The Essential Cytosolic Iron-Sulfur Protein Nbp35 Acts without Cfd1 Partner in the Green Lineage*

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In photosynthetic eukaryotes assembly components of iron-sulfur (Fe-S) cofactors have been studied in plastids and mitochondria, but how cytosolic and nuclear Fe-S cluster proteins are assembled is not known. We have characterized a plant P loop NTPase with sequence similarity to Nbp35 of yeast and mammals, a protein of the cytosolic Cfd1-Nbp35 complex mediating Fe-S cluster assembly. Genome analysis revealed that NBP35 is conserved in the green lineage but that CFD1 is absent. Moreover, plant and algal NBP35 proteins lack the characteristic CXXC motif in the C terminus, thought to be required for Fe-S cluster binding. Nevertheless, chemical reconstitution and spectroscopy showed that Arabidopsis (At) NBP35 bound a [4Fe-4S] cluster in the C terminus as well as a stable [4Fe-4S] cluster in the N terminus. Holo-AtNBP35 was able to transfer an Fe-S cluster to an apoprotein in vitro. When expressed in yeast, AtNBP35 bound 55Fe dependent on the cysteine desulphurase Nfs1 and was able to partially rescue the growth of a cfd1 mutant but not of an nbp35 mutant. The AtNBP35 gene is constitutively expressed in planta, and its disruption was associated with an arrest of embryo development. These results show that despite considerable divergence from the yeast Cfd1-Nbp35 Fe-S scaffold complex, AtNBP35 has retained similar Fe-S cluster binding and transfer properties and performs an essential function.

Proteins carrying iron-sulfur (Fe-S) clusters as cofactors are common in virtually all life forms. Fe-S proteins catalyze crucial steps in fundamental processes, including nitrogen fixation, respiration, photosynthesis, various metabolic pathways, and regulation of gene expression (1). The most common types of Fe-S clusters are the [2Fe-2S] and the cubane [4Fe-4S] form. Although Fe-S clusters can be assembled by chemical means, dedicated proteins for in vivo Fe-S protein maturation have been discovered over the past 10 years (2, 3). In plants assembly proteins have been localized to the plastids and mitochondria, the endosymbiotic organelles where photosynthesis and respiration take place, respectively (for review, see Refs. 4–6). The plastids contain all six proteins of the so-called SUF system plus NFU-type scaffolds. The mitochondrial matrix contains the ISC3 system (for iron-sulfur cluster assembly). Both systems appear to follow the same biochemical steps; (i) generation of persulfide by a cysteine desulphurase (the Nfs1-Isd11 complex in mitochondria; Cpnifs in plastids), (ii) transfer of persulfide and combination with iron to form an Fe-S cluster on a scaffold protein (ISU proteins in mitochondria; NFU2 in plastids), (iii) transfer of the Fe-S cluster from the scaffold protein to a target protein (mediated by chaperones in the ISC system).

Important Fe-S enzymes are also found in the cytosol and nucleus (3, 4). In plants, these include DNA repair enzymes, RNA polymerase I and III (7), xanthine dehydrogenase, abscisic aldehyde oxidase (AAO3), and cytosolic aconitase. How these proteins obtain their Fe-S clusters is currently not known. By analogy with yeast, it is presumed that the mitochondria are required for cytosolic Fe-S cluster assembly, but supporting experimental evidence has not yet been provided. Bioinformatic analyses have revealed that sequence relatives of the yeast CIA proteins (for cytosolic iron-sulfur cluster assembly) are also present in the genomes of the model plant species Arabidopsis thaliana and the green alga Chlamydomonas reinhardtii (4, 8). In yeast and humans the CIA machinery includes two P loop NTPases, Cfd1 and Nbp35, which form a heterotetrameric complex and function as Fe-S scaffolds, mediating the de novo assembly of an Fe-S cluster and its transfer to target apoproteins (9–12).

Cfd1 and Nbp35 are classified as Mrp-like proteins that belong to the Mrp/NBP35 subfamily of P loop NTPases (13). Although Cfd1 and Nbp35 share 49% sequence similarity, they can be distinguished as different types based on their N-terminal domains (Fig. 1B). A third type of Mrp-like protein, HCF101, was characterized in plastids and is involved in the assembly of Photosystem I, which has three [4Fe-4S] clusters (14, 15). Recently, we have identified a fourth type of eukaryotic Mrp-like protein associated with Fe-S protein assembly. This protein, named InD1, is localized in the mitochondria and is required for the effective assembly of respiratory Complex I, which contains eight Fe-S clusters, in the yeast Yarrowia lipolytica (16).

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§ The abbreviations used are: ISC, iron-sulfur cluster (assembly); At, Arabidopsis thaliana; CIA, cytosolic iron-sulfur cluster assembly; FL, full-length; GFP, green fluorescent protein; ΔN, deletion of N-terminal domain; NBP, nucleotide binding protein; Sc, S. cerevisiae.
Cytosolic Iron-Sulfur Protein Assembly in Arabidopsis

To initiate studies on Fe-S protein assembly in the plant cytosol and analyze how this process may depend on the organelar assembly systems, we investigated two uncharacterized Mrp-like proteins in Arabidopsis. Here we show that NBP35 (gene locus At5g50960) is essential for embryo development and is localized in the cytosol, whereas the gene product of At4g19540, designated NBP35, was localized in the mitochondria. AtNBP35 bound [4Fe-4S] clusters that were readily transferred to an apoprotein in vitro, consistent with an Fe-S scaffold function.

EXPERIMENTAL PROCEDURES

Plant Material and Yeast Strains—The A. thaliana lines, SALK_054678 and SALK_056204, were obtained from the Nottingham Arabidopsis Stock Centre. The presence and position of the T-DNA insert in the NBP35 gene (At5g50960) was confirmed by PCR. Segregation analysis showed that the T-DNA of the T-DNA insert in the At5g50960 gene (At5g50960) was confirmed by PCR. Segregation analysis showed that the T-DNA insert in the

FIGURE 1. Plant and algal genomes contain NBP35 but not CFD1. A, phylogenetic analysis of Mrp-like proteins in eukaryotes. Protein sequences containing the Mrp family signature in the model plant A. thaliana (At), the green alga Ostreococcus lucimarinus (Ol), the fungi S. cerevisiae (Sc), and V. lipolytica (Vl) and man (Hs) were aligned with T-COFFEE. Tree building and tree rendering were performed with PhyML and TreeDyn, respectively, based on the central portion of the proteins (including the Walker AB motifs and the Mrp domain). E. coli (Ec) Mrp served as an out-group. B, diagrams of the Mrp-like proteins showing domain structure and conserved motifs. Plant/algal NBP35 proteins have a C-terminal insertion of conserved sequence not found in other groups (wave pattern). AB, Walker A and B motifs; Mrp, Mrp family signature (InterPro IPR000808); C, (semi)conserved cysteine residues; MTS, mitochondrial targeting signal; cTP, chloroplast transit peptide; DUF59, domain of unknown function 59 (InterPro IPR002744).

In Vitro Fe-S Analysis—The full-length (FL) AtNBP35 coding sequence or a version lacking the first 54 codons (ΔN) was isolated from wild-type Arabidopsis cDNA and cloned into the pet15b expression vector (Novagen) in-frame with an N-terminal His tag. Protein overexpression and affinity purification were carried out following the manufacturer’s manuals. Chemical reconstitution of the Fe-S clusters was carried out in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI). In a typical experiment, 20–30 μM protein in 25 mM Tris-HCl, 150 mM NaCl, pH 8.0 (buffer A), was treated with 10 mM dithiothreitol (final concentration) at 15 °C for 1 h. The reconstitution was started by the addition of 10 mol eq of ferric ammonium citrate and Li2S followed by incubation at 15 °C for 3 h. To remove non-bound iron and sulfide, reconstituted AtNBP35 was passed through a Sephadex G25 gel filtration column using buffer A containing 1 mM dithiorthioleot (final concentration) at 15 °C for 1 h. The reconstitution was started by the addition of 10 mol eq of ferric ammonium citrate and Li2S followed by incubation at 15 °C for 3 h. To remove non-bound iron and sulfide, reconstituted AtNBP35 was passed through a Sephadex G25 gel filtration column using buffer A containing 1 mM dithiorthioleot (final concentration) at 15 °C for 1 h. The reconstitution was started by the addition of 10 mol eq of ferric ammonium citrate and Li2S followed by incubation at 15 °C for 3 h. To remove non-bound iron and sulfide, reconstituted AtNBP35 was passed through a Sephadex G25 gel filtration column using buffer A containing 1 mM dithiorthioleot (final concentration) at 15 °C for 1 h. The reconstitution was started by the addition of 10 mol eq of ferric ammonium citrate and Li2S followed by incubation at 15 °C for 3 h. To remove non-bound iron and sulfide, reconstituted AtNBP35 was passed through a Sephadex G25 gel filtration column using buffer A containing 1 mM dithiorthioleot (final concentration) at 15 °C for 1 h. The reconstitution was started by the addition of 10 mol eq of ferric ammonium citrate and Li2S followed by incubation at 15 °C for 3 h. To remove non-bound iron and sulfide, reconstituted AtNBP35 was passed through a Sephadex G25 gel filtration column using buffer A containing 1 mM dithiorthioleot (final concentration) at 15 °C for 1 h.
tein using vector pOL-GFP-S65C (27) and radiolabeling of yeast cells, protein extraction, and immunoprecipitation (18). Error bars represent the S.D. value.

RESULTS

NBP35 but Not CFD1 Is Conserved in the Green Plant Lineage—With the availability of recently sequenced genomes of plants and algae, we investigated the conservation of Mrp-like proteins and their domains in the green lineage. Three separate coding sequences for proteins with a Mrp family signature were usually found: one with a putative mitochondrial targeting sequence, designated INDL, the plastid HCF101, characterized by an N-terminal DUFS domain, and an NBP35 homolog with an N-terminal Fe-S cluster domain (Fig. 1). No other sequence relative that could correspond to yeast Cfd1 was found in any of the six plant, one moss, and four green algal genomes analyzed. In addition, the predicted NBP35 proteins in the green lineage differed from those in the fungi/Metazoa group in the C-terminal region after the highly conserved VENMS motif (Fig. 1B and supplemental Fig. S1). Most notably, the C-terminal CXXC motif, which is critical for Fe-S cluster binding to Cfd1 and Nbp35 in yeast,1 is absent from plant and algal NBP35 proteins. Instead, a ~35- amino acid insertion is found that is conserved in all Viridiplantae genomes investigated so far but that has no sequence similarity to other known protein motifs. As a consequence, only one C-terminal cysteine residue is found in all NBP35 proteins: Cys-234 in S. cerevisiae numbering, corresponding to Cys-220 in Arabidopsis NBP35. However, a number of semiconserved cysteine residues are found in the C-terminal 70-amino acid sequence of plant and algal NBP35 proteins. The absence of CFD1 genes in the green lineage suggests that the NBP35 protein may function as a homo-oligomer in the cytosol without a CFD1-like partner.

AtNBP35 Is a Soluble Cytosolic Protein—To determine the cellular localization of NBP35 (At5g50960) in the model plant Arabidopsis, the full coding sequence isolated from cDNA was fused in-frame to a C-terminal GFP sequence and transiently expressed using particle bombardment of Arabidopsis leaves. GFP fluorescence was found in the cytoplasm, which is appressed to the plasma membrane in the highly vacuolate, jigsaw-shaped epidermal cells (Fig. 2, A and B). Green fluorescence was also observed in the nucleus, which, however, may be due to diffusion, a recurrent problem in determining the localization of nuclear proteins as GFP fusions (28, 29). We, therefore, investigated the localization of endogenous AtNBP35 using cell fractionation and immunoblotting. Antibodies specific to AtNBP35 (see supplemental Fig. S2) recognized an ~37-kDa protein in total plant extracts (Fig. 2C), in agreement with the predicted molecular mass of 37,306 Da. The AtNBP35 protein was found in the soluble, cytosolic fraction but not in purified nuclei, mitochondria, or chloroplasts. We also investigated the localization of the INDL protein (At4g19540) using specific antibodies and obtained a signal in the purified mitochondrial fraction only, similar to the pattern observed for ISU1 (Fig. 2C). Note that these two proteins are not or are barely detectable in total extract due to their low abundance and the relatively minor contribution of mitochondria to total protein in plant extract. These data show that AtNBP35 is a soluble cytosolic protein that is spatially separated from the homologous AtINDL protein in the mitochondria.

Both the N- and C-terminal Domains of AtNBP35 Bind Fe-S Clusters—Because the characteristic C-terminal CXXC motif of the Cfd1/Nbp35 scaffold proteins is missing in plant and algal NBP35 proteins, it was of interest to analyze the capacity of the C-terminal domain to bind an Fe-S cluster. To this end, AtNBP35 was overproduced in Escherichia coli, either FL or lacking the N-terminal Fe-S cluster binding motif (ΔN). The proteins were purified by His-tag affinity purification (Fig. 3A, inset). The FL protein appeared brown in color, whereas the ΔN

FIGURE 2. Arabidopsis NBP35 is localized in the cytosol. A and B, the fusion gene AtNBP35-GFP was placed under the control of the 35S promoter and transiently expressed in Arabidopsis leaves using particle bombardment. Images of epidermal cells expressing GFP were obtained by confocal microscopy. C, immunolocalization of NBP35 and INDL in Arabidopsis cell fractions. Total proteins (Tot), cytosolic fraction (Cyt), and purified nuclei (Nuc), mitochondria (Mit), or chloroplasts (Chl) were separated by SDS-PAGE (30 μg of protein per lane) and labeled with specific antibodies. The purity of the cell fractions was verified with antibodies against the following marker proteins: mitochondrial Fe-S scaffold protein (ISU1), Rieske protein of the cytochrome f complex in chloroplasts (these organelles are not developed in cell culture; therefore, no signal is seen in total protein extract) (PETC), actin (cytosol/nuclear envelope) and histone 3 (nucleus).

1 D. J. A. Netz, unpublished information.
protein was virtually colorless except for a light yellow hue. UV-visible spectroscopy of the FL protein showed absorbance peaks at 320 and 415 nm, which were more than halved upon addition of dithionite (Fig. 3A). These spectral features are characteristic of a [4Fe-4S] cluster (30); however, the intensity of the signal indicated that only a minor fraction of the molecules contained a cluster (11). The UV-visible spectrum of ΔN AtNBP35 (as isolated) did not have a pronounced absorbance. We, therefore, incubated both FL and ΔN protein with excess iron and sulfide under reductive, anaerobic conditions to chemically reconstitute the Fe-S clusters. Quantification of protein-bound Fe and sulfide showed that FL AtNBP35 bound 5.7 ± 1.2 iron and 5.1 ± 0.8 acid-labile sulfide per polypeptide, whereas the ΔN protein bound 1.8 ± 0.7 iron and 1.7 ± 0.1 acid-labile sulfide per polypeptide (Fig. 3B, inset). Subtraction of these numbers indicate that the N-terminal domain bound a [4Fe-4S] cluster. The C terminus may bind either a [2Fe-2S] cluster or a [4Fe-4S] cluster bridging two polypeptides, assuming complete reconstitution in our samples. The second possibility is supported by the UV-visible spectrum of reconstituted ΔN AtNBP35 (Fig. 3B), which is typical of a cubane cluster. Further support for a cubane cluster was obtained by EPR spectroscopy, showing attenuation of the EPR signal of the reduced protein above 40 K (supplemental Fig. S3).

The EPR spectrum of FL AtNBP35 after reduction with dithionite is rhombic and is dominated by the sharp signal of the N-terminal cluster, with g values of 2.051, 1.928, and 1.86 (Fig. 3C). In contrast, FL yeast Nbp35 and its human counterpart huNbp35 have signals of 1.1 and 1.2. Comparison of the EPR signals of Arabidopsis NBP35, chemically reduced with 2 mm sodium dithionite, with the yeast and human Nbp35 proteins. EPR conditions: temperature, 10 K; microwave power, 2 milliwatts; microwave frequency, 9.459 GHz; modulation frequency, 100 kHz; modulation amplitude, 1.25 millitesla (mT). D, Mössbauer spectrum recorded at 80 K (no applied field) of reconstituted FL AtNBP35. The lines are the simulated quadrupole doublets with parameters (δ, ΔE, full width at half-maximum, all in mm/s): 0.43, 1.20, and 0.42 (85% of total intensity) and 0.34, 0.65, and 0.44 (15% of total intensity). The solid line is the sum of the simulated components. E, gel filtration of AtNBP35. His-tagged AtNBP35 (39.5 kDa) eluted in two peaks from a Superdex 200 column (GE Healthcare), corresponding to a dimeric form (calculated molecular mass of 98 ± 10 kDa) and a tetrameric form (188 ± 17 kDa).
mixed-valent \((Fe^{2.5+} - Fe^{2.5+})\) pairs in exclusively cysteine-coordinated \([4Fe-4S]^{2-}\) clusters (31). Because of the instability of \(\Delta N\) AtNBP35 upon concentration of protein for Mössbauer spectroscopy, the individual isomer shift and quadrupole splitting parameters of N- and C-terminal clusters could not be determined.

Gel filtration analysis of recombinant as isolated FL AtNBP35 showed that most of the protein occurred as a dimer, with some forming a tetramer (Fig. 3E). The chromatographic behavior of \(\Delta N\) AtNBP35 or the reconstituted proteins could not be analyzed due to instability during the gel filtration procedure. Taken together, these data indicate that AtNBP35 occurs as a homo-oligomer with a stable \([4Fe-4S]\) cluster in the N-terminal domain of each subunit and a C-terminal \([4Fe-4S]\) cluster shared between two protomers.

**Holo-AtNBP35 Can Transfer an Fe-S Cluster**—Next we investigated whether the *Arabidopsis* NBP35 protein can act as an Fe-S scaffold in that it can transfer an Fe-S cluster to a potential target protein *in vitro*. Following a previously described assay (11), chemically reconstituted and desalted FL AtNBP35 was added to apoLeu1, the apoform of yeast isopropylmalate isomerase. Cluster transfer was followed by the generation of enzymatic activity upon conversion of apoLeu1 to the \([4Fe-4S]\)-containing form. Cluster transfer to Leu1 occurred at a rate and efficiency comparable with that observed for yeast Nbp35 or the Cfd1-Nbp35 complex (11). At the earliest time point that we were able to measure (1.5 min), more than 80% of the maximal Leu1 activity was obtained. In contrast, chemical reconstitution with the same amounts of ferric ion and sulfide hardly led to activation of apoLeu1 (Fig. 4). The addition of 1 mM ATP/GTP (plus MgCl\(_2\)) had no effect on cluster transfer, as has been noted for ApbC (40) and Cfd1/Nbp35.4 These *in vitro* data suggest that a labile Fe-S cluster of AtNBP35 can be donated to a target Fe-S protein.

**AtNBP35 Binds Fe-S Clusters in Vivo but Cannot Replace Yeast NBP35**—To analyze the physiological relevance of the *in vitro* data, we expressed AtNBP35 in yeast cells (*S. cerevisiae, Sc*). First, we confirmed that the AtNBP35 protein was localized in the yeast cytosol (Fig. 5A). Second, we investigated whether Fe-S clusters were assembled on AtNBP35 by the yeast Fe-S cluster assembly machinery. For this we made use of a mutant, Gal-NFS1, containing a galactose-regulatable NFS1 gene encoding the only cysteine desulfurase in yeast. Yeast cells harboring an empty plasmid or a plasmid overexpressing AtNBP35 were used to prepare a cell extract (Ex) for immunoprecipitation with anti-AtNBP35 agarose beads (IP). Equal amounts of extract and precipitated protein were separated by SDS-PAGE and stained with Coomassie. Hc, heavy chain; Lc, light chain. The arrow indicates immunoprecipitated AtNBP35, C, \(^{55}\)Fe labeling and immunoprecipitation of AtNBP35 in wild-type and Gal-NFS1 yeast cells. Cells overproducing AtNBP35 were grown in the presence of glucose to down-regulate expression of NFS1, the gene for cysteine desulfurase. The \(^{55}\)Fe associated with AtNBP35 was measured by scintillation counting. The amounts of AtNBP35 and Nfs1 were visualized by immunoblotting (lower panels).
significant amount of $^{55}\text{Fe}$ (10 times over background) was associated with immunoprecipitated AtNBP35 (Fig. 5, B and C). Down-regulation of NFS1 expression led to a near loss of $^{55}\text{Fe}$ bound to AtNBP35 (Fig. 5C, last bar). The Nfs1-dependent $^{55}\text{Fe}$ binding to AtNBP35 indicates that the iron was part of an Fe-S cluster.

Next, we investigated whether AtNBP35 can replace the function of yeast Nbp35 using cells carrying a temperature-sensitive mutant copy of the endogenous NBP35 gene (32, 33). Upon shifting the cells to 37 °C, the mutant ScNbp35 protein becomes non-functional, leading to growth arrest after a few cell duplications. The growth of the temperature-shifted nbp35 mutant was rescued by expression of wild-type ScNBP35 from a plasmid but not by Arabidopsis NBP35 (Fig. 6). We then tested whether AtNBP35 could rescue a temperature-sensitive cfd1 mutant (33). Surprisingly, expression of AtNBP35 enabled slow growth of temperature-sensitive cfd1 mutant cells shifted to 37 °C. We also tested the functional requirement of the N-terminal domain of AtNBP35 for yeast complementation, as yeast Cfd1 lacks this domain. Truncated ΔN AtNBP35 did not complement the temperature-sensitive cfd1 mutant, although the protein was stably expressed (supplemental Fig. S2). Similar results were obtained with galactose-regulatable mutants of NBP35 and CFD1 (not shown). In summary, AtNBP35 is able to acquire Fe-S cofactors in the yeast cytosol but cannot substitute for the endogenous yeast Nbp35. In contrast, AtNBP35 can partially replace the function of Cfd1 in yeast.

AtNBP35 Is Ubiquitously Expressed and Essential for Embryo Development—To assess the role of NBP35 in Arabidopsis, we investigated the expression of AtNBP35 and analyzed available insertion mutants. Immunoblot analysis of vegetative and reproductive organs showed that AtNBP35 is ubiquitously expressed (Fig. 7A). This is in agreement with microarray data from the GeneVestigator data base (supplemental Fig. S4).

Comparison of the signal intensities in lanes with cell extract and known amounts of purified protein indicated that the abundance of AtNBP35 is less than 0.005% of SDS-extractable protein.

Two plant lines with insertions in the AtNBP35 gene were obtained from the Arabidopsis stock center. SALK_054678 contains a T-DNA insert in the 5′-untranslated region, and SALK_056204 contains a T-DNA insert at the 3′ end of the first intron (Fig. 7B). Homozygous plants were bred from the SALK_054678 line, but they had no detectable phenotype. Reverse transcription PCR using primers in the 5′- and 3′-untranslated region confirmed the absence of full-length NBP35 transcript (not shown). However, PCR products covering the start and end of the coding sequence were as abun-
Cytosolic Iron-Sulfur Protein Assembly in Arabidopsis

In plants and algae, Fe-S proteins are abundant in four cell compartments: plastids, mitochondria, cytosol, and nucleus. Biogenesis of Fe-S proteins in the latter two compartments has not been investigated in photosynthetic organisms despite the occurrence of unique and important Fe-S proteins. It has been proposed that in mammals alternative splicing mechanisms provide small amounts of ISC protein isoforms in the cytosol.

Another marked difference between plant/algal and the fungal/metazoan NBP35 proteins is the absence of the C-terminal C motif, which is replaced by an H11011/H11012 cluster which remain to be determined by site-directed mutagenesis. The presence of the C-terminal cluster is not required for dimer formation, as the as-isolated FL protein was able to form dimers (Fig. 3, A and B).

To initiate the functional analysis of AtNBP35, we performed an in vitro cluster transfer assay and complementation studies with yeast nbp35 and cfd1 mutants. Although the in vitro assay suggested similar cluster transfer properties of AtNBP35 and ScNbp35 (Fig. 4 and Ref. 11), expression of AtNBP35 could not rescue the growth defect of ScNbp35-depleted cells (Fig. 6). The reason for the lack of complementation is not known; Fe-S cluster assembly on AtNBP35 could be demonstrated in yeast, and control experiments confirmed stable expression of AtNBP35 in the yeast cytosol (Fig. 5). Surprisingly, the growth of ScCfd1-depleted cells was restored albeit partially by expression of FL AtNBP35 but not without the N-terminal domain.

Our studies presented here will be fundamental for further studies to unravel the precise molecular function of AtNBP35. Because we have shown that AtNBP35 is an essential gene (Fig. 7), embryo lethality has to be circumvented by the construction of partial or regulatable knock-down mutants, which is in progress in our laboratory. Moreover, it will be interesting to investigate the cellular role of other CIA homologs in plants, including AtNARI (41) and AtCIA1 (42), and to study their protein interactions with AtNBP35. The presence of NBP35 as a homologomer in the plant cytosol rather than the Cfd1-Nbhp35 complex shows that plants provide an insightful model system for Fe-S protein biogenesis and the compartmentalization of this process in eukaryotic cells.

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