leaf homogenate were described before (18).

**Subcellular Localization of GFP Fusion Proteins—**The plasmids TP/SufE-GFP or full-length/SufE-GFP were transformed into Arabidopsis protoplasts, and expressed proteins were observed under a confocal microscope, as described (22).

**Antibodies and Immunoblotting—**Cleaved CpSufE in 100 mM NaCl, 25 mM sodium phosphate, pH 7.5, was used to raise polyclonal antibody in rabbits at a commercial facility (PRF&L, Canadasis, PA). The CpNifS antibodies have been described (18). The Hsp70 antibody was purchased from Sigma. Rubisco antibody was purchased from AgriSera (Vannas, Sweden). Immunoblotting was performed as described (18). Band intensities were quantified with ImageJ software (National Institutes of Health, Bethesda, MD).

**RNA Blot Analysis—**Total RNA from different Arabidopsis tissues was prepared, electrophoresed, and probed with a 32P-labeled 900-bp CpSufE cDNA, essentially as described before (18). Band intensities were quantified with ImageJ software (National Institutes of Health, Bethesda, MD).

**RESULTS**

Identification of CpSufE and Sequence Characteristics—CpNifS is a Cys desulfurase (17, 18) required for iron sulfur cluster formation in the chloroplast (27). However, compared with most Cys desulfurases with a housekeeping role in Fe-S cluster formation, the Cys desulfurase activity of purified CpNifS is very low, despite a high selenocysteine lyase activity (18). When the in vitro reconstitution activity of purified CpNifS protein was compared with the activity of CpNifS in stromal extracts, it was concluded that CpNifS in stroma is about 50–80 times more active (27). Thus, a stimulatory activity must be present in plastids. We con-
considered that this stimulatory activity could at least in part be explained if plastids contain a homologue of SufE, a protein that stimulates the Cys desulfurase activity of the bacterial SufS proteins. A SufE-like sequence (At4g26500) was identified by a BLAST search within the Arabidopsis genome data base (TAIR; available on the World Wide Web at www.arabidopsis.org), using the E. coli SufE (29) as a query sequence. The genomic sequence of At4g26500 contains a single predicted exon. A cDNA containing the full coding sequence was obtained by reverse transcription-PCR. Sequence analysis of the cDNA confirmed the presence of a single uninterrupted coding sequence in the genome (not shown). The open reading frame is predicted to encode a full-length precursor protein of 371 amino acids (see Fig. 1A), including a putative chloroplast targeting peptide (66 amino acids) as predicted by the TargetP program (available on the World Wide Web at www.expasy.org; see Ref. 31). Because of the predicted location and similarity with SufE proteins, we named At4g26500 CpSufE, for chloroplastic SufE. The predicted mature polypeptide is 305 amino acids long, with a molecular mass of 33.6 kDa and an isoelectric point of 4.87. The mature size of the Arabidopsis CpSufE homologue is much larger than what was reported for the bacterial SufE proteins, which are about 15 kDa in size (29, 32). A sequence alignment was performed to determine the similarity of the putative Arabidopsis CpSufE with possible SufE homologues from
The rice and Arabidopsis protein similarity to the cyanobacterial SufE is absent from the prokaryotic homologues. The last 88 residues of this region correspond to the N-terminal half of the predicted mature protein. Localization of CpSufE in chloroplasts—The TargetP program predicted a cleavable transit sequence of 66 amino acids and a chloroplast localization of CpSufE. To examine the subcellular localization, we constructed fusions with GFP. In the first construct, the CpSufE transit peptide was fused to the N terminus of GFP; in a second construct, the full-length precursor CpSufE was fused to the N terminus of GFP. GFP alone expressed from the same constitutive promoter was used as a control. Constructs were separately introduced into Arabidopsis protoplasts, and the localization in cells was analyzed using confocal laser microscopy. Fluorescence corresponding to GFP expressed without a transit sequence was excluded from the chloroplasts as expected (Fig. 2A). In contrast, green fluorescence from the transit sequence fusion was localized to the chloroplast stroma, as indicated by the overlay of green fluorescence and red autofluorescence (Fig. 2A, middle). Green fluorescence from the full-length CpSufE coupled to GFP was localized to discrete locations in the chloroplasts (Fig. 2A, bottom).

The localization of CpSufE in chloroplast stroma was verified by immunoblotting (Fig. 2B). CpSufE antibody detected as little as 0.5 ng of purified CpSufE. The antibody detected a protein of 37 kDa in total leaf homogenate (TH), and this signal was quantitatively recovered in the chloroplast stroma fraction (St, Fig. 2B). The preimmun serum at the same dilution did not recognize any bands in these samples (data not shown). These data indicate the absence of CpSufE in stroma. Based on the band intensities, we estimate that the amount of CpSufE present in 20 μg of stromal protein is between 10 and 20 ng. Thus, the abundance of CpSufE in stroma is between 0.05 and 0.1% of total protein, similar to the abundance (0.06%) reported for CpNifS (27). In conclusion, the immunoblotting and GFP experiments strongly suggest a stromal localization of CpSufE.

Expression Analysis of CpSufE—Expression patterns can give clues about the function of a protein. To determine the CpSufE mRNA and protein expression patterns in different Arabidopsis tissues, we performed RNA blot and immunoblot analyses (Fig. 3) and quantified band...
CpSufE was used for all biochemical characterizations unless indicated otherwise. The protein was correctly cleaved and purified. The cleaved C-terminal sequence analysis yielded the sequence GSHMASS, which indicated that the protein was correctly cleaved and purified. The cleaved CpSufE was used for all biochemical characterizations unless indicated otherwise. The purified recombinant CpSufE eluted as a dimer from an analytic gel filtration column (Fig. 4C).

CpSufE alone did not show any activity in either the Cys desulfurase assay (Fig. 5C) or the SeCys lyase assay (Fig. 5B), both of which are activities displayed by CpNifS. To test if CpSufE changes the kinetic properties of CpNifS, the activities of CpNifS alone and of CpNifS plus CpSufE in a 1:1 molar ratio were assayed over a concentration range for both substrates (Table 2). CpNifS alone displayed a barely detectable level of Cys desulfurase activity ($V_{\text{max}} = 0.0013 \mu\text{mol/min/mg}$) but displayed high SeCys lyase activity ($V_{\text{max}} = 2.44 \mu\text{mol/min/mg}$), in agreement with our previous results (18). However, when CpSufE was added to CpNifS in a 1:1 molar ratio, we observed a 40-fold increase in the $V_{\text{max}}$ for Cys desulfurase activity compared with CpNifS alone. In contrast, the $V_{\text{max}}$ for SeCys lyase activity was reduced by 30%. The addition of CpSufE to CpNifS caused the $K_m$ for cysteine to decrease 2.5-fold. On the other hand, the $K_m$ for selenocysteine increased slightly (1.4-fold). Thus, the $V_{\text{max}}/K_m$ ratio of CpNifS was increased 100-fold by CpSufE. Titration experiments at saturating substrate concentration in which the amount of CpNifS was kept constant while CpSufE was varied indicated that the Cys desulfurase activity of CpNifS depended on the amount of CpSufE present. The stimulation by CpSufE reached an apparent saturation point at a CpSufE/CpNifS molar ratio of 5:1, at which point we observed a 60-fold stimulation (Fig. 5A).

CpNifS has 5 cysteines, one of which, Cys$^{388}$, is conserved and predicted to be required for Cys desulfurase activity (18), based on similarity with bacterial CsdB (36) for which a structure is published (37). To investigate if this cysteine in CpNifS is required for the activity of the protein, we purified a mutant, CpNifSC388S, in which the cysteine was altered to serine. Purified CpNifSC388S displayed the same absorbance spectrum and elution profile from a gel filtration column as the WT protein. Similar to what had been found for the CsdB Cys mutant (36), the mutant CpNifSC388S protein retained about 80% of its SeCys lyase activity (Fig. 5B). This indicates that under the assay conditions, the decomposition of selenocysteine does not depend on the active site cysteine and that the mutant CpNifSC388S enzyme was folded and active. However, the CpNifSC388S mutant protein had no detectable Cys de-
sulfurase activity anymore, neither alone nor with CpSufE present. Thus, the conserved cysteine 388 is essential for Cys desulfurase activity of CpNifS but not for SeCys lyase activity.

CpSufE has a single conserved cysteine, Cys<sup>65</sup>, in its mature sequence. To test the requirement of the thiol group for CpSufE activity, we expressed and purified a mutant in which the cysteine was changed to serine. Like the wild-type CpSufE, the mutant CpSufE<sub>C65S</sub> did not show activity by itself (Fig. 5, B and C). Only WT CpSufE and not CpSufE<sub>C65S</sub> displayed stimulation of CpNifS Cys desulfurase activity, indicating that the conserved cysteine 65 is essential for this function (Fig. 5C). Both CpSufE and CpSufE<sub>C65S</sub> had a similar small but noticeable negative effect on the SeCys lyase activity of CpNifS.

**The C65S Mutation in CpSufE Is Dominant Negative in Vitro**—We tested the effect of the presence of the CpSufE and CpNifS Cys mutants on the activity of WT CpNifS and WT CpSufE. Interestingly, the presence of CpSufE<sub>C65S</sub> severely inhibited the stimulatory activation of CpNifS by WT CpSufE in a concentration-dependent manner, thus showing a dominant negative effect in vitro. In marked contrast, even a 5-fold excess of mutant CpNifS<sub>C388S</sub> did not inhibit the activation of WT CpNifS by CpSufE (Fig. 6). A straightforward interpretation of these observations is that in these incubations, the mutant CpSufE<sub>C65S</sub> titrates WT CpNifS into an inactive complex, whereas mutant CpNifS<sub>C388S</sub> does not titrate away WT CpSufE from WT CpNifS. The difference may be explained by differences in affinity between mutant and WT proteins that in turn may be related to the ability to decompose cysteine or accept S.

**CpSufE Complexes with CpNifS**—The observed alterations of the catalytic properties of CpNifS by CpSufE prompted us to investigate if CpSufE and CpNifS form a complex. To address this question, we first performed a gel filtration analysis (Fig. 7). The native molecular weight of recombinant CpSufE and of the CpSufE<sub>C65S</sub> mutant was determined by comparing the elution time from a Superdex 200 gel filtration column with standards. In gel filtration experiments, CpSufE eluted in single peak at 18.5 min (Fig. 7A, trace 1). The same elution profile was seen for CpSufE<sub>C65S</sub> (not shown). The retention time of 18.5 min corresponds to an estimated molecular mass of ~70 kDa, indicating that purified recombinant CpSufE and the CpSufE<sub>C65S</sub> mutant are homodimeric proteins. CpNifS eluted as a single peak at 17.6 min (Fig. 7A, trace 2), which corresponds to a size of 86 kDa, in agreement with the mass expected for homodimeric CpNifS (18). When WT CpSufE and CpNifS (WT) were mixed in a 1:1 molar ratio and applied to the gel filtration column, we found most of the protein to elute in a peak with a retention time of 17.2 min (Fig. 7A, trace 3). Comparison with standards indicated an apparent molecular mass of ~110 kDa, indicating the formation of a complex that is significantly smaller than the added mass of two dimers together. The published structure for the homologous E. coli SufS protein suggests a very stable dimer structure for CpNifS (37). Indeed, the CpNifS dimer is very stable, and we have never observed monomers of this protein. We thus consider it likely that trace 3 in Fig. 7A represents a heterotrimer of two CpNifS subunits and one CpSufE subunit, which is in agreement with the estimated size of the complex. Indeed both CpSufE and CpNifS were found to be present in the peak as judged from SDS-PAGE, but CpSufE seemed less abundant in the peak fractions (Fig. 7B, gel 3), and the addition of more CpSufE

**FIGURE 5.** CpSufE stimulates Cys desulfurase activity 40-fold but reduces SeCys lyase activity of CpNifS. A, Cys desulfurase activity of CpNifS (2.5 μM) mixed with CpSufE in molar ratios ranging from 0 to 20 (CpSufE/CpNifS). B, selenocysteine lyase activities of proteins and protein combinations present at 2.5 μM. C, cysteine desulfurase activities of proteins and protein combinations present at 2.5 μM. Data are the means ± S.E. of three experiments.

**TABLE 2**

CpSufE changes kinetic properties of CpNifS

| Substrate | CpNifS | CpNifS + CpSufE |
|-----------|--------|-----------------|
|           | \(K_m\) | \(V_{max}\) | \(K_m\) | \(V_{max}\) |
| Cysteine  | 0.10 ± 0.02 | 0.0033 ± 0.0002 | 0.043 ± 0.011 | 0.055 ± 0.002 |
| Selenocysteine | 2.90 ± 0.36 | 2.44 ± 0.07 | 4.17 ± 0.24 | 1.76 ± 0.11 |
shifted the complex to a higher molecular weight (not shown). When the mutated CpSufE<sub>C65S</sub> was incubated with CpNiFS and loaded onto the gel filtration column, a peak with a retention time of 16.5 min was observed (Fig. 7A, trace 4), corresponding with a complex with an apparent size of ~150 kDa, which is in good agreement with the cumulative molecular mass of a CpSufE dimer and a CpNiFS dimer, a composition that is confirmed by SDS-PAGE analysis. These data indicate that the mutant CpSufE retains CpNiFS-binding ability and may have a higher affinity for CpNiFS than the WT CpSufE. When the cysteine mutant of CpNiFS was substituted for the WT protein and incubated with CpSufE, the same elution pattern was obtained that was observed with WT CpNiFS (data not shown). Therefore, the affinity of WT and mutant CpNiFS for CpSufE appears to be similar.

As mentioned above, the WT CpSufE and CpNiFS proteins appear to form a NiFS<sub>2</sub>-SufE trimer when incubated at lower concentrations, but this complex appears to shift to a heterotetramer when the CpSufE concentration is increased. A similar tetrameric complex was observed when the concentrations of both proteins were increased 10-fold (Fig. 7C). The formation of the putative trimeric and tetrameric complexes and the effect of cysteine on complex formation were further investigated by native PAGE (Fig. 7D). CpNiFS and CpSufE are both negatively charged and have a very similar charge/mass ratio. Therefore, we can expect that the mobility in native PAGE will be mainly determined by the size of the native protein complex. When individual proteins were loaded onto the native gel, CpNiFS migrated as a single band, presumably its dimeric form; WT CpSufE also migrated as a single band, presumably the monomeric form. When the WT CpSufE and WT CpNiFS were incubated in the absence of cysteine, the dimeric CpNiFS and CpSufE were observed, regardless of the presence of cysteine, and neither dimeric CpNiFS nor dimeric CpSufE was observed (Fig. 7D). A straightforward interpretation is that the mutant CpSufE<sub>C65S</sub> has a stronger affinity for CpNiFS or that the CpSufE<sub>C65S</sub>-CpNiFS complex is more stable. Finally, incubation of mutant CpNiFS<sub>C388S</sub> with mutant CpSufE<sub>C65S</sub> resulted in only the putative trimeric complex (band I), regardless of the presence of cysteine. These results are similar to those observed when the mutant CpNiFS<sub>C388S</sub> was incubated with WT CpSufE and may indicate that mutant CpNiFS<sub>C388S</sub> has a reduced binding ability to CpSufE. These native PAGE results confirm the presence of different CpSufE-CpNiFS complexes and also explain the dominant negative effect of CpSufE<sub>C65S</sub> when added to WT. The NiFS and SufE proteins form complexes even when the active site Cys residues in both proteins are changed to serine. Still, the active site residues in CpNiFS and CpSufE clearly affect the nature of the complexes that are formed. Furthermore, the effect of Cys on complex formation between the WT proteins suggests that conformational changes occur in CpNiFS upon the completion of a round of desulfuration, which in turn may facilitate a release of CpSufE from the complex. It thus appears that CpSufE is in dynamic exchange between a "free" CpSufE and several complexed states.

Investigating the Formation of the CpSufE-CpNiFS Complex—The formation of a CpSufE-CpNiFS complex was further investigated in nickel column coelution experiments. We used the His<sub>6</sub>-tagged CpSufE and incubated the protein with an equal amount of CpNiFS before loading onto a Ni-IDA column. The column was treated with a series of salt washes before elution with 1 M imidazole. Samples of the loaded and imidazole-eluted proteins were analyzed by SDS-PAGE (Fig. 8A). Wild-type CpNiFS was found to co-elute with the His-tagged wild-type CpSufE. Control experiments showed that CpNiFS did not bind to the nickel column by itself (data not shown). The same elution pattern was found for CpNiFS and His-tagged CpSufE regardless of whether the proteins had the wild-type sequences or were mutated at the essential cysteine residues. Therefore, in this nickel column binding assay, both CpNiFS and CpSufE are again shown to interact, and this interaction does not require the conserved Cys residues in either protein, which are however needed for Cys desulfurase activity. Another chloroplast protein, CplscA, which may serve as a chloroplast scaffold protein for Fe-S assembly (22), did not co-elute with CpSufE or with CpNiFS and CpSufE (data not shown).

The observation of the putative trimeric NiFS<sub>2</sub>-SufE complex in addition to the putative NiFS<sub>2</sub>-SufE<sub>2</sub> heterotetramer suggests the involvement of the CpSufE monomer in the formation of the complex. This hypothesis was further investigated by nickel column coelution experiments. We mixed dimeric His-tagged CpSufE with separately purified dimeric untagged (cleaved) CpSufE and CpNiFS, either with or without cysteine. The protein mixture was loaded onto a Ni-IDA column and eluted with 1 M imidazole to select for His-tagged CpSufE and any...
FIGURE 7. **CpSufE forms complexes with CpNifS.**

A, Superdex-200 gel filtration elution profiles ($A_{280}$). Trace 1, 15 μg of CpSufE; trace 2, 17 μg of CpNifS; trace 3, mixture of 15 μg of CpSufE and 17 μg of CpNifS; trace 4, mixture of 15 μg of CpSufEC65S and 17 μg of CpNifS. B, SDS-PAGE analysis of gel filtration fractions described in A. Aliquots of the indicated fractions were collected and separated on SDS-polyacrylamide gels, followed by staining with Coomassie Brilliant Blue. The peak fraction in each gel filtration run is marked by an asterisk. C, Superdex-200 gel filtration elution profile of a mixture of 200 μg of CpSufE and 200 μg of CpNifS. D, separation of CpSufE and CpNifS proteins and mixtures of CpSufE-CpNifS complexes on a 10% native PAGE gel. Proteins were visualized by staining with Coomassie Brilliant Blue.
proteins associated with it. The cleaved untagged CpSufE, which does not bind to the column by itself, and CpNifS were found to co-elute with His-tagged WT CpSufE, cleaved CpSufE (or CpSufEC65S), and CpNifS. The mixture of the three proteins was loaded and eluted from a Ni²⁺-nitrilotriacetic acid column as described under "Materials and Methods." The 1 M imidazole eluates were separated on a 12.5% SDS-polyacrylamide gel followed by staining with Coomassie Brilliant Blue. Purified His-tagged CpSufE, CpNifS, and mutant proteins were run on the same gel as controls. B, Ni²⁺-nitrilotriacetic acid column coelution of His-tagged CpSufE, cleaved CpSufE (or CpSufEC65S), and CpNifS. The mixture of the three proteins was loaded and eluted from a Ni²⁺-nitrilotriacetic acid column as described under "Materials and Methods." The 1 M imidazole eluates were separated on a 15% SDS-polyacrylamide gel. Group I, His-tagged CpSufE, cleaved WT CpSufE, and CpNifS. Group II, His-tagged CpSufE, cleaved CpSufEC65S, mutant, and CpNifS. C, a model for explaining the presence of cleaved CpSufE in the 1 M imidazole eluate as shown in B. E, CpSufE; S, CpNifS; His, His tag. The His-tagged CpSufE dimer and the cleaved CpSufE dimer split into monomers. One His-tagged CpSufE monomer and one cleaved CpSufE monomer both bind to a CpNifS dimer, forming a heterotetrameric complex that binds to the nickel column. D, a model for the formation of CpSufE-CpNifS complexes. A CpSufE dimer splits into two monomers. One of the CpSufE monomers binds to a dimeric CpNifS, leading to a trimeric NifS₂-SufE complex, which can acquire another CpSufE monomer, resulting in a tetrameric complex.

FIGURE 8. Separation of CpSufE-CpNifS complexes on a Ni²⁺-nitrilotriacetic acid column. A, Ni²⁺-nitrilotriacetic acid column coelution of His-tagged CpSufE and CpNifS. 100 µg His-tagged CpSufE (WT or mutant) and 100 µg of CpNifS (WT or mutant) were mixed and loaded on a 0.5-ml Ni-IDA-gelose column, followed by a series of washes as described under "Materials and Methods." The 1 M imidazole eluates were collected and separated on a 12.5% SDS-polyacrylamide gel followed by staining with Coomassie Brilliant Blue. Purified His-tagged CpSufE, CpNifS, and mutant proteins were run on the same gel as controls. B, Ni²⁺-nitrilotriacetic acid column coelution of His-tagged CpSufE, cleaved CpSufE (or CpSufEC65S), and CpNifS. The mixture of the three proteins was loaded and eluted from a Ni²⁺-nitrilotriacetic acid column as described under "Materials and Methods." The 1 M imidazole eluates were separated on a 15% SDS-polyacrylamide gel. Group I, His-tagged CpSufE, cleaved WT CpSufE, and CpNifS. Group II, His-tagged CpSufE, cleaved CpSufEC65S, mutant, and CpNifS. C, a model for explaining the presence of cleaved CpSufE in the 1 M imidazole eluate as shown in B. E, CpSufE; S, CpNifS; His, His tag. The His-tagged CpSufE dimer and the cleaved CpSufE dimer split into monomers. One His-tagged CpSufE monomer and one cleaved CpSufE monomer both bind to a CpNifS dimer, forming a heterotetrameric complex that binds to the nickel column. D, a model for the formation of CpSufE-CpNifS complexes. A CpSufE dimer splits into two monomers. One of the CpSufE monomers binds to a dimeric CpNifS, leading to a trimeric NifS₂-SufE complex, which can acquire another CpSufE monomer, resulting in a tetrameric complex.

Chloroplast Cysteine Desulfurase Activation

The Cys desulfurase activity of CpNifS is required for Fe-S formation in chloroplast stroma (27). However, purified CpNifS has only a very low level of Cys desulfurase activity by itself (18). In in vitro experiments, the newly identified chloroplast protein CpSufE interacts with CpNifS and stimulates the Cys desulfurase activity of CpNifS over 40-fold. Purified CpSufE enhances the Cys desulfurase activity of CpNifS by affecting both the substrate affinity and Vₘₐₓ. Indeed, the ratio of Vₘₐₓ/Kₘ was increased 100-fold by the addition of CpSufE to CpNifS. Furthermore, the CpNifS-dependent Fe-S reconstitution activity was found to be enhanced 20-fold by CpSufE. These findings provide at least a partial explanation for the 50–80-fold higher reconstitution ability of chloroplast stroma compared with CpNifS alone (27) and directly connect a SufE-like protein with Fe-S cluster formation. CpSufE and CpNifS should also be able to interact in vivo, because both proteins are localized in the stroma and have comparable expression patterns and expression levels. Thus, CpSufE appears to be an important regulator for both Cys desulfurase activity and Fe-S formation in plastids.

The SeCys lyase activity of CpNifS is not much affected by CpSufE. Therefore, plastids have the ability to decompose SeCys and thus prevent the specific and toxic incorporation of selenium in proteins, regardless of the activity of CpSufE. Indeed, overproduction of CpSufE (without CpSufE) provides a more effective strategy for increasing selenium tolerance (38). 

SufE proteins are evolutionarily conserved. Homologues are found in a wide variety of organisms, including prokaryotes and eukaryotes. All of these proteins have a conserved Cys residue that seems to function in 50–80-fold higher reconstitution ability of chloroplast stroma compared with CpNifS alone (27) and directly connect a SufE-like protein with Fe-S cluster formation. CpSufE and CpNifS should also be able to interact in vivo, because both proteins are localized in the stroma and have comparable expression patterns and expression levels. Thus, CpSufE appears to be an important regulator for both Cys desulfurase activity and Fe-S formation in plastids.

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SufE proteins are evolutionarily conserved. Homologues are found in a wide variety of organisms, including prokaryotes and eukaryotes. All of these proteins have a conserved Cys residue that seems to function in a sulfur transfer pathway, accepting the sulfur atom that is bound as
Chloroplast Cysteine Desulfurase Activation

sulfane sulfur to an active site Cys in a type II, SuF-like Cys desulfurase (29, 33). This mechanism seems to be conserved in the chloroplast, and the Cys residue of CpSufE was also found to be essential for efficient Cys desulfurase activity of CpNiFs. However, compared with bacterial proteins, the plant homologues have some unique properties. The Arabidopsis and rice SuF proteins have an extra C-terminal domain in their mature protein that shows good sequence similarity to E. coli BolA. The function of this BolA-like domain is at this point unclear. In E. coli, BolA is involved in the regulation of cell division in response to nutrition (34); however, the molecular mechanism by which BolA functions is not yet resolved. Recently, a comparison of data from genomic sequences, yeast-two-hybrid experiments, and three-dimensional structures, led to the hypothesis that the BolA protein may be a reductase interacting with a monothiol glutaredoxin (35). The bacterial BolA-glutaredoxin pair could be involved in defense against oxidative stress, targets of which are proteins or other organic compounds that are part of membranes or cell walls. The human glutaredoxin Grx2 was found to be an Fe-S protein probably functioning as a redox sensor (39). The 2Fe-25 cluster of holom-Grx2 is degraded under oxidative stress. The resulting apo-Grx2 becomes active, which causes a series of responses, including protein (de)glutathionylation, anti-apoptosis, and reduction of low molecular weight disulfides. This leads to the hypothesis that the CpSuF BolA domain may function as a redox sensor via interaction with a glutare- doxin. The Cys desulfurase activity of the CpNiFs-CpSuF complex and even the entire Fe-S biosynthetic machinery may thus be regulated in response to the redox state of the chloroplast stroma. In other studies, a glutaredoxin (Grx5) was reported to be directly required for Fe-S assembly in yeast and animals (40–42). A defect in Grx5 severely affected Fe-S cluster synthesis, but the wild type phenotype could be restored by overexpressing other components involved in Fe-S cluster biosynthesis. The function of Grx5 could be to regulate the redox state of important cysteine residues in mitochondrial Nfs1 and/or in the scaffold proteins (41). In analogy, it is therefore also feasible that a glutaredoxin interacting with the BolA domain of CpSuF is directly involved in chloroplastic Fe-S cluster biogenesis, by reducing cysteine residues in the active centers of CpNiFs, CpSuF, CplscA, or Nfu scaffolds. These recent findings raise interest in investigating the possible involvement of a glutaredoxin in plastidic Fe-S synthesis. However, the presence of multiple Grx candidates in chloroplasts adds complexity to addressing this question.

We found that CpSuF and CpNiFs interact dynamically in vitro, as judged from gel filtration, native PAGE, and affinity chromatography experiments. The two proteins may also interact in vivo in the chloro- plast stroma. When stromal proteins were separated by gel filtration and analyzed by immunoblotting, part of CpSuF (5–10%) eluted at a molecular mass of ~600 kDa, whereas most of the CpSuF appeared to be in the dimeric form (results not shown). Earlier, CpNiFs also was found to elute in part at this molecular mass (27), as did the putative Fe-S scaffold protein CplscA (22). Thus, all three proteins may be part of the same complex in vivo. The observations that CpSuF is present mostly in dimeric form in the stroma and that maximum stimulation of CpNiFs requires a 5-fold excess and not just an equal amount of CpSuF both may be taken to indicate that the interaction is transient in vivo. Remarkably, the mutation of the conserved cysteine to serine in either protein still allows interaction of CpNiFs and CpSuF, as measured by chromatography coelution, indicating that other amino acids are responsible for the binding. Interestingly, when present in excess, the mutant CpSuFEC65S has a dominant negative effect on the activity of CpNiFs, competitively inhibiting the activation by WT CpSuF (Fig. 6). This experiment further confirms the concept that the binding and formation of a two-component Cys desulphurase is a prerequisite for CpNiFs activation by CpSuF. In contrast to the dominant negative effect of the CpSuF mutant, an excessive amount of mutant CpNiFsC358S did not prevent WT CpNiFs from being activated by WT CpSuF, possibly because the CpNiFsC358S has a lower ability to bind to CpSuF than the WT protein, as suggested by the native PAGE experi- ments. Together, these observations may be explained by the following proposed reaction mechanism. CpNiFs binds the substrate cysteine. CpSuF monomers then bind to dimeric CpNiFs, and the binding affinity is increased after CpNiFs has decomposed the substrate Cys and has a sulfane sulfur bound to its active site, something the CpNiFs mutant cannot do, and therefore it does not act dominant negative. After transfer of the sulfur to the cysteine of WT CpSuF, the protein interaction becomes again reversible for the WT proteins, and CpSuF can leave and deliver the sulfur to downstream targets, such as scaffolding pro- teins in vivo. In vitro, this acceptor could be the dithiothreitol in the reaction mixture. The mutant CpSuF cannot receive the sulfur from CpNiFs and therefore is not released as easily, resulting in the observed dominant negative effect.

Purified CpSuF was observed to be a dimer in solution (Figs. 4 and 7), yet our experiments indicate that CpSuF enters complexes with CpNiFs as a monomer. In this context, it may be noted that bacterial SuF was observed to be mainly monomeric in solution (43). The proposed changes in affinity and the dynamic behavior of CpSuF during the catalytic cycle imply that conformational changes would occur in CpSuF and possibly CpNiFs. The active site cysteine in bacterial SuF is located such that the bound sulfane sulfur would be shielded from solvent interactions (43). The structure of SuF implies that conformational changes must occur as SuF accepts sulfur from SuS/CpNiFs (43). In the homologous plastid protein, this arrangement may help protect the sulfur from interaction with reactive oxygen, thus helping make Fe-S formation possible in this oxygendic environment.

In addition to CpSuF, homologues have been found in Arabidopsis for all of the bacterial SuF proteins, and all are predicted to be present in plastids. Therefore, it seems that in the course of evolution, plants have retained the entire SuF machinery, including SuFABCDE, for chloro- plast Fe-S cluster assembly (for a review, see Ref. 5). CpNiFs is the SuF-like protein, which is a Cys desulphurase supplying sulfur for the Fe-S cluster formation. CpSuF is the SuF-like protein, which is a Cys desulfurase activator as reported in this paper. CplscA is a SuF-like scaffold protein where a transient 2Fe-2S cluster is assembled (22). A similar pattern of gene expression was found for these proteins, supporting the possibility that they may function in the same biochemical process of Fe-S assembly (this work) (22). Chloroplastic SuFB, SuFC, and SuFD are homologues of bacterial SuFB, SuFC, and SuFD, respectively. They form a complex, which displays an iron-stimulated ATPase activity (23–25). However, a direct role of CpSuFBCD in the Fe-S formation still needs to be demonstrated. The SuF system in bacteria is responsible for Fe-S formation under oxidative stress and iron limitation (12). This may explain why plants contain a SuF-like system for biosynthesis of Fe-S clusters in chloroplasts, since they are oxygen-producing compartments. It has been suggested that the sulfur in SuF may be somewhat shielded. Besides SuF-like proteins, chloroplasts also contain Nfu-like scaffolds and the HCF 101 protein for Fe-S cluster biosynthesis (19, 26), suggesting that the Fe-S cluster biogenesis machinery in chloroplasts is more complex than any single operon in bacteria. Because of the barrier formed by the envelope, Fe-S clusters synthesized outside plastids cannot be easily supplied to chloroplasts, which are self-sufficient for the synthesis of their Fe-S clusters. Since plant plastids require a variety of Fe-S cluster types and have at least 20 Fe-S proteins, there is perhaps a
requirement for multiple-protein components. For instance, Nfu1–3 and CpslscA may be alternative scaffolds either for assembling different types of Fe-S clusters or for different physiological conditions. Among these many components of the plastid Fe-S cluster biosynthesis machinery, CpsNifs and CpsSufE hold a central and apparently essential position. Analysis of T-DNA insertion knock-out lines for CpsSufE indicates that knock-out of CpsSufE is seed-lethal. Offspring from a heterozygous CpsSufE knock-out gave rise to seeds that showed 100% germination but a 2:1 heterozygous/wild type ratio, with a complete absence of homozygous knock-outs.

In conclusion, the new results presented here reveal a new key component of the iron-sulfur biogenesis machinery in plant chloroplasts. These results are of significance, since still very little is known about how chloroplast iron-sulfur clusters are made, despite their vital importance for photosynthesis and assimilatory nitrogen and sulfur assimilation. Photosynthesis is the process that determines plant productivity and ultimately drives life on earth. Its importance for biology cannot be overstated. Also, plant iron status is of importance for human health, since billions of people worldwide are suffering from iron deficiency, and most of the dietary iron in developing countries is derived from vegetarian food sources.

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