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### Chloroplast HCF101 is a scaffold protein for [4Fe-4S] cluster assembly

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Oxygen-evolving chloroplasts possess their own iron-sulfur cluster assembly proteins including members of the SUF (sulfur mobilization) and the NFU family. Recently, the chloroplast protein HCF101 (high chlorophyll fluorescence 101) has been shown to be essential for the accumulation of the membrane complex Photosystem I and the soluble ferredoxin-thioredoxin reductases, both containing [4Fe-4S] clusters. The protein belongs to the FSC-NTPase ([4Fe-4S]-cluster-containing P-loop NTPase) superfamily, several members of which play a crucial role in Fe/S cluster biosynthesis. Although the C-terminal ISC-binding site, conserved in other members of the FSC-NTPase family, is not present in chloroplast HCF101 homologues using Mössbauer and EPR spectroscopy, we provide evidence that HCF101 binds a [4Fe-4S] cluster. <sup>55</sup>Fe incorporation studies of mitochondrially targeted HCF101 in *Saccharomyces cerevisiae* 

#### INTRODUCTION

Fe/S clusters (iron-sulfur clusters) are ancient cofactors of proteins required for various essential processes. As Fe/S clusters are able to transfer electrons and act as a catalyst they are important players in processes such as photosynthesis and respiration, nitrogen and sulfur metabolism, redox regulation and sensing, as well as gene expression and hence they are indispensable prosthetic groups of many proteins [1-3]. The biogenesis of such clusters from elemental iron and sulfur is an enzymatic process and requires a set of specialized proteins. As a first step sulfur is mobilized from the sulfhydryl group of cysteine, catalysed by a cysteine desulfurase, and elemental iron is relocated from cellular stores [4]. Synthesis of the Fe/S cluster takes place on specialized scaffold proteins, which assemble transiently bound clusters before their transfer to apoproteins. The components responsible for iron-sulfur protein maturation have been mostly characterized in bacteria and yeast and different systems for Fe/S cluster biogenesis have been identified. The Fe/S cluster assembly machinery is present in many bacteria and mitochondria [5,6]. The SUF (sulfur mobilization) system is active under iron-limiting and oxidative stress situations in bacteria and is also found in plastids [7]. The NIF machinery is dedicated to the assembly of nitrogenase in nitrogen-fixing bacteria [8]. In eukaryotes the mitochondrial Fe/S cluster machinery not only supports the organellular demand for the cofactor but is also required for the assembly of cytosolic and nuclear iron-sulfur proteins.

confirmed the assembly of an Fe/S cluster in HCF101 in an Nfs1-dependent manner. Site-directed mutagenesis identified three HCF101-specific cysteine residues required for assembly and/or stability of the cluster. We further demonstrate that the reconstituted cluster is transiently bound and can be transferred from HCF101 to a [4Fe-4S] apoprotein. Together, our findings suggest that HCF101 may serve as a chloroplast scaffold protein that specifically assembles [4Fe-4S] clusters and transfers them to the chloroplast membrane and soluble target proteins.

Key words: [4Fe-4S]-cluster-containing P-loop NTPase (FSC-NTPase), *Arabidopsis thaliana*, chloroplast, high chlorophyll fluorescence 101 (HCF101), iron-sulfur cluster assembly, scaffold protein.

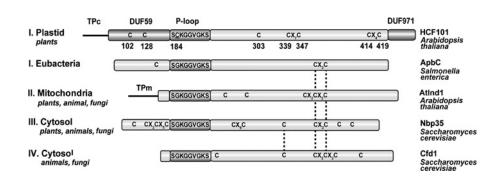
Additionally, proteins of the CIA (cytosolic ironsulfur protein assembly) machinery are essential for Fe/S cluster formation [9,10].

Several Fe/S cluster-containing proteins in chloroplasts need a specialized set of proteins for Fe/S cluster synthesis as the oxygen-sensitive biosynthesis process takes place within an organelle with a high oxygen concentration. Some of the participating proteins have been identified [11–14]. A candidate for sulfur mobilization in chloroplasts, CpNifS, has been shown to act as a cysteine desulfurase in vitro and in vivo [15,16]. This process seems to be stimulated by CpSufE [17]. For the second step in Fe/S cluster assembly the plastid scaffold protein NFU2 [NFU proteins are related to the C-terminal domain of NifU (nitrogen fixation, subunit U)] was demonstrated to carry a [2Fe-2S] cluster, which can be transferred to ferredoxin. As levels of PSI (Photosystem I) are decreased in mutant plants, NFU2 is proposed to be also involved in the assembly of [4Fe-4S] proteins, although the mechanism of maturation remains unclear [18,19]. Two further plastid scaffold proteins that carry a transient [2Fe-2S] cluster have been identified, CpIscA and two glutaredoxins, GrxS14 and GrxS16, all of which were able to assemble and transfer [2Fe-2S] clusters in vitro [20,21]. An IscA homologue in Synechocystis sp., has been shown to assemble a [2Fe-2S] cluster and to reactivate apo-adenylyl sulfate reductase, which requires a [4Fe-4S] cluster for activity [22]. However, to date, plastid candidates, which specifically assemble and transfer [4Fe-4S] clusters have not been described.

Abbreviations used: APO1, accumulation of Photosystem I 1; Cfd1, cytosolic iron-sulfur cluster deficient 1; DTT, dithiothreitol; DUF, domain of unknown function; Fe/S, iron-sulfur; FSC-NTPase, [4Fe-4S]-cluster-containing P-loop NTPase; FTR, ferredoxin-thioredoxin reductase; HCF101, high chlorophyll fluorescence 101; Ind1, iron-sulfur protein required for NADH dehydrogenase 1; IPTG, isopropyl β-D-thiogalactoside; ISC, iron-sulfur cluster; Nbp35, nucleotide-binding protein 35; NFS1, NifS-like; NIF, nitrogen fixation; PSI, Photosystem I; SUF, sulfur mobilization; WT, wild-type.

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#### Figure 1 Schematic alignment of cysteine residues in members of the FSC-NTPase superfamily in eukaryotes and eubacteria

The plastid (class I) and mitochondrial (class II) forms in *Arabidopsis* (HCF101 and AtInd1 respectively), the cytosolic class III and IV homologues (Npb35 and Cfd1 respectively) in yeast and the class I eubacterial homologue ApbC in *Salmonella enterica* are represented. Cysteine residues indicated with broken lines are highly conserved within the four classes, but are not present in the chloroplast HCF101 protein. TPm, mitochondrial transit peptide; TPc chloroplast transit peptide.

Apart from PSI, which contains three [4Fe-4S] clusters, a number of other chloroplast enzymes depend upon this cofactor, such as the heterodimeric FTR (ferredoxin-thioredoxin reductase) complex, the nitrite and sulfite reductases, and presumably the NAD(P)H dehydrogenase. Two plastid proteins, HCF101 (high chlorophyll fluorescence 101) and APO1 (accumulation of PSI), have been genetically described as essential and specific factors for assembly of [4Fe-4S]-cluster-containing protein complexes, although the molecular mechanism remained unclear [11,23]. Whereas APO1 function interferes with translation of PSI transcripts psaA and psaB, HCF101 exerts its function on a post-translational step [23]. In addition to the lack of PSI, the HCF101 mutants accumulate less heterodimeric FTR complex, whereas levels of [2Fe-2S]-cluster-containing proteins are not affected [23]. APO1 is only conserved among higher plants [11], whereas HCF101 belongs to the ancient and ubiquitously distributed protein family of soluble FSC-NTPases ([4Fe-4S]cluster-containing P-loop NTPases) [23,24]. Four classes of P-loop FSC-NTPases involved in Fe/S cluster biogenesis have been defined, members of which share a highly conserved protein signature and represent bacterial, plastid, mitochondrial and cytoplasmic proteins [23].

Plastid homologues and eubacterial forms with an N-terminal DUF59 [domain of unknown function 59; Pfam number PF01883 (http://pfam.sanger.ac.uk/)] domain belong to FSC-NTPase class I. The mature HCF101 protein, without the predicted transit peptide, contains an N-terminal DUF59 domain and eight cysteine residues along the sequence. All cysteine residues are conserved among higher plants but of the two cysteine residues located in the DUF59 domain only Cys<sup>128</sup> is highly conserved (see Supplementary Figure S1 available at http://www.BiochemJ.org/bj/425/bj4250207add.htm). The eubacterial representatives of class I can be divided further into subclasses, depending on the presence of the DUF59 domain and the positions of the cysteine residues. Of the eubacterial FSC-NTPase proteins, ApbC in Salmonella enterica is required for the maturation of the Fe/S cluster proteins present in the thiamine biosynthetic pathway [25-27]. Class II members are mitochondrially targeted eukaryotic proteins. Recently, Yarrowia lipolytica Ind1 (iron-sulfur protein required for NADH dehydrogenase 1) has been shown to function in the assembly of mitochondrial complex I, possibly acting as a scaffold protein for Fe/S cluster assembly [28]. This protein and the Arabidopsis homologue AtInd1 (also termed HCF101-L1) are localized in mitochondria and share conserved C-terminal cysteine residues [28,29]. Nbp35 (nucleotide-binding protein 35) and Cfd1 (cytosolic iron-sulfur cluster deficient 1), members

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of classes III and IV in yeast, have been shown to form a stable complex and to act in [4Fe-4S] cluster assembly in the cytosol [30–32]. They both bind a transient [4Fe-4S] cluster at the C-terminus and Nbp35 binds an additional [4Fe-4S] cluster at the N-terminus [32]. Homologous members of the FSC-NTPase family in *Arabidopsis*, yeast and prokaryotes, which belong to classes II–IV share several conserved cysteine residues (Figure 1) but, strikingly, these are not present in plastid HCF101 forms [26,28,30–32]. Furthermore, characteristic motifs for Fe/S cluster binding, such as CXXC are present in the N- and/or C-termini of cytosolic and mitochondrial proteins in eukaryotes and in eubacteria, e.g. in the *Salmonella* homologue ApbC, but remarkably are lacking in the plastid HCF101 form (Figure 1).

In this study we present a detailed biochemical and spectroscopic characterization of HCF101 *in vitro* and *in vivo*. As shown by UV–visible, EPR, and whole-cell Mössbauer spectroscopy HCF101 carries a [4Fe-4S] cluster that can be rapidly transferred to convert the apoprotein form of a yeast [4Fe-4S] protein into a catalytically active holoenzyme. <sup>55</sup>Fe radiolabelling experiments in yeast confirmed further the incorporation of an Fe/S cluster into HCF101 in a cellular context. Taken together, we propose a scaffold function for HCF101 that *in vivo* supports the assembly of the [4Fe-4S] clusters into chloroplast PSI and FTR complexes.

#### **EXPERIMENTAL**

#### Protein overexpression and purification

The complete cDNA of HCF101 was obtained from a cDNA library [33]. To express HCF101 without the predicted transit peptide the cDNA fragment corresponding to amino acid residues 64-532, including the stop codon, was cloned into the NdeI and BamHI restriction sites of pET23a<sup>+</sup> (Novagen). Plasmids were transformed into Escherichia coli strain BL21(DE3)/pLysS (Novagen) and cells were initially grown at 37 °C in LB (Luria-Bertani) broth. Overexpression was induced when the  $D_{600}$ reached 0.6 by the addition of 1 mM IPTG (isopropyl  $\beta$ -Dthiogalactoside) and cells were harvested after 5 h growth at 30°C. Cells were broken in a French press (Microfluidics) in 25 mM Tris/HCl, pH 8.1, containing 4 mM MgCl<sub>2</sub>, 10 % (v/v) glycerol and 1 mM DTT (dithiothreitol) unless otherwise stated. After removal of the membranes by centrifugation at  $22\,000\,g$  for 30 min, DNA was precipitated with the addition of 1% (w/v) streptomycin sulfate and centrifuged as above. The supernatant was applied to a HiPrep 16/10 DEAE-Sepharose FastFlow<sup>™</sup> IEX column (GE Healthcare) and eluted with a 0-0.5 M NaCl gradient in the same buffer as above. Fractions containing the purified protein were concentrated with Amicon ultracentrifuge units (Millipore) and the protein concentration was determined with the Bradford reagent (Roth). Proteins were visualized by SDS/PAGE on 15% Tris/glycine gels and were stained using Coomassie Brilliant Blue.

For Mössbauer analysis, BL21(DE3)/pLysS cells containing the respective plasmids were grown on M9 minimal medium. <sup>57</sup>Fe (Chemotrade) was prepared as described in [34] and added to a final concentration of 50  $\mu$ M <sup>57</sup>FeCl<sub>2</sub> 1 h before the induction of overexpression. Cells were harvested 7 h after induction and frozen rapidly in liquid nitrogen.

#### Site-directed mutagenesis of all cysteine residues of HCF101

All cysteine codons were replaced with serine codons in the expression vector using site-directed mutagenesis as described in [35]. The oligonucleotides used to insert the point mutations are listed in Supplementary Table S1 (available at http://www.BiochemJ.org/bj/424/bj424pppadd.htm). The presence of the mutated bases was confirmed by DNA sequencing.

#### Spectroscopic methods

UV–visible absorption spectra were recorded with a Jasco V-550 spectrometer in a 1-ml-diameter quartz cuvette sealed with a rubber stopper. The cuvette contained 1 mg/ml HCF101 protein in buffer A (Tris/HCl, pH 8.0, containing 150 mM NaCl).

Mössbauer data were recorded on a spectrometer with alternating constant acceleration. The minimum experimental line width was  $0.24 \text{ mm} \cdot \text{s}^{-1}$  (full width at half-height). The sample temperature was maintained at a constant level in an Oxford Instruments Variox cryostat and the <sup>57</sup>Co/Rh source (1.8 GBq) was at room temperature (20 °C). Isomer shifts are quoted relative to metal iron at 300 K. The zero-field spectra have been simulated by using Lorentzian doublets.

For EPR analysis purified HCF101 was transferred to EPR tubes (4.7 mm outer diameter, 0.45 mm wall thickness, Ilmasil-PN highpurity quartz; Quarzschmelze) directly after reconstitution and desalting. For reduction of the Fe/S cluster of HCF101 a freshly prepared solution of sodium dithionite in buffer A was added to a final concentration of 2 mM. After mixing the sample was capped with rubber tubing, stoppered with a Perspex rod and shock-frozen in liquid nitrogen. EPR spectra were recorded with a Bruker E500 ELEXSYS continuous-wave X-band spectrometer using a standard ER4102ST cavity and an ESR910 Oxford Instruments helium-flow cryostat. The magnetic field was calibrated with an ER035M Bruker NMR probe and the microwave frequency was measured with a HP5352B Hewlett Packard frequency counter.

#### Chemical reconstitution of Fe/S clusters and cluster transfer to apo-Leu1

Purified recombinant apo-HCF101 used for chemical reconstitution contained only approx. 0.2 mol of iron and sulfur per monomer. Before chemical reconstitution of the Fe/S clusters in an anaerobic chamber (Coy Laboratory Products), 60  $\mu$ M apo-HCF101 was reduced with 10 mM DTT in 25 mM buffer A for 1 h. A 5-fold molar excess of ferric ammonium citrate and lithium sulfide over the HCF101 monomer concentration was added and the sample was incubated for 30 min at 23 °C. Unbound iron and sulfide was removed by desalting on a 10 ml Sephadex G-25 column (GE Healthcare) equilibrated in buffer A. Protein concentration was determined with the Microbiuret method at 545 nm [36]. Iron was determined colorimetrically after treatment with 1 % (w/v) HCl at 80 °C for 10 min and addition of ferene as an iron chelator [37]. Acid-labile sulfur was determined using the Methylene-Blue method [37]. For Fe/S cluster transfer holo-HCF101 was mixed with recombinant reduced apo-Leu1 [32]. Aliquots (5  $\mu$ l) were analysed at various time points for Leu1 activity.

#### <sup>55</sup>Fe incorporation

The yeast strains used were Saccharomyces cerevisiae W303 and the galactose-regulable mutant Gal-NFS1 [6]. A construct was generated which fuses the mature HCF101 protein to the  $F_1$ - $\beta$ mitochondrial-targeting sequence and a C-terminal Myc tag by cloning into a pRS426 vector. The construct is expressed under the control of the TDH3 promoter. Additionally, the targeting sequence was excised in some experiments, allowing cytoplasmic expression of HCF101. The oligonucleotides used are listed in Supplementary Table S1. The plasmids were transformed into W303 or into Gal-NFS1 cells. Wild-type (WT) cells were cultivated for 40 h in SC (synthetic complete) minimal medium containing 2% (w/v) glucose. Gal-NFS1 cells were grown in 2% (w/v) galactose or glucose. The cells were cultured in ironfree minimal medium during the last 16 h. After washing and transfer to fresh iron-free minimal medium, approx. 0.5 g of cells were radiolabelled for 2 h with <sup>55</sup>FeCl<sub>3</sub>. Following radiolabelling, cells were resuspended in 0.5 ml of lysis buffer [10 mM Tris/HCl, pH 7.4, containing 2.5 mM EDTA, 150 mM NaCl, 10% (w/v) glycerol and 0.5 % Triton X-100] and disrupted with glass beads. Anti-Myc beads (20  $\mu$ l) were added to the lysates and rotated for 1 h at 4°C. After immunoprecipitation, beads were washed three times with lysis buffer and the amount of HCF101-Mycassociated radioactivity was measured by scintillation counting. Immunoblot analysis of the expressed fusion proteins was performed with anti-Myc and anti-Nfs1 antibodies [6].

#### RESULTS

#### Expression and purification of HCF101 from E. coli

In order to biochemically investigate the function of HCF101 the coding sequence of the mature protein, without the predicted transit peptide, was cloned into a pET23a<sup>+</sup> vector and overexpressed without a tag in the *E. coli* strain BL21(DE3)/pLysS. At 5h after induction with IPTG the total amount of HCF101 protein reached approx. 20% of the total soluble *E. coli* protein extract. The protein migrated at an apparent molecular mass of about 53 kDa (the calculated mass was 50.5 kDa). After purification using anion-exchange chromatography the protein was determined to be approx. 90% pure (Figure 2a). HCF101 was purified under standard aerobic conditions and the eluate was a brownish colour. UV–visible absorption spectra of the purified protein showed a prominent peak at 330 nm and a discrete shoulder at 420 nm indicating the presence of intact, as well as partially degraded, [4Fe-4S] clusters (Figure 2b).

#### Chemical reconstitution of HCF101

For a better investigation of the cluster type, the cofactor was chemically reconstituted by anaerobic incubation of the purified HCF101 protein with a 5-fold molar excess of iron and sulfide over the HCF101 protein concentration. UV–visible absorption spectra of the sample, and determination of the iron and sulfide content, showed that the intensity of the characteristic shoulder at 420 nm was increased by up to 4-fold as compared with the non-reconstituted (as isolated) protein (Figures 2b and 2c). Dithionite reduction resulted in approx. 50% bleaching of the absorbance at 400 nm, a behaviour normally associated

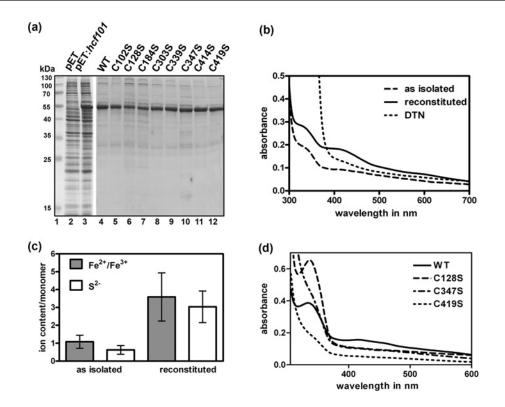


Figure 2 Purified HCF101 binds one [4Fe-4S] cluster per monomer in vitro

(a) SDS/PAGE and Coomassie Brilliant Blue staining of *E. coli* extracts and purified HCF101 proteins. *E. coli* extract containing overproduced HCF101, analysed 5 h after induction, is shown in lane 3 and an extract from cells containing the empty pET23a<sup>+</sup> vector is shown as a control in lane 2. Lanes 4–12 show the purified WT and the eight cysteine residue mutant HCF101 proteins after anion-exchange chromatography. Lane 1 contains molecular mass standards. (b) UV–visible absorption spectra of WT HCF101. A distinct shoulder at 420 nm is visible in the aerobically isolated sample (---) and after the chemical reconstitution under anaerobic conditions (solid line). The peak disappeared after reduction with 1 mM sodium dithionite (DTN; ---). (c) The iron and sulfur content of the purified HCF101 protein was determined before and after chemical reconstituted). This suggests binding of one [4Fe-4S] cluster per monomer after reconstitution. (d) Cysteine residues of HCF101 required for stable Fe/S cluster binding *in vitro* were identified. UV–visible absorption spectra of purified WT and mutated HCF101 proteins (C128S, C347S and C419S), lacking the shoulder at 420 nm, are shown. A protein concentration of 1 mg/ml was used in (b) and (d).

with [4Fe-4S] clusters [37a]. The reconstituted sample bound approximately four iron and four sulfur ions per monomer, indicating that HCF101 may bind a single [4Fe-4S] cluster per monomer (Figure 2c). The reconstituted Fe/S cluster proved to be oxygen-sensitive, as the 420 nm shoulder slowly decreased after exposure to air and was almost completely lost after 4 h (see Supplementary Figure S2 available at http://www.BiochemJ.org/ bj/425/bj4250207add.htm).

# Cysteine residues required for binding the [4Fe-4S] cluster in recombinant HCF101

As the location of the cysteine residues in HCF101 differs from all other members of the FSC-NTPase family and from typical Fe/S cluster-binding proteins, it was particularly challenging to investigate which cysteine residues were involved in binding the Fe/S cluster. As a first approach the ability to bind a cluster was analysed *in vitro*. For this purpose we replaced all eight cysteine residues with serine residues by site-directed mutagenesis. The recombinant proteins were expressed and purified as described for the WT protein. The typical brownish colour of WT HCF101 was not found in three of the mutant forms (C128S, C347S and C419S). UV–visible spectral analysis confirmed that the characteristic shoulder at 420 nm was completely absent in these mutant proteins (Figure 2d). All other recombinant proteins showed only a slightly diminished intensity of the shoulder at 420 nm indicating that less Fe/S cluster was associated with these

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mutant proteins after biosynthesis in *E. coli*, but that the Fe/S cluster was still able to bind HCF101 to a limited extent (results not shown). The involvement of at least three cysteine residues in the Fe/S cluster association to HCF101 is compatible with the stoichiometry of one cluster per monomer.

#### Whole-cell Mössbauer and EPR spectroscopy studies on recombinant HCF101

To elucidate further the nature of the cluster and the cysteine residues involved in binding we performed whole-cell Mössbauer and EPR spectroscopy (Figures 3a-3c). The zero-field Mössbauer spectrum recorded with intact E. coli cells after overexpression of WT HCF101 in 57Fe-enriched medium at 80K showed a superposition of two quadrupole doublets with an intensity ratio of 89:11 (Figure 3a, upper panel). The moderately low isomer shift  $(\delta = 0.50 \text{ mm} \cdot \text{s}^{-1})$  and quadrupole splitting  $(\Delta E_0 = 1.04 \text{ mm} \cdot \text{s}^{-1})$ s<sup>-1</sup>) of the major species were typical of [4Fe-4S] clusters in the diamagnetic  $(2^+)$  state [37b,38]. The iron sites, which formally were two Fe<sup>2+</sup> and two Fe<sup>3+</sup> ions, were hence indistinguishable because of complete valence delocalization. The 11% minority subspectrum, in contrast, had a very large isomer shift ( $\delta = 1.26$  mm  $\cdot$  s<sup>-1</sup>) and large quadrupole splitting  $(\Delta E_0 = 3.23 \text{ mm} \cdot \text{s}^{-1})$ , which are typical of high-spin Fe<sup>2+</sup> with six hard ligands. Such a component has been observed in whole cells previously and could be assigned to primary intermediates of iron metabolism [34,35].

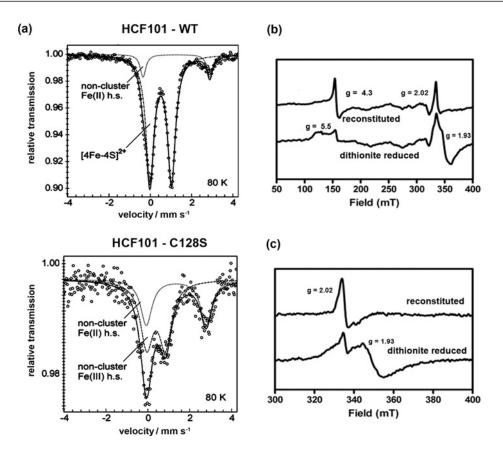


Figure 3 Spectroscopic analysis of HCF101

(a) Mössbauer spectroscopy of intact *E. coli* cells were performed after overexpression of HCF101 WT (upper panel) and HCF101 C128S (lower panel) in <sup>57</sup>Fe-enriched medium. Equal expression of protein was checked by SDS/PAGE. h.s., high spin. (b) Chemically reconstituted HCF101 was analysed by EPR spectroscopy before (top trace) and after reduction (bottom trace) with 2 mM sodium dithionite. EPR conditions; temperature 10K; microwave frequency, 9420 ± 1 MHz; modulation amplitude, 1.25 mT; modulation frequency, 100 kHz; microwave power 20 mW. (c) An experiment as performed in (b), but at 1.26 mW microwave power.

The Mössbauer spectrum of the control sample for the C128S mutant also showed that essentially there were two quadrupole doublets (Figure 3a, lower panel), which had rather broad lines and resembled neither the spectra known for haem enzymes nor those for Fe/S clusters [38]. The absolute intensity of the signal in the cells expressing the C128S mutant was much lower than the signal of cells expressing the WT protein. The major spectral species (61%) had a lower isomer shift and quadrupole splitting than expected for cubane Fe/S clusters  $(\delta = 0.39 \text{ mm} \cdot \text{s}^{-1} \text{ and } \Delta E_Q = 0.89 \text{ mm} \cdot \text{s}^{-1})$ . The values were close to those known for  $\gamma$ -FeOOH or similar oxidic species (with an isomer shift of  $\delta = 0.37 \text{ mm} \cdot \text{s}^{-1}$  and quadrupole splitting of  $\Delta E_Q = 0.57 \text{ mm} \cdot \text{s}^{-1}$ ) from iron precipitations [39]. Oxidized Fe<sup>3+</sup> clusters, which might be considered as possible alternative explanation, despite the isomer shift being atypically high, can be excluded because of the absence of the sizable EPR signals from such clusters (Figures 3b and 3c). The second component in the Mössbauer spectrum again represented a non-Fe-S Fe<sup>2+</sup> component ( $\delta = 1.36 \text{ mm} \cdot \text{s}^{-1}$  and  $\Delta E_Q = 2.86 \text{ mm} \cdot \text{s}^{-1}$ ). Thus signals from Fe/S clusters could not be detected in the Mössbauer spectrum of the mutated protein C128S.

Purified, reconstituted HCF101 showed a weak EPR signal at g = 2.02 from a [3Fe-4S]<sup>1+</sup> cluster (Figure 3c, upper trace). The integrated intensity amounted to no more than 1 % of the HCF101 molecules. Such a species could have been derived from the breakdown of a minor proportion of the [4Fe-4S]<sup>2+</sup> cluster, which could not be detected in the Mössbauer spectrum. Upon

reduction with dithionite a broad rhombic EPR signal with g values of 2.02 and 1.93 was observed (Figure 3c, lower trace), which was accompanied by a sharp isotropic (g = 2.003) radical signal, derived from dithionite breakdown products. The rhombic EPR signal had an integrated intensity of 0.05 spins per HCF101 in two different preparations. As Mössbauer spectroscopy (Figure 3a), iron and sulfide analysis and the extent of reduction as judged from visible spectroscopy (Figure 2b) indicated the presence of a stoichiometric amount of [4Fe-4S]<sup>1+/2+</sup> in HCF101 we were puzzled by the low intensity. Inspection of the low field region revealed a broad signal at g = 5.5 from a S = 3/2 form of the [4Fe-4S]<sup>1+</sup> cluster (Figure 3b, lower trace). This signal appeared upon reduction and was thus not from adventitiously bound high-spin ferric ions, like the sharp g = 4.3 signal in the upper trace of Figure 3(b), which disappeared upon reduction (Figure 3c, lower trace). Further analysis of the S = 3/2 signals was hampered by limitations imposed by solubility of the reconstituted HCF101 protein. It is likely that the g = 5.5 signal encompasses the majority of the [4Fe-4S]<sup>1+</sup> clusters in HCF101, as upon double integration such weak signals represent more spins than sharp S = 1/2 signals. Similar broad anisotropic S = 3/2 signals have also been observed for the [4Fe-4S]<sup>1+</sup> cluster of the nitrogenase iron protein [40], the activator of 2-hydroxyglutaryl-CoA dehydratase [41], the Fx cluster in the type I homodimeric photosynthetic reaction centre in heliobacteria [42] and the Cfd1/Nbp35 complex (D.J.A. Netz, A.J. Pierik and R. Lill, unpublished work). As to our

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knowledge S = 3/2 signals do not occur in natural [2Fe-2S]<sup>1+</sup> clusters, the presence of the g = 5.5 signal in dithionite-reduced HCF101 corroborates the presence of a [4Fe-4S]<sup>1+/2+</sup> cluster in HCF101 as shown by iron and sulfur analysis, UV–visible and Mössbauer spectroscopy.

#### HCF101 harbours an Fe/S cluster in vivo in S. cerevisiae

We next attempted to obtain further in vivo evidence for the presence of an Fe/S cluster on HCF101. As this is technically problematic in plant cells, we used S. cerevisiae and a <sup>55</sup>Fe radiolabelling assay. The coding sequence of mature, Myc-tagged HCF101 protein (either containing or lacking the  $F_1\beta$  ATPase mitochondrial presequence) was cloned into a yeast expression vector. The plasmids were transformed into WT yeast cells and were able to produce significant amounts of protein in both the mitochondria and the cytosol (Figure 4a, lower panel). Cells were subjected to radiolabelling with 55Fe, a cell extract was prepared and HCF101-Myc was affinity-purified via the Myc-tag. Strong radiolabelling of HCF101–Myc with <sup>55</sup>Fe was observed upon localization of the protein in mitochondria, but not in the cytosol (Figure 4a, upper panel). As the latter result was not due to the lack of stability of HCF101 (Figure 4a, lower panel), our finding suggests that the Fe/S cluster on HCF101 is not capable of forming when the protein is in the yeast cytosol. To analyse whether the 55Fe associated with mitochondrially targeted HCF101-Myc was indicative of the binding of an Fe/S cluster, we analysed the dependence of <sup>55</sup>Fe binding on the function of the cysteine desulfurase Nfs1, a central component of the mitochondrial assembly system. When Nfs1 was depleted in Gal-NFS1 cells, which contain a regulatable NFS1 gene [6], a 75 % decrease in <sup>55</sup>Fe incorporation was seen (Figure 4b). This result shows that the 55Fe incorporated into HCF101 is part of an Fe/S cluster. Taken together, the radiolabelling experiments in yeast show that HCF101 can also bind an Fe/S cluster in a eukaryotic system, yet its localization to mitochondria is required to achieve significant Fe/S cluster assembly.

#### HCF101 can transfer its Fe/S cluster to apo-Leu1

A common feature of scaffold proteins is their capability to transfer the bound labile Fe/S cluster to the apo-form of acceptor proteins [3,10,42]. We tested the putative scaffold function of HCF101 in vitro. It is at present not feasible to use apo-PSI complexes of higher plants to test the transfer of Fe/S cluster from HCF101. We therefore chose yeast apo-Leu1 as a model acceptor protein. Upon binding of a [4Fe-4S] cluster Leu1 exhibits isopropylmalate isomerase activity [32]. Apo-HCF101 was chemically reconstituted to its holoform (typically containing  $3.6 \pm 1.2$  Fe and  $3.0 \pm 0.8$  S per monomer) and mixed with apo-Leu1. Upon incubation of the two proteins under anaerobic conditions isopropylmalate isomerase enzyme activity developed within 2 min, with more than 90% conversion of apo-Leu1 into the active enzyme (Figure 5). In contrast, upon chemical reconstitution of apo-Leu1 with similar amounts of iron and sulfide instead of a transferable Fe/S cluster, a comparatively slow and inefficient generation of the Leu1 enzyme activity was observed. Even after 30 min only approximately one-third of the activity was detected compared with using HCF101 as a donor after 2 min. These results show that the HCF101-bound Fe/S cluster is labile and can be efficiently transferred to acceptor proteins. Hence, HCF101 fulfils a critical requirement for a scaffold function, i.e. the capacity to transfer a labile Fe/S cluster to a target protein under conditions when chemical reconstitution is inefficient.

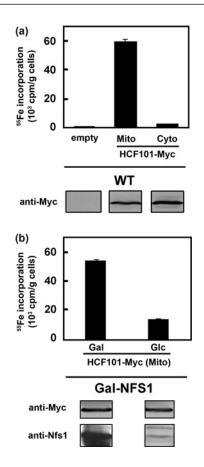


Figure 4 HCF101 can assemble an Fe/S cluster in yeast mitochondria

(a) W303 WT yeast cells were transformed with vectors pRS426-TDH3 encoding a Myc-tagged HCF101 with (Mito) or without (Cyto) an N-terminal F<sub>1</sub> $\beta$ -ATPase mitochondrial-targeting sequence or with the empty vector (empty). After growth in minimal medium with 2 % (w/v) glucose and lacking iron for 40 h, cells were radiolabelled with <sup>55</sup>Fe and incubated for 2 h. Cell lysates were prepared and HCF101 was affinity-purified using anti-Myc antibody beads. The amount of <sup>55</sup>Fe bound to HCF101–Myc was measured by scintillation counting. (b) Gal-NFS1 cells, in which the *NFS1* gene is under the control of a *GAL1–10* promoter, were transformed with pRS426-TDH3 encoding the mitochondrially targeted Myc-tagged HCF101. After growth in 2 % (w/v) glactose- (Gal) or 2 % (w/v) glucose (Glc)-containing iron-poor minimal medium for 40 h, <sup>55</sup>Fe binding were carried out as described above. The lower panels are immunostains of trichloracetic acid-precipitated cell lysates separated by SDS/PAGE (12.5 % gels) and probed with the indicated antibodies.

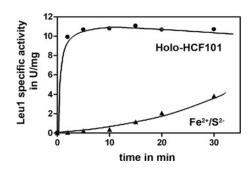


Figure 5 Holo-HCF101-mediated transfer of the [4Fe-4S] cluster to the apo-Leu1 target protein

Reconstituted and desalted holo-HCF101 (3.85  $\mu$ M) was mixed with reduced apo-Leu1 (2.45  $\mu$ M) at 23 °C in buffer A. Isopropylmalate isomerase activity was measured at the indicated time points ( $\bullet$ ). In a subsequent control experiment, activation of 2.45  $\mu$ M apo-Leu1 with ferric ammonium citrate and Li<sub>2</sub>S at concentrations identical with those present in HCF101 (15.4  $\mu$ M each) was assayed for isopropylmalate isomerase activity ( $\blacktriangle$ ).

In the present study, we biochemically investigated the molecular function of the plastid representative HCF101 of the FSC-NTPase superfamily. The protein was previously shown in cell biological experiments to function as an essential factor for assembly of PSI but the mechanism of action remained unknown. Levels of PSI and FTR complexes, harbouring three and one [4Fe-4S] clusters respectively, were significantly diminished in the HCF101 mutant although translation of PSI proteins was not affected [23]. Bacterial, cytosolic and mitochondrial members of the FSC-NTPase family have been described as binding to [4Fe-4S] clusters and their role in Fe/S cluster biogenesis in their particular cell compartments has been documented [26,31,43,44]. However, despite the overall striking conservation of the protein signatures, surprisingly the typical and conserved Fe/S clusterbinding motifs of all the other FSC-NTPase members was absent in HCF101 (Figure 1) [23]. Therefore it was still a matter of debate whether HCF101 had the ability to bind and transfer Fe/S cluster or if it had possibly acquired other functions in the course of evolution. Our results showed that heterologously expressed HCF101 in E. coli retained approx. one iron and one sulphur ion per HCF101 monomer after purification, and that after chemical reconstitution approximately four iron and four sulfur ions per HCF101 monomer were present. This is consistent with the binding of a [4Fe-4S] cluster. Indeed, UV-visible, Mössbauer and EPR spectroscopy of reconstituted HCF101 confirmed the presence of a [4Fe-4S] cluster. Interestingly, the reduced species of HCF101 was mainly present in the S = 3/2 form. This type of EPR signal has been encountered in many proteins which have solvent-exposed clusters [41]. The property of HCF101 to rapidly transfer of its cluster to apo-Leu1 occurs also supports the hypothesis that its cluster is solvent-exposed rather than buried in the protein. Taken together, in the present study we unequivocally demonstrated, by several methodologies, that despite, the lack of characteristic FSC-NTPase family binding motifs HCF101 harbours a labile [4Fe-4S] cluster.

In vitro analyses using site-directed mutagenesis in E. coli identified three cysteine residues that are essential for binding the Fe/S cluster. As expected the only cysteine residue that is also conserved in cyanobacteria, Cys<sup>128</sup>, was the most crucial for the assembly/stability of the cluster when expressed in E. coli. The other two cysteine residues involved in cluster binding, Cys<sup>347</sup> and Cys<sup>419</sup>, were present in the context of a second cysteine residue, namely as part of a  $CX_7C$  or a  $CX_4C$  motif respectively. These motifs might resemble metal-binding sites. The other two cysteine residues of these motifs are also likely to be involved in Fe/S cluster-binding and/or stabilization as the cluster of the respective purified mutant forms were more unstable compared with the WT protein (results not shown). In contrast with all other FSC-NTPase members a cysteine residue (underlined) is present in the highly conserved P-loop domain of the plant HCF101 (CKGGVGKS). This might indicate that there has been a change-of-function for this domain in the plant lineage, although the newly evolved cysteine residue in the P-loop is not essentially required for Fe/S cluster-binding (result not shown). Two Fe/S cluster-binding cysteine residues (Cys<sup>97</sup> and Cys<sup>131</sup>) have also been identified in the homodimeric protochlorophyllide oxidoreductase subunit BchL, a NifH-like protein sharing some sequence similarity with HCF101 [45]. One of these BchL cysteine residues (Cys<sup>131</sup>) is conserved in HCF101 (Cys<sup>303</sup>), yet might have lost its function as a cluster ligand.

Nevertheless, with the exception of Cys<sup>128</sup>, which is also conserved in cyanobacteria, all other cysteine residues are not preserved in other organisms (see Supplementary Figure S1) pointing to a specialized plastid form of class I FSC-NTPase proteins. Therefore, HCF101 may represent a divergent type of Fe/S cluster-binding protein, which presumably evolved as an adaptation to oxygen evolution in the chloroplast. So far almost nothing is known about the formation of [4Fe-4S] clusters in plastids and their insertion into PSI and other chloroplast complexes. To clarify the possible function of HCF101 regarding post-translational assembly of PSI, the *in vitro* cluster transfer properties of the [4Fe-4S] cluster on HCF101 was tested. As it is not feasible to establish an *in vitro* assay for the transfer of [4Fe-4S] clusters on the apo-PSI complex or to study Fe/S cluster transfer in a living plant cell, we used the yeast apo-Leu1 as a model target protein. HCF101 efficiently transferred its cluster to apo-Leu1 demonstrating that it binds its [4Fe-4S] cluster in a labile fashion. These results suggest that HCF101 could transfer its

fashion. These results suggest that HCF101 could transfer its Fe/S cluster to apoproteins in chloroplasts. Whether the HCF101 function is specific for [4Fe-4S] clusters, cannot be derived from in vitro experiments due to the limitations of the in vitro Fe/S cluster experiments. Nevertheless, it is generally accepted that the ability to rapidly and efficiently transfer Fe/S cluster(s) in vitro to acceptor apoproteins is an essential prerequisite for scaffold function of an Fe/S cluster-containing protein. As an alternative scaffold protein NFU2, which binds [2Fe-2S] clusters has been described in chloroplasts [18,19]. Somewhat surprisingly, Arabidopsis nfu2 mutants also have a PSI defect, even though these plants exhibit a much milder phenotype than hcf101 plants [19]. At present, it is unknown whether NFU2 can assemble [4Fe-4S] clusters by itself, or facilitates transfer of the assembled [2Fe-2S] clusters on to HCF101, thus forming transient [4Fe-4S] clusters.

Our biochemical studies suggest that HCF101 can assemble a [4Fe-4S] cluster and transfer it to apoproteins. It is thus conceivable that this protein fulfils a similar function to the Cfd1-Nbp35 complex, which performs a scaffold role in cytosolic and nuclear iron-sulfur protein assembly [32], or to the mitochondrial Ind1 involved in Fe/S cluster incorporation into respiratory complex I [28]. Strikingly, the motifs which transiently bind the Fe/S clusters in these latter proteins differ markedly from that in HCF101. Future studies therefore will have to uncover the precise structure of Fe/S cluster-bound HCF101. Moreover, whether Fe/S cluster assembly on HCF101 occurs spontaneously in the plastid has to be addressed, i.e. whether HCF101 serves as a true scaffold or whether Fe/S cluster assembly depends on other scaffolds such as NFU1, NFU2 and NFU3, or the SufA-type scaffolds [46], and functions as a so-called carrier for delivering the cluster to [4Fe-4S] apoproteins.

#### **AUTHOR CONTRIBUTION**

Serena Schwenkert, Daili Netz, and Jeverson Frazzon performed the biochemical analysis. Antonio Pierik recorded EPR spectra. Eckhard Bill performed Mössbauer spectroscopy. Roland Lill contributed to the planning and execution of some experiments. Jeferson Gross was involved in cloning. Jörg Meurer designed and supervised the study. Serena Schwenkert, Daili Netz, Roland Lill and Jörg Meurer wrote the paper with contributions from all authors.

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### SUPPLEMENTARY ONLINE DATA Chloroplast HCF101 is a scaffold protein for [4Fe-4S] cluster assembly

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	102 128
Arabidopsis HCF101	: EKDULKA SQIID DFGTD VS GFVKDGINEALGEVSFR ELTTPA VKDMFENKANEVUAA PWVKKVNUTMSA
	: KKUVLVA SQIID-DFGTD VSCGFVKDEISEALEEVSFR ELTTPACPIKDMFEEKANEVVAA PWVKKVNVTMSA
	: ESDVLKA_SQIIDPDFGTD_VSCGFVKDNIDEAQGEVSFR_ELTTPACPVKDMFEQKANEVVAL_PWVKNVEVTMSA
[10] A. B. M.	: BADVLKAVSQIID-DFGTDIVS_GFVKDIQINEALGEVSFRUELTFACPIKDMFEQKANEVVAMUPWVKNVNVTMSA
	: KKDVLGA_SQIIDPDFGTDTVTCGFVKETTVDESTGEVSFQTELTTPACPVKDMFEQQAKEKVSATPWVKGVNVKMTA
Selaginella	: GHDVLVA_STIID-DFGAD_VT_GFVKDQADKSSGEVAFR_ELTTPACFVKDMFEQQAKEKVAA_PWVTNVKVTMSA
	: EEQULAKURNVIDPDFGEDIVACGFVROLEVDASVGFVSFTUELTTPACPVKEMFOROSTOFVKELPWVRDVSIKLTA
Ostreococcus tauri	: ESEVLSKURRVIDPDFGEDIVNCGFVKAUVIDESAGSVLFAUELTFACFVKAEFEROAKAFVEEDDWVKRVSVTMTA
Micromonas	: BADVLNAPRNVIDPDFGEDTVNCGFVKDPRVSDAGDVTFTPELTTPACPVKEEFDRLSKQVVTAPEWAKSCNVNMTA
Galdieria maxima	: QKQVLEL_KNIEDPDLKQN_VELGFVQN_ERVAKEDGKYDVRFT_QLTTPACPIKEKFQNDAKEWVSS_LWVRNVE_DLRA
Cyanidioschyzon	: TEQLLSA KAVVDPDLGQDIVTLGFVKNI-QFGGDEHYGTVSFDVELTTPACFIKERFREECTRLAES PFVTRANVRLTA
Phaeodactylum	: QGEVLST KSVIDPDLGSDIVTLGFVQNKLDGRDVSFDVELTTPACFVKEQFQLDCQQLVQD PWTNNIQVTMTA
Thalassiosira	: QSQILAAISVINDPDLNADIVSLGFVQNLKIDESSNIVSLDIELTTPACPVKDLFVQQCQDIINGLAWTRGADVTLTS
	: EDQ LAAT RQVQEPELGGD VSRQMVKHIAICDGIVRCT ELTTPACPLKDQIRSEABAAVLAVPGVREVH EFTA
	: QEQVLEARKTVPEPDLKKD-VTLNM-RDIAIDGQN-SFT-VLTTPACPLKELIRNSCTEARHK-VSGTAVV-INMT
	: SRS LEI RPVEDPELRKS VELNM RNVKIDGGKVSFT VLTTPACPLREFIVED COKAVKK PGVTDVS EVTA
Synechococcus	: AAALLEALRPVQD-ELRRS.VELNM.RDRVEPGRVAFT.VLTTPACELREFIVEECKAALRQ-APIEAID.TVTA
	: EPQURDATRMVVD_VAGRD_VSAGYVSGEIHAGEVAFQ_QFRPESADYLKQLQEQCAQVIGA_PGVERVT_NMSG
Salmonella	: RAMVAGT ANFQH TLKHN TTLKA HHVAWMDDT HVE VMPFVWNSAFEVLKEQCSAD LR TGAKAIDWKLSY
Arabidopsis-L1	:MATVAL RS RRRELHAAHISA
Chlamydomonas-L1	:MGWRQGAAEWLLSNAASGWRGAASAAALAAGGASGTARAGAPAGASRGA :
Homo sapiens	
Yarrowia lipolytica	:MRGFRFIAPFORSIATISKLOPITANFHSSPALRSHENPIGFFRSP :MENGDIPEDANEH_PGPQSESAGKSDS_AG_PNQEA_AT
Arabidopsis-L2 Chlamydomonas-L2	:MASSASAAPTGEVPDNANQHOPGTASDQAGKSASAGOPNQEAQA1 :MASSASAAPTGEVPDNANQHOPGTASDQAGKSAAQAGOPNQEAQA1
Homo sapiens	:
Saccharomyces Nbp35	
Drosophila	
Homo sapiens	
Danio rerio	
Saccharomyces Cfb1	
	184
Arabidopsis HCF101	: QPAKPIFAGQLPFGLSRTSNILAVS <mark>SCKGGVCKSTVA</mark> VNLAYTLAGMCARVGIFDADVYGPSLPTVVNPSSRIJEMN
Oryza sativa	: OPARPAYAGELPEG MOKHSNI WAWSKORCEWERSHVAMMITAYTITAGMEAU MEHEDADWARFRITADMISPONKLIVMN
Populus	
	: OPARPVFAGOLPAG - HOTHSNIHE/WSS(CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
	: QPAKPLIADDVPAGHKKVSNIVAVSSCKGGVGKSTVAVNLAYSLAQMCARVGIFDADIYGPSLPTMVSPEVKVIQMN
Selaginella	: QPAKALAAEGLPRSDQNVSNILAVSSCKGGVGKSTVAVNLAYSLAGMCAKVGIFDADVYGPSLPTMVSPELRVLQMV
Volvox	: OPAKALAAEGLPRSDONVSNIIAVS3CKGGVGKSTVAVNLAYSLAGMGAKVGIFDADVYGPSLPTMVSPELRVLQMV : OPFXPLLPESGRPGGDAKWRFIIVS3CKGGVGKSTVSVNLAYTLAQMGAKVGIFDADVYGPSLPTMVNPEIKVIEMD
Ostreococcus tauri	: UPARNDAPE-IVEGERRWSHILL#VSSCAGGVGASIISVRIMATIMAMIGAMWGITD/#DVFGPSTPID/ISPDVPVIEND
	: QPVTNDMPD-AVEGLKGVRHILAVSSCKGGVCKSWTSVNLAYTIRMMCAXVGIFDADVFGPSLETWTSPSQAVIQMD
	: NEINRAQAGDRPDNKYKHIIAYASCKGGVGKSTVAVNLAFTIIKLCGKVGIMDADIYGPSDPTDVQPSNKIVQYK : OTPSAAAPEAGGSRDPISOVSNIVLVTSAKGGVAKSTTAVNLAFVLARLCARVGILDADIYGPSDPINVNPSHNEKRIR
Thalassiosira	: QPSVQETATLGWSQVGAVIAVSSCKGGVGKSTTAVNLAFSLQRLGATVGIFDADVVGPSLPTMITPODDVRFV : QPTAAPSDAPLGWSQLGAVIAVSSCKGGVGKSTTAVNLAFALESLGAXVGIFDADVVGPSLPTMVTP©DDVVFV
Chloroflexus	: NVRRPAGIPEQSA POWANVIAWAAGKGGVCKSTVAANLAVALAQMCAQVGLLDADVEGPSIPEMLGVRGQPMAVS
Cytophaga	: QPTAAPSDAPLGWSQIGAVIAVS3CKGGVGKSTTAVNLAFAUESLCAXVGIFDADVYGPSLPDMVTPODDNVRFV : NVRPAGIPEGSAIPGVANVIAVAACKGGVGKSTTANLAVAJAQWCAQVGLDADVSGPSLPIMGVGQPAVS : ADVTTGRFNSGPVIPGWNNIAVSCKGGVGKSTTANLAVAJSKSGAXVGLDADISGPSIPTMFDVDDVRPNVI : ETPQKSLPDRTGISGVNNIAVSCKGGVGKSTAVNVAVALSKSGAXVGLDADISGPDFTMGDADAOIVVR : ETPRSPSLPNRQSISGVNNIAVSCKGGVGKSTSVSVNVAVALAQSCARVGLDADISGPDFTMGDADAOIVVR
	: ETPQQKSLPDRTGISGVKNIIAVSSCKGGVGKSTVAVNVAVALAQTCAKVGLLDADIYGPNDPTMIGLADAQIVVR
Synechococcus	: ETPRSPSLPNRQSPPGYRNIIAISSGKGGVGKTSVSVNVAVALAOSCARVGLUDADIYGPNVPLMLGLODRSLVVQ
Magnetococcus	: NPQQQAEPL
Salmonella	: NIATLKRVKNOPGINGVKNIIAVSSCKCCVCKSSTAVNLALALAAEGAKVGVLDADIYGPSIPTMLGABDORPTSP
Arabidopsis-L1	: YKFSSASAGGRTTELREHGYKDIIAYASGKGGYGKSSTAVNLAVALANKCELK-IGLLDADVYGPSVPIMMNINQKPQVNQ
Chlamydomonas-L1	: AASRGAAAAGPQKKLG <mark>L</mark> KDVQHTVATTSAKGGVGKSTTAVNVAVAMATRLGLR-VGLLDADVHGPSIPTLMNLRGKPELDK
Homo sapiens	: QRRTQIMSRGLPKQKPHEGVKQVHVWASGKGEVGKSTTAVNIMLAIMANDSSKAHGIMDVDV/GPSVEKMMNLKGNPELSQ
Yarrowia lipolytica	: ASAPRIPRKTTRRPEPLAGVKKTUVVSSAKGGVGKSTVSVNTALSLAKRELRVGLLDVDLEGPSIPTMFGLSGEPRMTH
Arabidopsis-L2	: -APKGPDPDLVAIAERMSTVKHKILVLSGKGGVSKSTFSAQISFALAGMDHQVSLMDIDICGPSIEKM GLSGQEIHQS
Chlamydomonas-L2	: -APKGPDPDLAAIAARVSRVKHKULVLSGKGGVGKSTVSAQLAFALARREFEVGLLDIDICGPSVPKMUGLSGQETHSS
Homo sapiens	: GAGATPDTAIEEIKEKWKTVKHKULVLSCKGGVCKSTFSAHLAHGLAEDENTQ-IAHLDIDICGPSIPKINGLSGEQVHQS
Saccharomyces Nbp35	: -APKGPDPDLVAIAERSSTVKHKHLVLSGKGEVGKSTFSAQUSFALAGKDHQVGIAMDIDICGPSTFKMGGLSGQEHQS : -APKGPDPDLAAIAARMSRVKHKHLVLSGKGEVGKSTVSAQLARACFEVGIADIDICGPSTFKMGLSGGEHKS : GAGATPDTAIEEIKEKWKTVKHKHLVLSGKGGVGKSTFSAHLAHGLAEDENTQ-IALLDIDICGPSTFKINGLSGEVHQS : L-PKGPDPDIPLITDNISGTEHKHLVLSGKGGVGKSTFAAMSWAISADEDLQ-VGAMDIDICGPSTFKINGLSGEVHQS :
Drosophila	:mldkvknvivvlsgkggvgkstvstqlslalrkngfkvglldidlcgpsvpvllglegrdifqc
Homo sapiens	:meaaaepgnlagyrhiilylogkgyykstistelalalrhagkkvgildydlogpsiprmlgogravhq :MDGSGKGNUDQYKHVLYLSCKGGVGKSTITTELALAFRHAGKKVGILDYDLCGPSIPRMLSVGKPEVHOC
Danio rerio	:MDGSGKGNUDQVKHVLVLSGKGGVGKSTITTELALAFRHACKKVGILDVDLCGPSIPRMLSVGKPEVHO :MEEQEIGVPAASLAGIKHIILLSGKGGVGKSSVTTOTALTLCSMCFKVGVLDIDLTGPSIPRMFGLENESIYQG
Saccharomyces Cfbl	MEDQLIGYPANDAGIANIHILILGGAGGWEADDVIIQTELTICSMEPAWEVNDIDTELYSTERWPGLENESUYQG

<sup>1</sup> These authors contributed equally to this work.

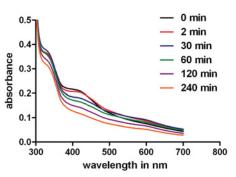
<sup>&</sup>lt;sup>2</sup> To whom correspondence should be addressed (email joerg.meurer@lrz.uni-muenchen.de).

Arabidopsis HCF101	303 : PEKKTIIPTEYMCYKINSFCEAGQGRAIMRGPMVSGVINOLITTTENGELDYLVIDMPPGTGDIOLITICOVAP
Oryza sativa	: PESRSILPTEYLGVKMVSFGFAGQGRAIMRGPMVSGVINOLITTTDWGELDYLVIDMPPGTGDIHLTLCQVAP
Populus	: PEKRTIIPTEYLGWKLVSFGEAGOGRAIMRGPMVSGVIDOLLTTTENGELDVLVIDMPPGTGDIOLTLCOVVP
Vitis vinifera	: PEKRSHIFTEYLCVKLVSFCEAGQGRAMRCPMVSGVINOLLTTTENGELDVLVIDMPPGTCDIOLILCOVVP
Physcomytrella	: PETRAIIPTEYLGVKLVSFGYAGQGSADMRGPMVSGVINOFLTTTDWGELDYLVIDMPPGTGDIOLTLCQVVP
Selaginella Volvox	: EDTKQIIPTEYLCVKIVSFCEAGQGTAIMRGPMVSGVINOLITTDWGELDYLIIDMPPGTCDIOLITLCOVVP : PATKAIFPTEYECVKVVSFCEAGQGSAIMRGPMVSGLIQOMITTAAWGELDYLVVDFPPGTCDIOLILCOTVS
Ostreococcus tauri	: KETGTIKPVEYECVKVVSFCBAGQGSAINRGPMVSGLINOLLTTTDWGELDYLIIDMPPGTCDV0LTLCCVVP
Micromonas	: KETGSUTPTEYECVGIVSFCEAGQGSAUMRGPMVSGLINOMLTTTAWCDHDYLIIDMPPGTCDVOLTICOVLP
Galdieria maxima	: DGRIIPLEYENVKLMSFGYINPESAIMRGPMIANMMNOLITETDWCSLDYLVIDMPPGTCDIQLTICOTVS
Cyanidioschyzon	: LTPDGLIMPETRACWKINASFCERINSDPADLRCHAMSSITETOEIIOOTDWCSIDDUIDUUPGCACDIALIUPGOVIK
Phaeodactylum	: GRQVAPLORNGWRLMSFGYVNDGSAVMRGPMVTQLDDOFLSVTHWGADDYDIDDMPPGTGDIODTLTOKUN
Thalassiosira Chloroflexus	: GRQHAPLERGDWSLMSFGYVNEGSATMRGPMVTQLLDOFLSLTNWCALDYLINDMPPGTCDTQLTLSORUN : DANGQPM-MLPLSNHGIXVMSVGFLID-ESQPVIWRGPMVSQLLROFLYQVAWAPLDYLIIDMPPGTCDVALTIAOSLP
Cytophaga	FNENCK DTHER TEOV OWNERSTOPPICED - AFCAVILIDED ACCARDOFT CD COMPANIES MDDCT CD THE DEVOTED
Nostoc punctiforme	STETGDI-LEPAFNHCVKLVSMCFLID-RDQPVLWRGPMLNGVLROFLYQVQWGELDYLIVDMPPGTCDAOLTITOAVP
Synechococcus	: KREDGGEDIFPLENYGVKMVSMCLUVG-RDQPVIWRGPMLNGVIROFLYQVQWGELDVLIVDMPPGTGDAQUTLVQAVP
Magnetococcus	: EKGQKVTEMBKYGVKIMSMGSFMP-EDTPMIWRGPMVGMAVECLIRDIDWGEMDYLVIDIPPGTGDAQLTLTOKVP
Salmonella	: DGTHWARINSHCHATNSICHAWT-DDNAMWICHWASKAWMOMOETLWPDWDWWWWWWICHCACONAN
Arabidopsis-L1	: DMKWHEVENYEWKEVSMELUVE-KDAPL/WKEPAV/MSAUAKMTKGVD/VEDUDIL/V/D/D/1926//CDA0USHS0/NUK
Chlamydomonas-Ll Homo sapiens	: SGTGALMLPKENYRVKTMSFGFFLE-GDEPVVWRGPMVNNAFDKM/FGTEWCLDVLVVDMPPGTGDAOIN/GGR/P : SNLWRPLLNYGIACMSMGFLVE-ESEPVVWRGLM/MSA/EKL/RQVDWGGLDYLVVDMPPGTGDVOLSVSON/P
Yarrowia lipolytica	: EGKLIPMSKFCHOVMSMCFLVD-PNKAVAWRCLLVQKALECLLQDVDMCTLDVLVMDLPPGTCDV0LTIACTVK
Arabidopsis-L2	: NLGWSPVYVEDNLGVMSIGFMLPNSDEAVIWRGPRKNGLIKOFLKDVYWGEIDYLVVDAPPGTSDEHISIVOYLL
Chlamydomonas-L2	: NLGWSPVYVEDNDCVMSIGFMLPNSDEAVIWRGPRKNGLIKOFMKDVWGEIDYLVVDAPPGTSDEHISIVOYML : GAGWSPVYVEDNDAVMSIGFMLPNPDEAVIWRGPRKNGLIKOFMKDVDWGELDYLVVDAPPGTSDEHITIT <u>OCL</u> O
Homo sapiens	: GSGWSPVYVEDNEGVMSVGFLLSSPDDAVIWRGPKKNGMIKOFURDVDWGEVDYDIVDTPPGTSDEHUSVVRYLA
Saccharomyces Nbp35	: NSGWTPVYVTDNPATMSIQYMLPEDDSAIIWRGSKKNLLIKKFIKDVDWDKLDYLVIDTPPGTSDBHISINKYMR : ddgwvpvytdes-qtlavmsigfilknredpviwrgpkktmmirafitdvrwdaldyliidtopgtsdehitvmealk
Drosophila Homo sapiens	: ddgwypvytaes-qtHavmeigflikhredpviwigskktmmirdflitdvrwdeidylidetsgentsgentvmeelk : drgwapvfldre-qsisimsvgfllekpdeavvwrgskknalikgfvsdvawgeldylvvdtppgtsdehmaticalr
Danio rerio	: DSGWVPVYADPQQQQHALMSIAFLLEDSDEAVIWRG>KKTALIGOFVSDVAMCELDIMAVDTPPGTSDEHDAVLONIR
Saccharomyces Cfb1	: DSGWVPVYADPQQQQHALMSIAFILEDSDEAVIWRGPKKTALIGOPVSDVAWGELDILLVDTPPGTSDEHLAVLENUR : PEGWQPV-KVETNST-GSLSVISLGFLLGDRGNSVIWRGPKKTSMIKOFISDVAWGELDVLLIDTPPGTSDEHISIABEIR
	339 347
Arabidopsis HCF101	
Oryza sativa	: LTAAVIVTTPCKLAFIDVAKGVRMES-KIKVPCVAVVENMCYFDADGKRFYPFCQCSGAQVVQQFGIP : LTAAVIVTTPCKLAFIDVAKGVRMES-KIKVPCVAVVENMCHFDADGKRYYPFCRCSGSQVVQQFGIP
Populus Vitis vinifera	: LTAAVIVTTPOKDAFIDVAKGVRMESKUKVPCVAVVENMOHEDADGKRYYPECRESGSQVVQQFGIP : LTAAVIVTTPOKDAFIDVAKGVRMESKUKVPCVAVVENMOHEDADGKRYYPECRESGSQVVQOFGIP
Physcomytrella	: LTAAVIVTTPOKLAFIDVAKGVRMESKLKVPCVAVVEAMCHEDADGKRYYPFCRCSGSQVVQQFGIP : LTAAVIVTTPOKLAFIDVAKGVRMESKLKVPCIAVVEAMCFFEGDDKRYYPFCKCSGSKVVEQFGIP
Selaginella	TTAAVIVITECKLAFIDVAKGVRMESKUKVPCLAVVENMOYEDADGKRYYPECRCSGKOVVOOFGIS
Volvox	: FSAAVIVTTPCKLAFIDVAKGIRMFAKLVVPCVAVVENMSYFEADGKRFFPFCQGSGERIQRDFGLP
Ostreococcus tauri	: ITAAVVVTTPOKLAFIDVEKGVRMFAKLAVPCVSVVBNMSYFEVDGVKHKPFGEGSGAKICEOYGVP
Micromonas	: ITAAVVVTTPOKLAFIDVEKGVRMFSKLRVPOVAVVENMSYFDGDDGKRYKPFGEGSGQRICDDYGVP
Galdieria maxima	: LDAAVIVTTPQQLSFQDVIKGIQMEGKVSVPCVALVENMAYEPNDIPDKRYYD-CHCKSQKIANDYGIP : ATAAVVVTTPQRLAFADVVKGIQLLDKMAVPPIAVVESMAYEVAPDTGKRYDL-CKCHSARISREFGIR
Cyanidioschyzon Phaeodactylum	: ATAAVVVTTPORTAFADVVKCLQ/LDKMAVEPTAVVESXAY:VAPDTGKTYDLFCKCHSARISREFCIR : TTAAVIVTTPORTSFADVVKCVEMEDTVNVPCHAVVENVAYMESADPEKIQIFCACHRDRISQQWGIE
Thalassiosira	: ITAAVIVTPOELSEVDVERGVEMEDTVNVPCTAVVENMAYLEREETEMIRIEGPCHKRRUSEOWGIE
Chloroflexus	: MAGKRYDISCSCGAER AOOLCM
Cytophaga	: VTGAIVVTTPCKVAIADAQRGLQMERQPQVNVPVDGVIENMAWET-PAELPENKYYIFCKDGGKELAEKFDVP : MAGAVIVTTPCTVALDDSRKGLRMEQQMNVPVDGLVENMSYFIPPDQPDKHYDIFCSGGGSKTAAELGVP
Nostoc punctiforme	: MAGAVIVTTPOTVALIDSRKGIRMEQQ_NVPVLGLVENMSYFIPPDQPDKHYDIFCSCGGSKTAAELGVP
Synechococcus	A LAC
Synechococcus Magnetococcus	A LAC
Synechococcus Magnetococcus Salmonella	: LAGAVIVTTPOSVALLDSRKGINMERQLGVPILGIVENMSYFIPPDLPDRQYDIFGSEGGETTARELGVP : LSGVVIVSTPODVALADVRKGINMEKKVEVPVLGIIENMSY/LCTEGGHRAEIFSEGAEKEAANSGAT : VTGAVVVTTPODIALTDAKKGIVMEKVEVPVLGVENMSMHIGSKGGHHREIFFGGAQKLAEKHTQ
Synechococcus Magnetococcus	: LSGAVIVTTPQSVALLDSRKGINMERQLGVPILGIVENMSYFIPPDLPDRQYDIFGSEGGETARELGVP : LSGVVIVSTPQDVALADVRKGINMEKKVEVPVLGIIENMSY/LCTEGGHRAEIFSEGAEKEAANSGMT : VTGAVVVTTPQDIALDDAKKGIVMEKVEVPVLGVENMSMHIGSKGGHHPIFGTGGACKAEKYHTQ : LSGAVIVSTPQDVALADANRGISMEDKVRVFILGVENMSCFVCPHCNEPSFIFGKEGARTAAKKGIK LSGAVIVSTPQDVALADANRGISMEDKVRVFILGVENMSCFVCPHCNEPSFIFGKEGARTAAKKGIK
Synechococcus Magnetococcus Salmonella Arabidopsis-L1	: LAGAVIVITEQSVALDSEKGINMER-OUGVEUGVEMMSYILEPDLEDRQVDIEGSEGGTTARELGVE : LSGVVIVSTEQDVALDVEKGINMEKKVEVFVLGIIENMSYLLTEGGHEREISH GAEKEAANSGMT : VTGAVIVTEPOIALTDAKKGIVMEKVEVFULGVENMSGMIGSNCGHHEFFETGGAQKLAKKHKGY : LSGAVIVSTEQDVALDADANGISMEDKVEVFILGVENMSCVCFHCNEPSFIFCKEGARRTAAKKGK : LSGAVIVSTEQDVALDVER-AQMILKUEVFILGVENMSCVCFHCNEPSFIFCKEGARRTAAKKGK : LSG
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica	: LAGAVIVTTPQSVALLDSRKGLNMERQLGVPILGIVENMSYTIPPDLPDRQYDIFGSEGGETTARELGVP : LSGVVIVSTPQDVALADVRKGINMEKKVEVPVUGITENMSYTLGTEGHRAEIFSHGGAKKAANSGMT : VTGAVVVTTPQDIALTDAKKGIVMEEKVEVPVUGIVENMSMHIGSNGGHHEPIFGTGAQKLAEKYHTQ : LSGAVIVSTPQDVALADANRGISMD
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2	: IAGAVIVTTPCSVALLDSRKGINMERQUGVEILGTVERMSYTIPPDLPDRQYDIFCSEGGETTARELGYP : LSG
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2	: IAGAVIVTTPCSVALLDSRKGINMERQUGVEILGTVERMSYTIPPDLPDRQYDIFCSEGGETTARELGYP : LSG
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2 Homo sapiens	: IAGAVIVTTPCSVALLDSRKGINMERQUGVEILGTVERMSYTIPPDLPDRQYDIFCSEGGETTARELGYP : LSG
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2	: IAGAVIVTTPCSVALLDSRKGINMERQUGVEILGTVERMSYTIPPDLPDRQYDIFCSEGGETTARELGYP : LSG
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2 Homo sapiens Saccharomyces Nbp35	: IAGAVIVTTPCSVALLDSRKGINMERQUGVEILGTVERMSYTIPPDLPDRQYDIFCSEGGETTARELGYP : LSG
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2 Homo sapiens Saccharomyces Nbp35 Drosophila Homo sapiens Danio rerio	: IAGAVIVTTPCSVALLDSRKGINMRQUGVELCGVERMSYTLFPDLPDRQYDIFCSEGGETTARELGVP : LSG
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2 Homo sapiens Saccharomyces Nbp35 Drosophila Homo sapiens Danio rerio	IAGAVIVTTPOSVALDSKGINMERQIGVEILGTVERMSYITPDLPDRQYDIFOSEGGETTARELGYP       IAGAVIVTTPOILADURKGINMEKRVEVPUIGTIENMSYILETEGHRAFISH GAEKEANSGAT       VTGAVIVTPOILADURKGINMEKVEVPUIGTIENMSYLLETEGHRAFISH GAEKEANSGAT       ISGAVIVTPOILADARGISMDKVRVPIIGIVENMSC-VCPHCNEPSFIFOKEGARTARKGIK       ISG
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2 Homo sapiens Saccharomyces Nbp35 Drosophila Homo sapiens Danio reric Saccharomyces Cfb1	: hGG
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2 Homo sapiens Saccharomyces Nbp35 Drosophila Homo sapiens Danio rerio Saccharomyces Cfb1 Arabidopsis HCF101	<pre>IAGAVIVTTPCSVALDSRKGINMERQIGVEILGVERMSYIJPDLPDRQVDFCSEGGTTARELGVP ILSGAVIVTTPCDIALDSRKGINMEKKVEVFVIGIIENMSYLLTEGGHREITSTCGAGKEANSGMT VTGAVIVTPCDIALDDRKGINMEKVEVFVIGUENMSYLLTEGGHREITSTCGAGKEANSGMT ILSGAVIVSTPCDIALDDRKGINMEKVEVFUIGUENMSCTVCPHCNEPSFIFCKEGARRTAAKKGIK ILSGAVIVSTPCDIALDDRK-AQMILKURVFILGIVENMSCTVCPHCNEPSFIFCKEGARRTAAKKGIK ILSGAVIVSTPCDIALDDRK-AQMILKURVFILGIVENMSCTVCPHCNEPSFIFCKEGARRTAAKKGIK ILSG</pre>
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2 Homo sapiens Saccharomyces Nbp35 Drosophila Homo sapiens Danio reric Saccharomyces Cfb1	<pre>: AGAVIVTTCQVALDSRKGINME Q'GVEILGVENMSYILPDLPDRQVDFCSEGGTTARELGVE : LSGAVVVTTCQIALDDRKGINME KVEVFVIGILENMSYLLTEGGHRAEISH GAEKEANSGMT : VTGAVVVTTCQIALDDRKGINME KVEVFVIGILENMSYLLTEGGHRAEISH GAEKEANSGMT : LSGAVVVTTCQIALDDRKGINME KVEVFVIGVENMSCLVENCGGHREFFCTGAQKARKYHTQ : LSGAVVVTTCQIALDDRKARME KVEVFVIGVENMSCLVENCGGHVEHFCTGGVERAADYGND : TTG</pre>
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2 Homo sapiens Saccharomyces Nbp35 Drosophila Homo sapiens Danio rerio Saccharomyces Cfb1 Arabidopsis HCF101 Oyyza sativa Populus Vitis vinifera	<pre>IAGAVIVTTPQSVALDSRKGINMRR-OGGVFLGTVERMSYILPDLPDRQVDFCSEGGTTARELGYP ILSGAVIVTTPQDIALDDRRGINMRKVEVFVLGTIENMSYLLTEGGHRAITSH GACKAARSGMT VTGAVIVTPQDIALDDRRGINMEKVEVFVLGVENMSYLLTEGGHRAITSH GACKAARSGMT ILSGAVIVSTPQDVALDDRRGISMDKVRVFLLGVENMSCVCPHCNEPSFIFCKEGARRTAAKKGLK ILSGAVIVSTPQDVALDDRRGISMDKVRVFLLGVENMSCVCPHCNEPSFIFCKEGARRTAAKKGLK ILSGAVIVSTPQDVALDDRRGISMDKVRVFLLGVENMSCVCPHCNEPSFIFCKEGARRTAAKKGLK ILSG</pre>
Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Homo sapiens Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2 Homo sapiens Saccharomyces Nbp35 Drosophila Homo sapiens Danio reric Saccharomyces Cfb1 Arabidopsis HCF101 Oryza sativa Populus Vitis vinifera Physcomytrella	<pre>IAGAVIVTTPOSVALDSRKGINMRRQUGVELGTVERMSYITPDLPDRQYDFCSRGGTTARELGYP ILSGNVVTTPOILADDRKGINMRKVEVPUIGTIENMSYLLTEGGHREFFFTGGAGKKANSKT VTGAVIVSTPOILADDRKGINMEKVEVPUIGVENMSGLVOPHONEPSFIFCKEGARTAAKKGK ILSGAVIVSTPOILADDARKGINMEKVEVPUIGVENMSC-VOPHONEPSFIFCKEGARTAAKKGK ILSGAVIVSTPOILADDARKGINME</pre>
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Arabidopsis HCF101     : NS DEEFLLHPATVRNDRSAQS-VDEWTGEQKVLYGDVAEDIEPEDIEPEGIAPMGNYAVSITWPDGFSQIAPYDQL       Oryza sativa     : DS DEEFLLHPATVRNDRSAQS-VDEWTGEQKUQY GDVPEDIEPEEIRPMGNYAVSITWPDGFSQIAPYDQL       Vitis vinifera     : DS EEEFLLHPATVRNDRSAQS-VDEWTGEQKLQY ADVPEDIEPEEIRPMGNYAVSITWPDGFSQIAPYDQL       Selaginella     : GT TEEFLLHPATVRNDRSAQS-VDEWTGEQKLQY ADVPEDIEPEEIRPMGNYAAUSITWPDGFSQIAPYDQL       Selaginella     : GT TEEFLLHPATVRNDRSAKS-IDEWTGEQKLRY GDVREDIEPEEIRPMGNYAAUSITWPDGFSQIAPYDQL       Soloscoccus tauri     : GEN DEEFLLHPATVRNDRSAKS-IDEWTGEQKLRY GDVREDIEPEAIQPLGNYAANINFWPGFSQIAPYDQL       Soloscoccus tauri     : GEN		
Populus:DSEEEFLLHPATVRRNDRSAQS-VDEWTGEQKLQYADVPEDIEPEEIRPMGNYAVQITWPDGFSQIAPYDQLVitis vinifera:DSEEEFLLHPATVRRNDRSAQS-VDEWTGEQKLQYADVPEDIEPEEIRPMGNYANSITWPDGFSQIAPYDQLSelaginella:: <td:< td="">::<td:< td="">:<td::< t<="" td=""><td></td><td>: NSDEEFLLHPATVRRNDRSAQS-VDEWTGEQKVLYGDVAEDIEPEDIRPMGNYAVSITWPDGFSQIAPYDQL</td></td::<></td:<></td:<>		: NSDEEFLLHPATVRRNDRSAQS-VDEWTGEQKVLYGDVAEDIEPEDIRPMGNYAVSITWPDGFSQIAPYDQL
Vitis vinifera: DS:EEEFLLHPATVRRNDRSAQS-VDEWTGEQKLQYADVPEDIEPEEIRPMGNYAVSITWPDGFSQIAPYDQLPhyscomytrella: GT:TEEFLLHPATVRRNDRSAKS-IDEWTGEQKLRY:DVAEDLAPESIRPMGNYAAAINNPDGFSQIAPYDQLSelaginella: GT:EEPFLHPATVRRNDRSAKS-IDEWTGEQKLRY:DVAEDLAPESIRPMGNYAAVINSPDGFNQVASYDQLVolvox: G:EEFFLLPPVVVRRNDRSAKS-IDEWTGEQKLRY:DVPQDARPAAINPLGNYAVQISWSDGFNQVASYELLStreococcus tauri: GENN:DKAFWITAKNVRLSDESARVKSSDESPRLLNGAPIPDDIAPVEAHVVGNYAVQISWSDGFNQVASYELMicromonas: DQQCIAWNENNKVEWSPWELRNA'SAS-DGESKKADFLMDGVTPVPDDIAPVEAHVVGNYAVQISWDGFSQVATFAQIGaldieria maxima: DQQCIAWNENNKVEWSPWELRNA'SAS'-DEETGRMLKK:SVDDN'YPMQMMNVGNYAVQISWDGGSVATFAQIPhaeodactylum: DQCSTDEEHVATLPPAELRRA'CR'AAC-VEELTGRQLLDPNSVDDN'YPMQMMNVGNYALSVMSDGRQS'MPMERPPhaeodactylum: DINATISPAELRRG'CR'AAC-VEELTGRQILVPSSVSKKAPNMVPTGNYALSVDWSDGHRSLYPYRQIChloroflexus:Synechococcus:Salmonella:Arabidopsis-L1:Homo sapiens:Saccharomyces Nbp35:Drosophila:Homo sapiens:Drosophila <td:< td="">Homo sapiens:Saccharomyces Nbp35:Source:Source:Source:Source:Source:Source:Source:Source:Source:Source:Source:Source:Source:Source:Source:<!--</td--><td></td><td></td></td:<>		
Physcomytrella     : GTTEEFLLHPATVRRNDRSAKS-IDEWSGEQKLRYTDVAEDLAPESIRPMGNYAAAINWPDGFSQIAPYDQL       Selaginella     : GTEEFFLUPATVRRNDRSAKS-IDEWGEQKLRYGDVREDIRPEATQPLCNYAVMISWPDGFNQVAPYDQL       Volvox     : GETEFLUPATVRRNDRSAKS-IDEWGEQKLRYGDVREDIRPEATQPLCNYAVMISWPDGFNQVAPYDQL       Ostreococcus tauri     : GENNDKAFWITAKNVRLSDESARVKGSDESPDLLGAPTPDDIAPVEMAVVQISWSDGFNQVASVELL       Ostreococcus tauri     : GENNDKAFWITAKNVRLSDESARVKGSDESPDLLGAPTPDDIAPVEMAVVGNYAVQISWSDGFSQVAFFAQI       Galdieria maxima     : DQQGIAWNENNKVEWSPWELNAGSCASC-VDEFTGKRHWKSVDRNVKPLQIQTAGNYAFSVIWSDGHQSIYPFERV       Cyanidioschyzon     : DHMEERIQPAALRRACCAAC-VDECTGKQILDPNSVDDNIYPMOMNVONYALAVWSDGHQSIYPFERV       Cyanidioschyzon     : DAHSEFFLEPALRRACCAAC-VEELTGRQILDPSVSDKIAPRNMVPTGNYALSVDWSDGHRSIYPYRQI       Thalassiosira     : DIQNATISPAELRRACCAAC-VEELTGRQILPSVSDKIAPRNMVPTGNYALSVDWSDGHRSIYPYRQI       Chloroflexus     :ATISPAELRRGCRAAC-VEELTGRQILPSVSDKIAPRNMVPTGNYALSVDWSDGHRSIYPYRQI       Salmonella     :ATISPAELRRGCRAAC-VEELTGRQILPSVSDKIAPRNMVPTGNYALSVDWSDGHRSIYPYRQI       Chlamydomonas-L1     :	Populus	: DSEEEFLLHPATVRRNDRSAQS-VDEWTGEQKLQYADVPEDIEPEEIRPMGNYAVQITWPDGFSQIAPYDQL
Selaginella     : GTEEPFYLHPATVRRNDRSAKS-IDEWTGEQKLRYGDVREDIEPEAIQPLGNYAVMISWPDGPNQVAPYDQL       Color     : GFEEPFYLHPATVRRNDRSAKS-IDEWTGEQKLRYGDVREDIEPEAIQPLGNYAVMISWPDGPNQVAPYDQL       Gatrecoccus     : GFNEEFFLLPPVVVRENDTSATS-IDEWTGEQKLRYDEVPQDARPAAINPLGNYAVQISWSDGFNQVASYELL       Micromonas     : LRVQLADEGGMPFYVRGCDVRRSDKSATA-DGESKKADPLNDG-VPVPDDIAPVEAHVVGNYAVQISWPDGFSQVATFAQI       Cyanidischyzon     : DQQGIAWNENNEVEWSPWELRNA'SSAS'-VDEETGKRHWKSVDRNVRPLQUQTAGNYAFSUIWSDGHQSIYPFERP       Phaeodactylum     : GVGSTDEEHVATLPPAELRRA'GR'AA'C-VEELTGRQILUPSVDSNVRPLQYMNNVGNYALSVWSDGHQSIYPFERP       Chloroflexus     :       Cytophaga     :       Sandoncoccus     :       Arabidopsis-L1     :       Chlamydmonas-L2     :       Homo sapiens     :       Prosophila     :       Homo sapiens     :       Drosophila     :       Homo sapiens     :       Drosophila     :       Homo sapiens     :       Drosophila     :       Homo sapiens     :       Danio revio     :		
Volvox: G: ETEFLLPPVVVRENDTSATS-IDEWTGQRK-R: DEVPQDARPAAINPLGNYAVQISWSDGPNQVASYELLOstreococcus tauri: GENN: DKAFWITAKNVRLSDESARVKGSDSPDRLLNGAFIPDDIAPVEMSVIGNYAMSITWPDGLSQVAAPSTELMicromonas: LuVQLADEGGMPFVVRGCVVRSDKSATA-DGESKKADPLMGVPYDDIAPVEMSVIGNYAMVQISWPDGPQVAAPGIGaldieria maxima: DQQGIAWNENNKVEWSPWELRNA'S CAS' - VDEFTGKRHWK SVDRNVKPLQIQTAGNYAFSVIWSDGHQSLYPPERVCyanidioschyzon: DHMEERIQPAALRRA'R CAAC' - VDEFTGKRHWK SVDRNVKPLQIQTAGNYAFSVIWSDGHQSLYPPERVPhaeodactylum: GVGSTDEEHVATLPPAELRRA'R CAAC' - VEELTGRQILVPNSVDNIYPMQMMVVGNYALAVMSDGHQSLYPPERVChloroflexus:	Physcomytrella	: GTTEEFLLHPATVRRNDRSAKS-IDEWSGEQKLRYTDVAEDLAPESIRPMGNYAAAINWPDGFSQIAPYDQL
Ostreococcus tauri     : GENNDKAFWITAKNVRLSDESARVKGSDESPDRLLNG - APIPDDTAPVEMSVIGNYAMSITWPDGLSQVAAFSTL       Micromonas     : LRVQLADEGGMPFYURGCDVRRSDKSATA-DGESKKADFLMGGVTPVPDDIAPVEAHVVGNYAVQISWPDGFSQVATFAQI       Galdieria maxima     : DQQGIAWNENNKUWSPMELIRNASSS-VDEFTGKRHWKSVDRNVKPLQIQAGNYAPSVUWSDGHQSIYPFERV       Cyanidioschyzon     : DHMEERIQPAALRRACRCAAC-VDECTGKQLLDP - NSVDDNYKPLQIQAGNYALSVDWSDGHQSIYPFERV       Chaedactylum     : GVGSTDEEHVATLPPAELRRACRCAAC-VEELTGRQILUPSSVSDKIAPRNMVPTGNYALSVDWSDGHRSIYPYRQI       Chloroflexus     :ATISPAELRRGCRCAAC-VEELTGRQILUPSSVSDKIAPRNMVPTGNYALSVDWSDGHRSIYPYRQI       Cytophaga     :ATISPAELRRGCRCAAC-VEELTGRQILUPSSVSDKIAPRNMVPTGNYALSVDWSDGHRSIYPYRQI       Salmonella     :ATISPAELRRGCRCAAC-VEELTGRQILUPSSVSDKIAPRNMVPTGNYALSVDWSDGHRSIYPYRQI       Chlamydomonas-L1     :	Selaginella	: GTEEPFYLHPATVRRNDRSAKS-IDEWTGEQKLRYGDVREDIEPEAIQPLGNYAVMISWPDGFNQVAPYDQL
Micromonas     :     LRVQLADEGGMPFYVRGCDVRRSDKSATA-DGESKKADFLMDGVTPVPDDIAFVEAHVVGNYAVQISWPDGFSQVATFAQI       Galdieria maxima     :     DQQGIAWMENNRVEWSPWELRNASSAS-VDEFTGKHHMKSVDRNVRPLQTQTAGNYAFSTUMSDGHQSIYPFERV       Cyanidioschyzon     :     DHMERTQPALRRARCAA-VDECTGKQLLDP-SVDDNTYPMQMNVGNYALAVWSDGHQSIYPFERV       Phaeodactylum     :     GVGSTDEEHVATLPPAELRRACROAAC-VEELTGRQILVP-SSVSDKTAFRNMVPTGNYALSVDWSDGHRSIYPYRQT       Chloroflexus     :     DIQNATISPAELRCGRCAAC-VEELTGRQILVP-ASISSSVKPLNMSPTGNYALSVDWSDGHRSIYPYRQT       Chloroflexus     :     :       Synechococcus     :     :       Synechococcus     :     :       Arabidopsis-L1     :     :       Homo sapiens     :     :       Chlanydmonnas-L2     :     :       Homo sapiens     :     :       Immodes     :     :       Prosophila     :     :       Homo sapiens     :     :       Drosophila     :     :       Homo sapiens     :     :       Drosophila     :     :       Homo sapiens     :     :       Drosophila     :     :	Volvox	: GETEFLLPPVVVRENDTSATS-IDEWTGQRK-RDEVPQDARPAAINPLGNYAVQISWSDGFNQVASYELL
Galdieria maxima     :     DQQGIAWNENNKVEWSPWELRNA CSOASC -VDEFTGKRHWKSVDRNVKPLQIQTAGNYAFSVIWSDGHQSLYPFERV       Cyanidioschyzon     :     :     :       Phaeodactylum     :     :     :       Phaeodactylum     :     :     :       Cyophaga     :     :     :       Cytophaga     :     :     :       Salmonella     :     :     :       Arabidopsis-L1     :     :     :       Chanydomonas-L1     :     :     :       Homo sapiens     :     :     :       Saccharomyces Nbp35     :     :     :       Drosophila     :     :     :       Homo sapiens     :     :     :       Saccharomyces Nbp35     :     :     :       Drosophila     :     :     :     :       Bonio rerio     :     :     :     :       Superior S	Ostreococcus tauri	: GENNDKAFWITAKNVRLSDESARVKGSDESPDRLLNGAPIPDDIAPVEMSVIGNYAMSITWPDGLSQVAAFSTL
Cyanidioschyzon     :       Phaeodactylum     :       GVGSTDEEHVATLSPARLRRACKCAAC-VEELTGRQILUPSSVSDKIAPRNMVPTONYALSVDWSDGHRSLYPYKQI       Chloroflexus     :       Cytophaga     :       Nostoc punctiforme     :       Synechococcus     :       Arabidopsis-L1     :       Chlamydomonas-L1     :       Homo sapiens     :       Saccharomyces Nbp35     :       Drosophila     :       Homo sapiens     :       Drosophila     :    Danio rerio     : <td></td> <td></td>		
Phaeodactylum     :     GVGSTDEEHVATLPPAELRAACROAC-VEELTGRQILVP-SSVSDKTAFRNMVPTGNYALSVDWSDGHRSLYPYRQI       Thalassiosira     :     DIQNATISPAELRACROAC-VEELTGRQILNPASISSSVRPLNMSPTGNYALSVDWSDGHRSLYPYRQI       Chloroflexus     :	Galdieria maxima	: DQQGIAWNENNKVEWSPWELRNACSCASC-VDEFTGKRHWKSVDRNVKPLQIQTAGNYAFSVIWSDGHQSLYPFERV
Thalassiosira     DIQNATISPAELRCGROALC-VEELTGKQILNPASISESVKPLNMSPTGNYALSVDWSDGHRSLYPYRQI       Chloroflexus		: DHMEERIQPAALRRACRCAAC-VDECTGKQLLDPNSVDDNIYPMQMMNVGNYALAVNWSDGHQSIMPWERF
Chloroflexus : Cytophaga : Nostoc punctiforme : Synechococcus : Magnetococcus : Arabidopsis-L1 : Chlamydomonas-L1 : Homo sapiens : Yarrowia lipolytica : Arabidopsis-L2 : Chlamydomonas-L2 : Saccharomyces Nbp35 : Drosophila : Homo sapiens : Saccharomyces Nbp35 : Saccharomyces Nbp	Phaeodactylum	: GVGSTDEEHVATLPPAELRRACROAAC-VEELTGRQILVPSSVSDKIAPRNMVPTGNYALSVDWSDGHRSLYPYRQI
Cytophaga     :       Nostoc punctiforme     :       Synechocccus     :       Magnetococcus     :       Salmonella     :       Arabidopsis-L1     :       Chlamydomonas-L1     :       Homo sapiens     :       Chlamydomonas-L2     :       Romo sapiens     :       Imagina (Construction)     :       Chlamydomonas-L2     :       Homo sapiens     :       Imagina (Construction)     :	Thalassiosira	: DIQNATISPAELRRGCRCAAC-VEELTGKQILNPASISESVKPLNMSPTGNYALSVDWSDGHRSLYPYRQI
Nostoc punctiforme Synechococcus Magnetococcus Salmonella Arabidopsis-L1 Chlamydomonas-L1 Yarrowia lipolytica Arabidopsis-L2 Chlamydomonas-L2 Endianydomonas-L2 Saccharomyces Nbp35 Drosophila Homo sapiens Saccharomyces Nbp35 Drosophila Homo sapiens Saccharomyces Nbp35	Chloroflexus	
Synechococcus     :       Magnetococcus     :       Salmonella     :       Arabidopsis-L1     :       Chlamydomonas-L1     :       Homo sapiens     :       Arabidopsis-L2     :       Chlamydomonas-L2     :       Saccharomyces Nbp35     :       Drosophila     :       Bomo sapiens     :       Saccharomyces Nbp35     :       Prosophila     :       Bomo sapiens     :       Saccharomyces Nbp35     :       Saccharomyces Nbp35     :       Chono sapiens     :       Saccharomyces Nbp35     : </td <td>Cytophaga</td> <td></td>	Cytophaga	
Magnetococcus     :       Salmonella     :       Arabidopsis-L1     :       Chlamydomonas-L1     :       Homo sapiens     :       Arabidopsis-L2     :       Arabidopsis-L2     :       Chlamydomonas-L3     :       Arabidopsis-L2     :       Chlamydomonas-L2     :       Bomo sapiens     :       Saccharomyces Nbp35     :       Drosophila     :       Homo sapiens     :       Sacharomyces Nbp35     :		
Salmonella : Arabidopsis-Ll : Chlamydomonas-Li : Yarrowia lipolytica : Arabidopsis-L2 : Chlamydomonas-L2 : Saccharomyces Nbp35 : Drosophila : Danio rerio :	Synechococcus	
Arabidopsis-L1 : Chlawydomonas-L1 : Homo sapiens : Arabidopsis-L2 : Chlawydomonas-L2 : Saccharomyces Nbp35 : Drosophila : Homo sapiens : Saccharomyces Nbp35 : Drosophila : Homo sapiens : Saccharomyces Nbp35 : Drosophila : Homo sapiens : Hom		
Chlamydononas-L1   :     Homo sapiens   :     Arabidopsis-L2   :     Arabidopsis-L2   :     Bomo sapiens   :     Saccharomyces Nbp35   :     Drosophila   :     Homo sapiens   :     Bomo sapiens   :	Salmonella	
Homo sapiens : Yarrowia lipolytica : Arabidopsis-L2 : Chlamydomonas-L2 : Bomo sapiens : Saccharomyces Nbp35 : Drosophila : Homo sapiens : Danio rerio :	Arabidopsis-L1	
Yarrowia lipolytica Arabidopsis-L2 : Chlamydomonas-L2 : Saccharomyces Nbp35 : Drosophila : Danio rerio :	Chlamydomonas-L1	
Arabidopsis-L2     :       Chlamydomonas-L2     :       Homo sapiens     :       Saccharomyces Nbp35     :       Drosophila     :       Homo sapiens     :       Janio rerio     :	Homo sapiens	
Action (abg) (abg)		
Homo sapiens : Saccharomyces Nbp35 : Drosophila : Danio rerio :		•
Saccharomyces Nbp35 : Drosophila : Homo sapiens : Danio rerio :		•
Drosophila : Homo sapiens : Danio rerio :		•
Homo sapiens : Danio rerio :	Saccharomyces Nbp35	•
Danio rerio :		
Saccharomyces Cfbl :	Danio rerio	•
	Saccharomyces Cfb1	:

#### Figure S1 Multiple sequence alignment of the FSC-NTPase family

Representative protein sequences of different organisms are shown subdivided according to the proposed four FSC-NTPase classes [23]. Organism names are coloured according to the class affiliation. Green background represents plants and algae taxa belonging to the class I. Yellow background represents eubacterial class I proteins. Grey background corresponds to class II members. Blue and magenta congregate taxa belonging to the class III and class IV respectively. Note that algae and plants class I proteins have a C-terminal extension corresponding to the DUF971 domain (COG3536). The very N-terminal and C-terminal regions are not shown in the alignment as they show little conservation. Amino acids coloured with a black background are 100% conserved. Amino acids coloured with a grey background and written with black characters are >40% conserved. Cysteine residues conserved in at least two sequences are depicted with a blue background. Numbers above the conserved cysteine residues in plants and algae correspond to the position of the residue in the sequence of the *Arabidopsis* HCF101 protein.



## Supplementary Figure S2 Oxygen sensitivity of the reconstituted HCF101 protein

Purified HCF101 protein was reconstituted as described in the Experimental section in the main paper. Decay of the shoulder at 420 nm in the presence of oxygen was monitored by UV–visible spectroscopy.

# Supplementary Table S1 Oligonucleotides used for site-directed mutagenesis and cloning

For details please see the Experimental section in the main paper. for, forward primer; rev, reverse primer.

Designation	Sequence
C102S-for	5'-GGAGCTGACAACACCCGCATcTCCAGTCAAAGAC-3'
C102S-rev	5′-GAAACAATATCTGTCCCAAAATCAGGATC-3
C128S-for	5'-CATCATCGCTGTTTCTAGTTctAAGGGTGGTG-3'
C128S-rev	5'-GTGTTGTCAGCTCCAAACGGAACGAAACCTC-3'
C184S-for	5′-GATATACAACTGACCTTATctCAGGTTGCGC-3′
C184S-rev	5'-CTAGAAACAGCGATGATGTTCGAAATTC-3'
C303S-for	5'-CTCAAAACTTAAGGTGCCTT <i>ct</i> GTTGCTGTTGTG-3'
C303S-rev	5′-TAAGGTCAGTTGTATATCACCAGTTCCAG-3′
C339S-for	5'-CTCAAAACTTAAGGTGCCTTctGTTGCTGTTGTG-3'
C339S-rev	5′-GCACCTTAAGTTTTGAGAACATCCTTACAC-3′
C347S-for	5'-GCTGTTGTGGAGAATATGTctCACTTTGACGC-3'
C347S-rev	5'-CATATTCTCCACAACAGCAACGCAAGGC-3'
C414S-for	5'-CGTTCCAGGATCTTGGTGTATcTGTAGTGCAAC-3'
C414S-rev	5'-CACCAAGATCCTGGAACGTTCTGGCAACG-3'
C419S-for	5′-GTGTATGTGTAGTGCAACAATctGCCAAGATAC-3′
C419S-rev	5′-TGTTGCACTACACATACACCAAGATCCTGGA-3′
INCORP-for	5'-GTAGAATTCTCAGCTCAAGCTAGTAGTAGTGTTGG-3'
INCORP-rev	5'-GTAGAATTCGACTTCGACTGGAGACAATGGAGG-3'

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## SUPPLEMENTARY ONLINE DATA Chloroplast HCF101 is a scaffold protein for [4Fe-4S] cluster assembly

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	102 128
Arabidopsis HCF101	: EKDULKA SQIIDEDFGTDEVSCOFVKDGINEALGEVSFREELTPACEVKDMFENKANEVWAAEPWVKKVNUTMSA
Oryza sativa	: KKDVLVALSQIID-DFGTDIVS_GFVKDIEISEALEEVSFRIELTTPACFIKDMFEEKANEVVAALPWVKKVNVTMSA
Populus	: ESDVLKALSQIID-DFGTDIVS_GFVKDINIDEAQGEVSFRUELTTPACFVKDMFEQKANEVVALLPWVKNVEVTMSA
[35] A. B. M.	: EADVLKALSQIID-DFGTDIVS_GFVKDIQINEALGEVSFR-ELTTPACFIKDMFEQKANEVVAM_PWVKNVNVTMSA
	: KKDVLGAVSQIID-DFGTDVTCGFVKETVDESTGEVSFQVELTPACPVKDMFEQQAKEKVSAVPWVKGVNVKMA
Selaginella	: GHDVLVALSTIID-DFGADIVT-GFVKDIQADKSSGEVAFR ELTTPACFVKDMFEQQAKEKVAALPWVTNVKVTMSA
Volvox	: EEQULAK RNVID DFGED VACGFVROI EVDASVGFVSFT ELTTPACFVKEMFOROSTOFVKE PWVRDVS KL A
Ostreococcus tauri	: ESEVISK RRVID DFGED VN GFVKALVIDESAGSVIFA ELTTPAC VKAEFEROAKAFVEE DWVKRVSVTMA
Micromonas	: EADVINATENVID-DFGEDTVNCGFVKDIRVSDAGDVTFTTELTTPAC-VKEEFDRLSKQVVTATEWAKSCNVNM-A
Galdieria maxima	: QKQVLELKNIED-DLKQNIVELGFVQN-ERVAKEDGKYDVRFT-QLTTPACPIKEKFQNDAKEWVSS-LWVRNVE-DLRA
Cyanidioschyzon	: TEQLLSA KAVVD DLGQDIVTLGFVKNI-QFGGDEHYGTVSFDVELTTPACPIKERFREECTRLAES PFVTRANVRLA
Phaeodactylum	: QGEVLST KSVIDPDLGSD VTLGFVQNKLDGRDVSFDVELTTPACPVKEQFQLDCQQLVQD PWTNNIQVTM A
Thalassiosira	: QSQILAA SVIND DLNAD VSLGFVQNKIDESSNIVSLD ELTTPAC PVKDLFVQQ QDI NG AWTRGAD VL S
Chloroflexus	: EDQ LAA ROVQEPELGGD VSRQMVKHAICDGIVRCT ELTTPACPLKDQIRSEAEAAVLAVPGVREVH EF A
Cytophaga	: QEQVLEA KTVPE DLKKD VTLNM RDAIDGQN SFT VLTTPACPLKELIRNS TEA HK VSGTAVV INMT
Nostoc punctiforme	: SRSILEI RPVED ELRKS VELNM RNVKIDGGKVSFT VLTTPACPLREFIVED QKAVKK PGVTDVS EV A
Synechococcus	: AAA LEA RPVQD ELRRS VELNM RDRVEPGRVAFT VLTTPACPLREFIVEECKAA RQ APIEAID TV A
Magnetococcus	: EPQVRDA RMVVD VAGRD VSAGYVSGEIHAGEVAFQ GRPESADYLKQLQEQ AQV GA PGVERVTVMSG
Salmonella	: RAMVAGT ANFQH TIKHN TILKA HHVAWMDDT HVE VMPFVWNSAFEVLKEQ SAD LR TGAKAIDWKL Y
Arabidopsis-L1	:
Chlamydomonas-L1	:MGWRQGAAEWLLSNAASGWRGAASAAALAAGGASGTARAGAPAGASRGA
Homo sapiens	:
Yarrowia lipolytica	:MRGFR IAP QRSIAIISRLQPITANFHSSPALRSHENPIGPKSP
Arabidopsis-L2	:MENGDIPEDANEHCPGPQSESAGKSDSCAGCPNQEACAT
Chlamydomonas-L2	:MASSASAAPTGEVPDNANOHCPGTASDQAGKSAACAGCPNQSICAT
Homo sapiens	:MEEV HD PGADSAQAGRGAS QG PNQRL AS
Saccharomyces Nbp35	
Drosophila	:
Homo sapiens	:
Danio rerio	1
Saccharomyces Cfb1	1
	184
	: QPARPIFAGQLPFGFSRISNIH VSSCKGGVCKSTVAVNLAYTLAGMCARVCTFDADV GPSIETMVNPESRIFEMN
	: OPARPAYAGELPEG TONISMITA VSSCKCGVCKSTVAVNLAYTLAGMCARVCTFDADV-CPSIPTAVSPDARLTVNN
	: OPAKPIFAGOLPFGUSRISNITAVSSCKGGVGKSTVAVNLAYTLAGMCARVGIFDADVYGPSLPTMVNPSSRIJEMN : OPARPAYAGELPEGLOKISNITAVSSCKGGVGKSTVAVNLAYTLAGMCARVGIFDADVFGPSLPTMVSPBNRLLVMN : OPARPVYAGOLPGGLOTISNITAVSSCKGGVGKSTVAVNLAYTLAGMCARVGIFDADVYGPSLPTMVSPBNRLJEMN
Vitis vinifera Physcomytrella	
Selaginella	: OPAKPLIADDVPAGUKKVSNIVAVSSCKGGVGKSTVAVNLAYSLAQMGARVGIFDADIYGPSLPTMVSPSVKVLOMN : OPAKALAAEGLPRSLONVSNIIAVSSCKGGVGKSTVAVNLAYSLAGMGAKVGIFDADVYGPSLPTMVSPSLRVLOMV : OPPKPLLPESGRPGG-UAKVRHIIAVSSCKGGVGKSTVSVLAYTLAQMGAKVGIFDADVYGPSLPLMVNPSIKVLEMD : OPASNDABF_TVEG-UPSVGHUIAVSSCKGGVGKSTVSVLAYTLAQMGAKVGIFDADVYGPSLPLMVNPSIKVLEMD
Volvox	: QPPKPLLPESGRPGG-DAKWRHIIAVSSCKGGVCKSTVSVNLAYTLAQMCAKVGIFDADVYGPSIPLMVNPDIKVJEMD
Ostreococcus tauri	: OPARNDAPE-TVEGIRRVSHIIAVSSCKSOVKSIVSVILATIAAMCAKVGILDADVYGPSLETMISPDVPVLEMD
Micromonas	: QPARNDAPE-TVEG - FRWSH: IAVS:CKGGVCKSTTSVNLAYTLAMMCAKVGILDADVYCPS:PTM_SPDVPVIEMD : QPVTNDMPD-AVEG - LKGVRH: IAVS:SCKGGVCKSTTSVNLAYTLRMMCAKVGILDADVYCPS:PTMTSPEQAVLOMD : NEINRAQAGDRPINKWKH: IAVS:SCKGGVCKSTVAVNLAFT_TKLCGKVGIMDADIYCPS:PTMTSPEQAVLOMD : PTSAAAPEAGGSRDPISOVSN:VLVTSSKGGVCKSTVAVNLAFT_TKLCGKVGIMDADIYCPS:PTMTVPSHNEKRIR : OPSVCAFTNSOUGAULIVSSCKGGVCKSTVAVNLAFT_TKLCGKVGIMDADIYCPS:PTMTVPSHNEKRIR
Galdieria maxima	: NEINRAQAGDRPPNKVKHUIAVASCKGGWGKSTVAVNLAFTLTKLCGKVGIMDADIVGPSHEIIVQPSNKIVQYK
Cyanidioschyzon	: NEINRAQAGDRPDNKVKHITAVASCKGGVCKSTVAVNLAFTITKLCGKVGIMDADIYGPSLPILVQPSNKIVQYK : OTPSAAAPEAGGSRDPLSOVSNIVLVTS <mark>AKGGVAKST</mark> TAVNLAFVLARLCARVGILDADIYGPSLPIMVNPBHNEKRIR
Phaeodactylum	: OF SAMAP BAGGS REFLOWS. ULTISANS WARS TAVIDATE WARS AN OUT DADITION THAT AND AND THE SAME STATE OF S
Thalassiosira	: QPTAAPSDAPLGMSQIGAVIAVSSCKGGVGKSTTAVNLAFADESLGAKVGIFDADVYGPSDPTMVTPDDDNVRFV
Chloroflexus	: QPTAAPS - DAPLG - MSCIGAVIAVSSCKGGVCKSTTAVNLAFALESLCAK - VGIFDADVYGPSIPTMYTPBDDNYFFY : NVRRPAGIPEQSA - IPGYAN IAVAACKGGVCKSTVANLAVALAQMCAQ - VGLDADVFGPSIPTMYDPDDNYFFY : ADVTTGRPNSGPV - IPHYKN IAVSSCKGGVCKSTITANLAVALSKSCAK - VGIDADISGPSIPTMFDVDVPNVI : ETPQCKSLPDRTG - ISGYKNIIAVSSCKGGVCKSTVAVALAQTCAK - VGLDADISGPSIPTMFDTMDDDAQTVR : ETPRSPSLPNRQS - IGOVRNIIAISSCKGGVCKTSVSVNAVALAQSCAR - VGLDADIYGPN
Cytophaga	: ADVTTGRFNSGPVUPHVKNIIAVSSCKGGVGKSTITANLAVALSKSCARVGIIDADISGPSIPTMFDVDVPNVI
	: ETPQQKSLPDRTGISGVKNIIAVSSGKGGVGKSTVAVNVAVALAQTCAKVGLLDADIYGENDPTMUGLADAQUVVR
Synechococcus	: ETPRSPSLPNRQSHPGWRNIIAISSGKGGVGKTSVSVNVAVALAQSGARVGLLDADIYGPNVPLMIGLODRSLVVQ
Magnetococcus	: NPQQQAEPL
Salmonella	: NIATLKRVKNOPGHNGVKNILAVSSGKGGVCKSSTAVNLALALAAEGAKVGVLDADIYGPSIPTMLGAEDORPTSP
Arabidopsis-L1	: YKFSSASAGGRTTELRUHGVKDIIAVASGKGGVGKSSTAVNLAVALANKCELK-IGLLDADVYGPSVPIMANINQKPQVNQ
Chlamydomonas-L1	A A SRGA A A A GPOKKI, GIK DWOHING INTERAKCEWEKSINT ANNAL VALLEVTRI, GLR - Weining International Company of the
Homo sapiens	: QRRTQIMSRGLPKQKPIECVKQ/IVVASGKGGVCKSTTAVNLALALAANDSSKAIGLLDVDVYGPSVFKM/NLKGNPELSQ : ASAPRIPRKTTRPEPTACVKKTVVSSAKGGVCKSTVSVNTALSLAKRCLRVGLLDVDIFGPSIPTMFGLSGEPRMTH : -APKGPDPDLVAIAERMSTVKHKILVISGKGGVGKSTPSAQISFALAGMDHQVGLMDIDICGPSIPKMIGLEGQEIHQS
Yarrowia lipolytica	: ASAPRIPRKTTRRPEPHAGVKKTIVVSSAKGGVGKSTVSVNTALSLAKRELRVGLLDVDIFGPSIPTMFGLSGEPRMTH
Arabidopsis-L2	: -APKGPDPDLVAIAERASTVKHKILVLSGKGGVGKSTFSAQ SFALAGMDHQVGLMDIDICGPSIPKM GLEGQETHQS
Chlamydomonas-L2	- APKGPDPDLAAIAARMSRWKHKULWLSGKGGWCKSWVSAOLAFALARREFE - WGUUDIDTCGPSVPKWUGLDGOETHSS
Homo sapiens	: GAGATPDTAIEEIKEKMKTVKHKTIVLSGKGGVGKSTFSAHLAHGLAEDENTQ-IALLDIDICCPSIPKIMGLEGEQVHQS
Saccharomyces Nbp35	A DECEMBER AND A DECEMBER AND A DECEMBER AND AND ADDRESS AND ADDRESS AD
Drosophila	:vglldidlegpsvgyllglegrdifqc
Homo sapiens	:meaaaepgnlagvrhiilvlsgkggvgkstistelalalrhagkkvgildvdlcgpsiprmlgacgravhqc
Danio rerio	:MDGSGKGNDDQVKHVLUUSGKGGVGKSTITTELALAFRHACKKVGILDVDLCGPSIPRMLSVGKPEVHQC
Saccharomyces Cfb1	:MEEQEIGVPAASLAGIKHIILIISGKGGVGKSSVTTQTALTICSMGFKVGVLDIDLTGPSLPRMFGLENESIYQG

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Arabidopsis HCF101	: PEKKTIIPTEYMGVKLVSFGEAGQGRAIMRGPMVSGVINCLITTEWGELDYLVIDMPPGTGDIQLTLCQVAP
Oryza sativa	: PESRSILPTEYLCVKMVSFGEAGQGRALMRGPMVSGVINQLLTTTDWCELDYLVIDMPPGTGDIHLTLCQVAP
Populus	: PEKRTIIFTEYLGVKLVSFGFAGQGRAMMRGPMVSGVIDOLLTTTENGELDYLVIDMPPGTGDIOLTLCOVVP : PEKRSIIFTEYLGVKLVSFGFAGQGRAMMRGPMVSGVINOLLTTTENGELDYLVIDMPPGTGDIOLTLCOVVP
Vitis vinifera Physcomytrella	: PEKRSIIFTEYLCVKLVSFGBAGQGRAIMRGPMVSGVINOLITTTEWGELDYLVIDMPPGTGDIOLTLCOVVP : PETRAIIFTEYLCVKLVSFGVAGQGSAIMRGPMVSGVINOFLTTTDWGELDYLVIDMPPGTGDIOLTLCOVVP
Selaginella	: EDTKQIIPTEYLGVKLVSFGFAGQGTAMMRGPMVSGVINCILTTTDWGELDVLIIDMPPGTGDIOLTLCQVVP
Volvox	: PATKAIFFTEYEGVKVVSFGFAGQGSATMRCPMVSGLIQOMLTTAANGELDYLVVDFPPGTGDIQLTLCQTVS
Ostreococcus tauri	: KETGTIKPVEYEGVKVVS <mark>F</mark> GBAGQGSALM <mark>RGPMVSGLINOLLTTTDWGELDVLIIDMPPGTGD</mark> VQLTLCQVVP
Micromonas	: KETGSITPTEYEGWGIVSFGEAGQGSAIMRGPMVSGLINOMITTAWGDDVLIIDMPPGTGDV0LTICOVLP
Galdieria maxima Cyanidioschyzon	: DGRIIPLEYENVKLMSFGYINPESAIMRGPMLANMMNOLITETDWCSLDYLVIDMPPGTCDIOLTICOTVS : LTPDGLMVFLTRAGVKLMSFGYINSDPAVLRGPMVSSLLTOLTOOTDWCSLDYLLVDLPPGTCDIOTTGOVLK
Phaeodactylum	: GROVAPLORN WRINSEGYVNDGSAVMRCPMVTOLIDOFTSVTHACADDYDTDDMPPGTCDIOFTTVKVN
Thalassiosira	: GRQTAPLERGDVSLMSFGYVNEGSATMRGPMVTQLLDOFUSLTNWCAHDYLIMDMPPGTGDIQLTUSORLN
Chloroflexus	: DANGOPM-MIPLSNHCHKVMSVGFID-ESOPVIWRCPMVSOF ROFIYOVANAPHDVITID) PCCCPVALITA SIP
Cytophaga	: ENENGKPTHIPIEQYCVKIISICELSP-AESAVVWRCPMASSALREFISDCDAGELDAMEFDMPPGTSDIHEIVOTVP
Nostoc punctiforme Synechococcus	: STETGDI-UEPAFNHGVKLVSMGFLID-RDQPVIMRGPMINGVIROFUYQVQMGELDYLIVDMPPGTGDAGLTUTOAVP : KREDGGEDIFFLENYGVKVVSMCLUVG-RDQPVIMRGPMINGVIROFUYQVQMGELDYLIVDMPPGTGDAGLTUVOAVP
Magnetococcus	: EKGQKVT:M:KYGVKINSMG5FMP-EDTPMIWRGPNVGMAVECLLRDIDWGELDYLVIDLPPGTGDA0LTITCKVP
Salmonella	: DGTHMAPIMSHCHATNSICYIVT-DDNAMVWRCPMASKA MOM QETLAPDHDYLVIDMPPGTCDICHTAON P
Arabidopsis-L1	: DMKMIPVENYGVKCMSMCLUVE-KDAPLVMRGPNVMSA-AKMTKGVDMCDLDLUVVDMPPGTGDAOISISON-K : SGTGALMLEKENYRVKTMSFGFFLE-GDEPVVMRGPNVNNAPDKMLFGTEMCLLDVLVVDMPPGTGDAOIN-GCR-P
Chlamydomonas-L1	: SGTGALMLPKENYRWKTMSFGEFLE-GDEPV/WRGPM/NNAFDKM/FGTEWELLDVLV/DMPPGTGDACIN/GCRIP
Homo sapiens Yarrowia lipolytica	: SNLWRPLINYCHACMSMGRUVE-ESEPVVWRGLWVMSATEKLURQVDWGOLDYLVVDMPPGTODVOLSVSON P : EGKLIPMSKFCHQVMSMGRUVD-PNKAVAWRCLUQKAUEOLUQVDWCTLDVLVMDLPPGTODVOLTIACTIK
Arabidopsis-L2	• NLGWS P//WWODN I/G////SIT/elo//T/PNSDEA///W///elo//W///////////////////////////
Chlamydomonas-L2	: GAGWSPVYVEDNHAVMSIGFMLPNPDEAV WRGPRKNGLIKOFLKDVDWGELDYLVVDAPPGTSDEHITITOCLO
Homo sapiens	: GSGWSPVYVEDNEGVMSVGFLLSSPDDAVIWRGPKKNGMIKOFLRDVDWGEVDYLIVDTPPGTSDEHLSVVRYLA
Saccharomyces Nbp35	
Drosophila Homo sapiens	: NSGWPvfldre-qharsiftromPEDSahwakSkNLikkFbV0vdkDrhvDrPofsbenistkkek : ddgwpvfldre-qharsiftlknredpviwfgpkkmmirfltdvrwdeldylidtopgtadehiweelk : drgwapvfldre-qaisimsvgfllekpdeavvwrgpkknalikofvsdvawgeldylvvdtppgtadehmaticalr
Danio rerio	: DSGWVPVYADPQQQQDALMSIAFILEDSDEAVIWRGPKKTALIGOFVSDVAWGELDILLVDTPPGTSDEHDAVLENLR
Saccharomyces Cfb1	: PEGWQPV-KVETNST-GSISVISLGFLLGDRGNSVIWRCPKKTSMIKOFISDVANGELDVLLIDTPPGTSDEHISIABELR
	339 347
	: LTAAVIVITECKEAFIDVAKGVRMESKIKVPCVAVVENMCEDADGKRYYPFGKCSGSEVVKQFGIP : LTAAVIVITECKEAFIDVAKGVRMESKIKVPCVAVVENMCYFDADGKRFYPFGQCSGAQVVQQFGIP
Oryza sativa Populus	: TTAAVIVYTPCKDAFTDVAKCVRMESKIKVPCVAVVENMCHEDADGKRYYPECRCSGSOVVOOFGIP
Vitis vinifera	: TADADGKRYYPCCRCSGSO/VOOFCP
Physcomytrella	: LTAAVIVTTPOKLAFIDVAKGVRMESKIKVPCIAVVENMOFFEGDDKRYYPEGKCSGSKVVEQFGIP
Selaginella	: LTAAVIVTTPOKLAFIDVAKGVRMPSKIKVPCLAVVENMCYFDADGKRYYPFGRESGKQVVQQFGIS
Volvox Ostreococcus tauri	: FSAAVIVITEOKIAFIDVAKCIRMEAKIVVPCVAVVENMSYFEADGKRFPFFCQCSGERIQRDFGLF : ITAAVVVTTEOKIAFIDVEKGVRMEAKIAVPCVSVVENMSYFEVDGVKHKPFCECSGAKICEQYGVP
Micromonas	: ITAAVVVTTPOKLAFIDVBKGVRMESKIRVPCVAVVENMSYFDGDDGKRYKPFGECSGQRICDDYGVP
Galdieria maxima	: DAAVIVTTPOOLSFODVIKGIOMFGKVSVPCVALVENMAYFEPNDIPDKRYYLFGHCKSOKIANDYGIP
Cyanidioschyzon	: ATAAVVVTTPCRDAFADVVRGTOLLDRWAVPPTAVVESVATFVAPDTGKRIDLFGRCHSARISREFGIR
Phaeodactylum	
Thalassiosira Chloroflexus	
Cytophaga	. VTGATVVTTPOKVATADAORGIOMEROPOVNVPVIGVTENMAWET-PAELPENKYYTECKDGGKETAEKEDVP
Nostoc punctiforme	MAGAVIVTTPOTVALUDSRKGURMEOOMNUPVLGUVENMSYFTPPDOPDKHYDIFCSGGGSKTAAELGVP
Synechococcus	: LAGAVIVTTPCSVALLDSRKGDNMERQLGVPIDGIVENNSYFIPPDLPDRQVDIFCSEGGETTARELGVP
Magnetococcus	: DSGWIVSTPODVALADVRKGINMFKKVEVPVIGITENMSYNDCTECGHRAEI-SHEGAEKEAANS OF
Salmonella Arabidopsis-L1	· LSGAVTVSTPODVALADANDGISMEDKVPVPTLGIVENMSCEVOPHONEPSETECKEGAPETAAKKGIK
Chlamydomonas-L1	: LSGAALVSTPQDVALIDVRRCAQMFLKLRVPLLGLIENMAYHRCGKCGHVEHIFCTGGVERAAADYGMD
Homo sapiens	TTGAVIVSTPCDIALMDAHKCARMERRVHVPVLCLVONMSVEOCPKCKHKTHTECADGARKLAOTLGLE
Yarrowia lipolytica	
Arabidopsis-L2 Chlamydomonas-L2	PTGIDGAILVTTPOEVSLIDVRREUSFCKKVGVFVLGVVENNSGLSOPLDIVACSEVFDSSGGAERACREMGVP VGGLGGGAAAVIVTTPOEVSLIDVRREVNFCRKVGLFVLGVVENNAGLRPPGAQLRLRAEVFRE-GGAARMCADMGVP TAHIDGAVLITTPOEVSLQDVRREINFCRKVKLFIIGVVENNSGSICPKCKKESQIFPPTTGAELACODLEVF ESGIDGAVVTTPOEVALIDVRREINFCRKVKLFIIGVVENNSGSVCPNCKGE-SQIFPPTTGAELACCODLEVF
Homo sapiens	TAHIDGAVIITTPOOLADURADURADURAC
Saccharomyces Nbp35	: ESGIDGALVVTTPCEVALLDVRKEIDFCKKAGINILGLVENNSGFVCPNCKGESQIFKATTOGGEALCKELGIK
Drosophila	: evgchgaivttpgevalddvrke tfcktg/nilgivenmsg/vcphctsctni/ssnggvslatyaqvp : pyqplgaivvttpgavsvgdvreltfcrktglrvmgivenmsg/tcphctectsv/sr/ggeelaqlagvp
Homo sapiens	: pyqplga.vvttpgavsvgdvrfeltfcrtglrvmgivenmsgitcphctectsv:sr:ggeelaglagvp : KHRVDGNVTVTTPQAVSTGDVRREITFCKKTNIKIHGVENNSG:VCPHcSECSNISK:GGEELAKLTCSA
Danio rerio Saccharomyces Cfbl	: KHKYDGSILVVIIIOANSIGUVRKEIIFKKINVKHUGVSNSG-VOHOSESILISKGGEEARLISA : YSKPDGGIVVIIIOANSIGUVREINF <mark>C</mark> KKVDIKIEGIENSG-VOHOSE
	414 419
	: HEFDERETESASGESETEEVISDEL-SDVARTEQDEGVCVVQQCAKIRQQVSTAVTYDKYLKAIRVKVP
Oryza sativa Populus	: H FD PIRT SASG T I EVVAD Q-GDV KT QN GV VQQ AKIRQQVSTAVSYDRSIRAIRVKVP : H FD PIRT SASG G M EVAD Q-GEV KI QN GI VQQ AKIRQQVSTAVTYDKSIKAIKVKVP
Vitis vinifera	: HTFD:PIRPTISASG.SCH.BUARD.G.GSD.RT.QH.GIC.V.QQCAKIRQQVSTAVTYDKFIKAIRVKVP
Physcomytrella	: HIFE DIRELSAAG TON EVVVD Q-GQVANIESD VGVCVVQQCAKLRQAVSTAVMYDKAINAIRVKVP
Selaginella	: NIFEFPIREARLYKALSAAGDSCTEEVVHDEQ-GDVARSESEIGVCVVQQCAKIRQQVSTAVTYDDAMRAIKVKVP
Volvox	: NUVRFPIVPDUSAAGGOTORPLVVADPT-SATAARMDUGAAVVREVAKMAGRPARQAVYYDPQKDVISVQLP : NULQVPIVPDUSACGOTORPLVURDPT-CETSSRYQEVAATVVREVAKLNNGKKPRVDIDPGYDGAFRVEIP
Ostreococcus tauri Micromonas	: N LOYPEVPD SACG TCR-LVIRD T-CETSSRYQEVAATVVREVAKLNNGKKPRVDIDPGYDGAFRVEIP : N FOYPEVPD SACG TCR-LVIVD A-GDVSTIYGAVAAKVVQEVAKLQAGPKGSLALDTEGVAGVDGA
Galdieria maxima	: FVESFPLDD-CRWSDNCIPAVLALSE-SKISQLYQSDASAVVQQIAKNAFGN-GKRIPQVFFDSDKCIIVISCN
Cyanidioschyzon	: STFQTPTWENAAGDTCTPVTITLPETSEIFQCYRRIAENIVQEGARVRFG-AVPIPQARWDADHREIVVQLQ
Phaeodactylum	: HSFSTEPLNKTAANGDNCTEFULEFED-SPPAKIYQEDASAVVSEVAKTKFAKSMRPSVQYDAESHLLQVSQN
Thalassiosira Chloroflexus	: HTYSVDFMGQHAQNG SCT FTHDN K-SPQADIYRQHAKSVVSEVAKIKFCTGKGGRPSVSYDVEKSILRVDDG : VIGO PFGMSVREGG NOO-AVISDAP-DAYADIRE ARQVAARISVLQYAMV
Cytophaga	: LIGQIPLVQGIRESGIMCKPAVIN-LD-KIT-QA-KELAETVAQQVAIRNASLAETRKVEIKV
Nostoc punctiforme	: LLGCVPLEISTRVGGDSCVPIVVGDPD-SASAKALTATALTTAGKVSVAALT
Synechococcus	: L'GREPPEIAURQGGDACQPIVISQPE-SASAQALRQDAKTDAGRVSMLALGAG
Magnetococcus	: FUGHIPISEDURKDSDACKPIVVARPD-SPQ.QQVLEHARNVVSKLQDGAGAPKMPKIVIE
Salmonella Arabidopsis-L1	: LIGOYPEHISUREDLORGTETVVSREE-SEFTAIYRELADRVAAQLYWQGEVIPGEIAFRAV
Chlamydomonas-L1	: DIGETPERSIREGSDECOPVOISSEG-SIVSKA QDIAQNOVKGLKELKEN-PDNEIQMKLNVPHSSSS : VIIGQVEDHVDIQTRSDACTPVVAAEPG-GAL/GAYVGIAERIHAKLLAL
Homo sapiens	: VLGDIPLHLNIREASDICOPIVFSOPE-SDEAKAYLRIAVEVVRRLPSPSE
Yarrowia lipolytica	: VIGNVELDPQICSQS KOVPANSGGVQAKYYDKIAEGVAEQ GV
Arabidopsis-L2	: FIGKVPMDPQLCKAAEQCKSCFEDNKC-LISAPALKSIQKVVPSTVMTE
Chlamudamana	
Chlamydomonas-L2 Homo sapiens	: LIGRIPLDGIGAAAAA RSVIPEAAG-AAGADAVKGGLEGVAP
Chlamydomonas-L2 Homo sapiens Saccharomyces Nbp35	: L GR PLDL GKNCDK QSFF DA D-SPATLA RS IQR QEF NLHQSKEENLISS
Homo sapiens Saccharomyces Nbp35 Drosophila	: L GRVPLDL GKNCDK-QSFF DAD-D-SPATLAVRS IQR QEF NLHQSKEENLISS : F GSVPLDR GKSCDM-ESF DNYD-SPASSAVLNVVEA RDAVGDV
Homo sapiens Saccharomyces Nbp35 Drosophila Homo sapiens	: L'GRVPLDL'GKNORCQSFF DA D-SPATLAVRS IQR QEF NLHQSKEENLISS
Homo sapiens Saccharomyces Nbp35 Drosophila Homo sapiens Danio rerio	: L GRVPLDL GKNCDK-QSFF DAD-D-SPATLAVRS IQR QEFONLHQSKEENLISS : F GSVPLDR GKSCDM-ESF DNYD-SPASSAVLNVVEA RDAVGDV

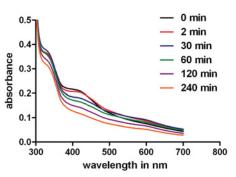
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Arabidopsis HCF101 : NSDEEFLLHPATVRRNDRSAQS-VDEWTGEQKVLYGDVAEDIEPEDIRPMGNYAVSITWPI Oryza sativa : DSDEEFLLHPATVRRNDRSAQS-VDEWTGEQKVQYGDIPEDIEPEEIRPMGNYAVSITWPI	GFSQIAPYDQL
Oryza sativa : DSDEEFLLHPATVRRNDRSAQS-VDEWTGEQKVQYGDIPEDIEPEEIRPMGNYAVSITWPI	
Populus : DSEEEFLLHPATVRRNDRSAQS-VDEWTGEQKLQYADVPEDIEPEEIRPMGNYAVQITWPI	
Vitis vinifera : DSEEEFLLHPATVRRNDRSAQS-VDEWTGEQKLQYADVPEDIEPEEIRPMGNYAVSITWPI	
Physcomytrella : GTTEEFLLHPATVRRNDRSAKS-IDEWSGEQKLRYTDVAEDLAPESIRPMGNYAAAINWPI	
Selaginella : GTEEPFYLHPATVRRNDRSAKS-IDEWTGEQKLRYGDVREDIEPEAIQPLGNYAVMISWPI	
Volvox : GETEFLLPPVVVRENDTSATS-IDEWTGQRK-RDEVPQDARPAAINPLGNYAVQISWSI	GFNQVASYELL
Ostreococcus tauri : GENNDKAFWITAKNVRLSDESARVKGSDESPDRLLNGAPIPDDIAPVEMSVIGNYAMSITWP	
Micromonas : LRVQLADEGGMPFYVRGCDVRRSDKSATA-DGESKKADFLMDGVTPVPDDIAPVEAHVVGNYAVQISWPI	
Galdieria maxima : DQQGIAWNENNKVEWSPWELRNASSASS-VDEFTGKRHWKSVDRNVKPLQIQTAGNYAFSVIWSI	GHQSLYPFERV
Cyanidioschyzon : DHMEERIQPAALRACCAAC-VDECTGKQLLDPNSVDDNIYPMQMMNVGNYALAVNWSI	
Phaeodactylum : GVGSTDEEHVATLPPAELRRACCAAC-VEELTGRQILVPSSVSDKIAPRNMVPTGNYALSVDWSI	
Thalassiosira : DIQNATISPAELRRGCRCAAC-VEELTGKQILNPASISESVKPLNMSPTGNYALSVDWSI	GHRSLYPYRQI
Chloroflexus :	
Cytophaga :	
Nostoc punctiforme :	
Synechococcus :	
Magnetococcus :	
Salmonella :	
Arabidopsis-L1 :	
Chlamydomonas-L1 :	
Homo sapiens :	
Yarrowia lipolytica :	
Arabidopsis-L2 :	
Chlamydomonas-L2 :	
Homo sapiens :	
Saccharomyces Nbp35 :	
Drosophila :	
Homo sapiens :	
Danio rerio :	
Saccharomyces Cfb1 :	

#### Figure S1 Multiple sequence alignment of the FSC-NTPase family

Representative protein sequences of different organisms are shown subdivided according to the proposed four FSC-NTPase classes [23]. Organism names are coloured according to the class affiliation. Green background represents plants and algae taxa belonging to the class I. Yellow background represents eubacterial class I proteins. Grey background corresponds to class II members. Blue and magenta congregate taxa belonging to the class IV respectively. Note that algae and plants class I proteins have a C-terminal extension corresponding to the DUF971 domain (COG3536). The very N-terminal and C-terminal regions are not shown in the alignment as they show little conservation. Amino acids coloured with a black background are 100 % conserved. Amino acids coloured with a grey background and with white characters are > 60 % conserved. Amino acids coloured with a grey background and written with black characters are > 40 % conserved. Cysteine residues conserved in at least two sequences are depicted with a blue background. Numbers above the conserved cysteine residues in plants and algae correspond to the position of the residue in the sequence of the *Arabidopsis* HCF101 protein.

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## Supplementary Figure S2 Oxygen sensitivity of the reconstituted HCF101 protein

Purified HCF101 protein was reconstituted as described in the Experimental section in the main paper. Decay of the shoulder at 420 nm in the presence of oxygen was monitored by UV–visible spectroscopy.

# Supplementary Table S1 Oligonucleotides used for site-directed mutagenesis and cloning

For details please see the Experimental section in the main paper. for, forward primer; rev, reverse primer.

Designation	Sequence
C102S-for	5'-GGAGCTGACAACACCCGCATcTCCAGTCAAAGAC-3'
C102S-rev	5′-GAAACAATATCTGTCCCAAAATCAGGATC-3
C128S-for	5'-CATCATCGCTGTTTCTAGTTctAAGGGTGGTG-3'
C128S-rev	5'-GTGTTGTCAGCTCCAAACGGAACGAAACCTC-3'
C184S-for	5′-GATATACAACTGACCTTAT <i>ct</i> CAGGTTGCGC-3′
C184S-rev	5'-CTAGAAACAGCGATGATGTTCGAAATTC-3'
C303S-for	5'-CTCAAAACTTAAGGTGCCTTctGTTGCTGTTGTG-3'
C303S-rev	5′-TAAGGTCAGTTGTATATCACCAGTTCCAG-3′
C339S-for	5'-CTCAAAACTTAAGGTGCCTTctGTTGCTGTTGTG-3'
C339S-rev	5′-GCACCTTAAGTTTTGAGAACATCCTTACAC-3′
C347S-for	5'-GCTGTTGTGGAGAATATGTctCACTTTGACGC-3'
C347S-rev	5′-CATATTCTCCACAACAGCAACGCAAGGC-3′
C414S-for	5'-CGTTCCAGGATCTTGGTGTATcTGTAGTGCAAC-3'
C414S-rev	5'-CACCAAGATCCTGGAACGTTCTGGCAACG-3'
C419S-for	5′-GTGTATGTGTAGTGCAACAATctGCCAAGATAC-3′
C419S-rev	5'-TGTTGCACTACACATACACCAAGATCCTGGA-3'
INCORP-for	5'-GTAGAATTCTCAGCTCAAGCTAGTAGTAGTGTTGG-3'
INCORP-rev	5'-GTAGAATTCGACTTCGACTGGAGACAATGGAGG-3'

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