Review

Fe-S Protein Synthesis in Green Algae Mitochondria

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Abstract: Iron and sulfur are two essential elements for all organisms. These elements form the Fe-S clusters that are present as cofactors in numerous proteins and protein complexes related to key processes in cells, such as respiration and photosynthesis, and participate in numerous enzymatic reactions. In photosynthetic organisms, the ISC and SUF Fe-S cluster synthesis pathways are located in organelles, mitochondria, and chloroplasts, respectively. There is also a third biosynthetic machinery in the cytosol (CIA) that is dependent on the mitochondria for its function. The genes and proteins that participate in these assembly pathways have been described mainly in bacteria, yeasts, humans, and recently in higher plants. However, little is known about the proteins that participate in these processes in algae. This review work is mainly focused on releasing the information on the existence of genes and proteins of green algae (chlorophytes) that could participate in the assembly process of Fe-S groups, especially in the mitochondrial ISC and CIA pathways.

Keywords: mitochondria; algae; iron-sulfur; ISC machinery; CIA machinery

1. Introduction

Iron (Fe) is an essential micronutrient for all aerobic organisms. However, it is highly toxic in the free state and can cause oxidative stress and damage to cellular macromolecules. For this reason, organisms developed mechanisms to regulate the content of Fe, responding to the deficiency or increase of the metal. Fe is present in numerous proteins that participate in energy metabolism, such as those that are involved in the mitochondrial and chloroplastic electron transport chains [1].

Both Fe and S are two compounds that are important for the synthesis of Fe-S groups and ferrosulfoproteins. These groups are inorganic cofactors that are present in numerous proteins that participate in different metabolic pathways such as photosynthesis and respiration, previously mentioned, but also in the regulation of gene expression, protein translation, maintenance of DNA integrity, and in metabolic pathways related to the assimilation of nitrogen, sulfur and iron, and amino acid metabolism [2–4].

Although there are numerous works that characterized the function and regulation of genes and proteins that participate in the production of Fe-S groups in bacteria, yeasts, and humans, little is known about the occurrence and function of these genes in photosynthetic organisms, especially in algae. The objective of this work is to identify the distinctive characteristics of the Fe-S group synthesis pathway in chlorophytes mitochondria. For this, we have reviewed the importance, incorporation, and uses of Fe and S in these organisms and also made a survey on the presence of possible genes and proteins that would be involved in the ISC pathway of Fe-S synthesis in these algae.
2. Fe and S Uptake and Metabolism in Algae

Fe-S clusters are among the most ancient cofactors used by living organisms. Life originated in water, where originally its constituent elements were abundant and easily accessible. However, with the evolution of photosynthetic organisms, the atmosphere and the oceans filled with oxygen, and the bioavailability of the elements radically changed. Chlorophytes have to incorporate the substrates for the synthesis of Fe-S centers, Fe and S, from the surrounding medium. In primeval times, Fe was present as the soluble ferrous (+2) ion. However, in oxygenic environments, Fe is oxidized to ferric (+3) ion, which forms insoluble oxides and hydroxides [5]. Land plants developed two different strategies to solubilize the metal: reduction and chelation. Both are energy expensive, but it’s the price to continue taking advantage of the chemical properties of Fe for biological reactions. Most work regarding Fe metabolism in chlorophytes was done with the model algae Chlamydomonas reinhardtii because it is easy to grow in a synthetic defined medium, and its genome has retained genes from the last common ancestor from both the plant and animal lineages. By homology searches in genomic and transcriptomic databases, particularly from algae grown in metal deficient conditions, with known yeast, animal, and plant transporters as baits, the repertoire of Fe and other metal transporters in Chlamydomonas and other algae has been analyzed [6]. C. reinhardtii incorporate Fe by a reduction-based strategy. Insoluble ferric oxides and other salts are solubilized by the activity of ferric reductases on the plasma membrane. Chlamydomonas has one gene of the NOX family of ferric reductases, FRE1, orthologous to the Fre1/Fre2 and FRO2 genes involved in Fe uptake in yeast and Arabidopsis thaliana, respectively. FRE1 is localized to the plasma membrane and is induced by Fe deficiency, coordinately with FEA1, FEA2, FOX1, and FTR1 [7]. FOX1 and FTR1 are similar in molecular functions to the Fet3p/Ftr1p pair in yeast, and perform ferroxidation and high affinity Fe uptake in Chlamydomonas, respectively [6,8]. FOX1 is a multicopper oxidase with the highest similarity to human ceruloplasmin and hephaestin, and this fact explains the copper-dependency for Fe assimilation in this alga, like in yeast [9]. The permeases like FTR1 are abundant in algal genomes and are thought to channel ferric ions to the cytoplasm, intimately associated with the multicopper oxidases; however, some chlorophytes do not express these proteins, nor they have the corresponding genes (see below). Chlamydomonas cells have an extracellular space between the wall and the plasma membrane that houses proteins involved in nutrient assimilation [10]. FEA are algal-specific proteins secreted to this periplasmic space. Although the exact biochemical and molecular role of these proteins remains to be determined, they are the major secreted proteins during Fe deficiency and are most probably involved in Fe assimilation. Chlamydomonas lacking cell wall secrete FEA proteins to the medium and are more sensitive to Fe deficiency compared to algae with cell walls that retain FEA proteins [7].

Recently Ostreococcus tauri has been proposed as a new model green alga for Fe metabolism studies [11]. This alga grows in open oceans where Fe scarcity is more prevalent than in C. reinhardtii growing niches. The most striking characteristic in O. tauri Fe uptake is that it does not seem to involve Fe reduction, because it lacks the classical components. O. tauri, and other green algae like Ostreococcus lucimarinus or Micromonas sp., do not have genes for the FOX1/FTR1 pair [6], and although they encode ferric reductases, they are not induced by Fe deficiency [11]. Additionally, there is no clear connection between copper and Fe metabolism, and, instead, zinc seems to play an important role in regulating iron uptake [11]. Several other genes coding for potential Fe uptake transporters have been detected in O. tauri, including one gene from the ZIP family, related to the Arabidopsis IRT1 high affinity Fe uptake transporter, and a gene from the algal FEA family, which seems to include potential transmembrane and Fe binding motifs [11]. Once iron enters the algal cell, it must be trafficked to the different organelles, and when in excess, transported to sites of storage. Major sinks for Fe are chloroplasts and mitochondria. However, rather little is known about transport of Fe across these membranes. The mitochondrial solute carrier (MSC) family is a large family of intracellular
plants involve the transport of multiple substrates. Mrs3p/Mrs4p from yeast belong to this family and are involved in Fe transport across inner mitochondrial membrane [12]. MITs from rice and Arabidopsis are also members of the same family and were recently related to Fe traffic into the mitochondria [13,14]. A protein similarity network of the MSC family led to the identification of a cluster of proteins that includes Mrs3p/4p, plant MITs and predicted iron transporters from algae [14].

Sulfur, the second element in Fe-S clusters, and an essential macronutrient present in proteins, lipids, carbohydrates, and several metabolites, has also suffered a great change in its most abundant chemical form in the environment. In ancient oceans, it was mainly present as sulfide (S\(^2\)-), while with the advent of oxygenic photosynthesis, it was oxidized to sulfate (SO\(^4\)-). Primary producers have to invest energy in its reduction to sulfide in order to be able to incorporate it to biomolecules. The sulfate anion (SO\(^4\)-) is the preferred source of S, but Chlamydomonas also has the ability to acquire sulfur from organic compounds. This is achieved by the secretion of two arysulfatases (ARS) to the periplasmic space, capable of cleaving sulfate anions from esterified organic sulfate [15,16]. The high affinity sulfate transporters SULTR2 (plant type H\(^+\)/SO\(^4\)- cotransporter) and SLT1 and SLT2 (bacterial and animal type Na\(^+\)/SO\(^4\)- cotransporters) on plasma membrane mediate SO\(^4\)- uptake [17]. Once inside the cell, SO\(^4\)- must be reduced in order to be incorporated in biomolecules. But because SO\(^4\)- is a relatively inert molecule, the first step prior to assimilation is the activation by the enzyme ATP sulfurylase to render adenosine phosphosulfate (APS). In the pathway leading to reduction, the S of APS is reduced to sulfite by APS reductase, and sulfite is further reduced to sulfide by sulfite reductase. Finally, sulfide is incorporated into the amino acid skeleton of O-acetylserine (OAS) by OAS thiolylase to form cysteine [18], which could be further transformed into methionine, the methyl donor S-adenosylmethionine (AdoMet), and the antioxidant glutathione [19]. The assimilation process is essentially similar to that of Arabidopsis, and in all photosynthetic organisms, sulfate reduction occurs in the plastids, but ATP sulfurylases are also present in the cytosol, and plant ATP sulfurylases have a different evolutionary origin than those from green algae [20]. Cysteine, the source of S for the ISC Fe-S cluster biosynthesis, can be synthesized in the cytosol, plastids, and mitochondria in nearly all plant species. Current studies suggest a scenario in an Arabidopsis leaf where chloroplasts generate sulfide via reductive sulfate assimilation, the mitochondria provide the bulk of OAS, and the cytosol produces most of the cysteine [21]. It is unknown where the main synthesis of cysteine occurs in green algae, but C. reinhardtii, Coccomyxa subellipssoidea and Volvox carteri genomes contain several genes coding for OAS thiolylases [22].

Studies in Chlamydomonas have shown that transcript levels and activities of transporters and enzymes participating in the S assimilation pathway, such as ARS, SULTR2, SLT1, SLT2, ATP sulfurylase, APS reductase, and OAS thiolylase, increase under S deficiency conditions, and are highly regulated by the demand for reduced sulfur and by environmental conditions [23].

3. Synthesis of Fe-S Clusters

The most common and simplest forms of Fe/S clusters are of the (2Fe-2S) and (4Fe-4S) type, but also (3Fe-4S) forms or more complex clusters containing additional heavy metal ions are known [24,25]. Specifically, the iron-sulfur group biogenesis pathways include several proteins involved in apoprotein maturation in different cellular compartments [26].

Aerobic eukaryotic organisms depend on mitochondria to synthesize Fe-S groups, while photosynthetic aerobic organisms also synthesize Fe-S groups in chloroplasts [4,27]. Although mitochondria can assemble their own Fe-S proteins, they play a crucial role in the biogenesis of cytosolic and nuclear ferrosulfoproteins [26,27].

It was described in plants, by studies carried out mainly in A. thaliana, that there are three metabolic pathways for the assembly of Fe-S groups: (i) the SUF pathway (mobili-
zation of sulfur) in chloroplasts, (ii) the CIA pathway of assembly of Fe-S groups in cytosol, and (iii) the ISC pathway, mitochondrial iron-sulfur cluster [3,27]. The SUF and ISC machines carry out the synthesis of Fe-S groups in three basic stages. In the first stage, S is obtained from the reaction catalyzed by a cysteine desulfurase, NFS, and combined with Fe on a scaffold protein for de novo synthesis of (2Fe-2S) groups. In a second stage, the Fe-S group is released from the scaffold with the aid of chaperones and co-chaperones, and bound by a transfer protein. At this point, the (2Fe-2S) group can be inserted into apoproteins, trafficked to the late-acting machinery for (4Fe-4S) group synthesis, or, in the ISC pathway, used for export of a yet unknown sulfur-containing species to be utilized by the CIA system. The third stage is less known and comprises the conversion of (2Fe-2S) into (4Fe-4S) groups, and the insertion into apoproteins by dedicated targeting factors [27,28].

There are numerous works on the characterization of the synthesis pathway of Fe-S groups in mitochondria mediated by the ISC complex [29]. It has been reported that this complex is a heterodecamer composed of five proteins, NFS1, the scaffold protein ISCU, ISD11, an acyl-carrier protein (ACP), and frataxin [29]. NFS1 is a pyridoxal phosphate-dependent mitochondrial cysteine desulfurase. This protein produces S from alanine [30]. It was shown that in plants, specifically in Arabidopsis, there are two isoforms, AtNFS1, located in mitochondria and AtNFS2, with chloroplastic localization [3,31–33]. ISD11 is a member of the LRY protein family, which lacks orthologs in prokaryotes but is highly conserved in yeast, humans, and plants [34–36], while IscU is a scaffold protein homologous to ISU from plants [37]. Three genes encoding ISU1-3 were found in Arabidopsis (At4g22220, At1g01020, At4g04080) [31,38,39], while ACP is an acyl group carrier protein, homologous to Arabidopsis AtACP1-3 [40]. Finally, frataxin is a highly conserved protein from prokaryotes to eukaryotes [41,42]. This protein has been involved in various processes such as Fe homeostasis, respiration, heme metabolism, oxidative stress, and transfer of persulfide groups [43–49].

3.1. Cysteine Desulfurases

As mentioned above, the first step in the synthesis of Fe-S groups in mitochondria and chloroplasts is catalyzed by a cysteine desulfurase [50]. These enzymes are found in the three kingdoms of life, representing a conserved group of proteins with several essential functions, such as the formation of Fe-S groups and the synthesis of biotin, thiamine, molybdenum cofactor, thionucleosides in tRNAs, and lipoic acid [50–53].

Cysteine desulfurases belong to the class V group of aminotransferases and are classified into two main classes (I and II) based on their sequence similarity [50,54]. A particularly recognized structural difference between type I and type II is the catalytic loop, which contains a nucleophilic cysteine. This catalytic loop of type I cysteine desulfurases is longer than that of type II enzymes and, therefore, it is considered to be structurally more flexible than that of type II enzymes [55–57].

Class I of cysteine desulfurases contains the consensus sequence SSGSACTS and group II, the sequence RXGHHCA. In general, the group I contains the enzymes with sequence similarity to NifS and Isc and those of class II, those enzymes related to SufS and CsdA [50]. The resolution of the crystal structures of the IscS cysteine desulfurases of Escherichia coli (EclscS) and Thermotoga maritima (TmNifS), showed that these proteins form homodimers of around 90 kDa, also showing high structural similarity [55,58]. Each monomer is composed of a major domain in which the PLP cofactor is bound to a conserved lysine, and a minor domain that houses the active site cysteine [55,58,59].

The first cysteine desulfurases were described in the nitrogen-fixing bacteria Azotobacter vinelandii, called IscS and NifS. IscS is the cysteine desulfurase of the ISC system for the synthesis of Fe-S centers, while NifS is exclusively involved in the maturation of the enzyme nitrogenase [50,60]. In bacteria like E. coli, three genes were found that code for cysteine desulfurases: IscS, SufS (CsdB) and CsdA (CSD) [50]. On the other hand, in humans and in S. cerevisiae, there is a single gene that codes for a type I cysteine desulfurase
called Nfs1 [61, 62]. In humans, there are two isoforms of Nfs1 located in the cytosol and mitochondria, generated by the differential use of codons at the beginning of translation [61]. In yeast, due to differential cleavage of the signal peptide, Nfs1 is mainly localized in mitochondria but is also found in the cytosol and nucleus [63]. The deficiency of Nfs1 in both organisms produces a decrease in the activity of Fe-S proteins generating serious problems in growth [62, 64].

Few cysteine desulfurases have been described in higher plants, such as those from *A. thaliana* and *Glycine max* [65, 66]. In *Arabidopsis*, the presence of AtNFS1, located in mitochondria, and AtNFS2, present in chloroplasts was described [31, 33, 66–68]; while in *G. max* the presence of four cysteine desulfurases was reported [65]. The analysis of the amino acid sequences of AtNFS1 showed that this protein belongs to class I of cysteine desulfurases [33, 68]. Homology modeling studies showed that AtNFS1 exhibits the characteristic folding of class I cysteine desulfurases such as IscS from *E. coli* [32]. AtNFS1 and AtNFS2 are essential for the plant since homozygous mutants are lethal, and the reduction in the abundance of these proteins produces severe consequences in development. Furthermore, there is an additional cysteine desulfurase called ABA3 involved in the formation of the molybdenum cofactor in the cytosol, which is not related to the biosynthesis of Fe-S and groups [69].

Currently, the genes and proteins that would participate in the synthesis of Fe-S groups in algae have not been identified or characterized. Thus, we performed a bioinformatic search for sequence identity for Nfs1 and Nfs2 in Chlorophytes using the Uniprot Database [70] and the *A. thaliana* sequences as a query. The search results showed a total of eighteen sequences for Nfs1 and ten sequences for Nfs2 in green algae. Nfs1 homologs present a percentage identity between 62.2 and 70.6% with respect to AtNFS1, with a sequence length between 406 to 488 amino acid residues (Table 1). These sequences were analyzed in order to verify the conservation of critical amino acids for the function of the enzymes. For Nfs1 homologs, the residues belonging to the active site of the enzyme are almost completely conserved (T124, H152, K153, N203, E204, K225, Y386, according to the numbering of AtNFS1) as well as the catalytic residue Cys 377 and R403, involved in substrate binding. The active site of AtNFS2 has also almost all conserved residues belonging to the active site (T144, H172, H173, N224, V225, Q252, K275), a catalytic cysteine at position 418 (C418) and an arginine that binds the substrate (R433, all positions according AtNFS2 numbering). The exceptions are those enzymes belonging to *Chlorella variabilis* and *Chlamydomonas eustigma*, where V225 is replaced by a methionine, and in the protein from *Monoraphidium neglectum*, where we found a threonine in that position.

The presence of multiple cysteine desulfurases was previously described in other organisms such as *Bacillus subtilis* and other gram-positive bacteria. However, this had not been described in photosynthetic organisms [71]. We found two putative Nfs1 proteins in *C. reinhardtii*, *C. variabilis*, *O. tauri* and *M. commoda*, whereas one Nfs1 was identified in all the other algae species (Table 1). The presence of multiple NFSs in these algae could be a strategy to distribute sulfur towards different metabolic pathways. It would also be of great interest to study the possible participation and functions of these multiple mitochondrial cysteine desulfurases in the synthesis pathways of Fe-S groups in the four algae species mentioned above. On the other hand, since one of the *B. subtilis* cysteine desulfurases was reported to have reactivity towards cysteine in the presence of a sulfide transferase (SufU), it is possible that some of these desulfurases also interact with other proteins to fulfill their biological function.

To analyze the cell location, we use the Deep-Loc1.0 server [72]. The proteins found in *C. sorokiniana*, *M. conductrix*, *T. obliquus*, *V. carteri* f. nagariensis, *O. tauri* (2), *R. subcapitata*, *C. primus*, *M. commoda* (2), *C. reinhardtii* (2) and *C. variabilis* (2), would present mitochondrial localization while in *B. prasinos* the protein would be located in plastids. The analysis for the sequences of *A. protothecoides*, *C. subellipsoidea*, *M. commoda*, *M. neglectum*, and *M. pusilla* showed a cytoplasm localization.
Table 1. Orthologs proteins involved in Fe-S cluster synthesis in mitochondria of Chlorophytes.

| ISC Proteins | Function | NFS | ISU | NFU | ISD11 | HSCA | HSCB | ACP | FRATAxin |
|--------------|----------|-----|-----|-----|-------|------|------|-----|----------|
| Arabidopsis thaliana genes | At5g65720.1 | At4g22220 | At3g20970 (NFU4) | At1g51390 (NFU5) | At5g61220 | At4g37910 (HSCA1) | At5g09590 (HSCA2) | At5g06410 (mtACP) | At2g44620 (mtACP1) | At1g65290 (mtACP2) | At5g47630 (mtACP3) | At4g03240 |
| UniProt | O49543-1 | O49627-1 | Q9LIG6-1 | Q9C8J2 | Q8L9E3-1 | Q9GUM2 | Q9LDZ0 | Q8L7K4 | P53665 | O80800 | Q9FGJ4 | Q9ZR07 |

Identified Protein Sequences

| Organism | Identified Protein Sequences |
|----------|-------------------------------|
| Auxenochlorella protothecoides | A0A087SBH3 (c) RMZ55380.1 (c) A0A087SPF0 A0A087SND2 XP_011400182 A0A087SCC7 RMZ53064.1 (c) |
| Bathycoccus prasinos | K8FF08 (p) XP_007511837.1 K8F7J7 K8F1V7 XP_007510865 K8FET8 XP_007511602.1 (p) |
| Chlamydomonas eustigma | GAX73632.1 A0A250XNZ5 A0A250XIU3 GAX75929 A0A250WTW6 GAX72625.1 (c) |
| Chlamydomonas reinhardtii | PNW80545.1 PNW87417.1 XP_001693712.1 A0A2K3D318 A0A2K3D340 A8IZ28 A8IZU0 (c) A8IK66 Q6UKY4 A0A2K3D5B5 |
| Chlorella variabilis | A0A2P6U3X4 | PRW56423.1 A0A2P6TLS6 A0A2P6U0I2 A0A2P6TM18 A0A2P6TMK0 (c) A0A2P6TBX9 |
| Chlorella variabilis | XP_005843562.1 (c) XP_005842897.1 (c) XP_005850086.1 (c) E1ZCG7 (c) E1ZMD2 XP_005845092 (c) E1Z6T8 |
| Chloropicon primus | A0A5B8MHP9 | QDZ2066.1 | A0A5B8MCM2 | A0A5B8MHC3 | QDZ23877 | QDZ21836.1 |
| Coccocmyxa subellipsoidea | I0Z0B3 (c) XP_005646971.1 I0YUZ0 (c) I0Z768 I0YWN6 I0YSD5 XP_005644009.1 (c) |
| Dunaniella salina | KAF5838253 KAF5839176 (c) KAF5838281.1 KAF5830450.1 KAF5832491.1 KAF5832703.1 KAF5833575.1 |
| Gonium pectorale | KXZ49215.1 | A0A150GM18 | KXZ55931 (c) A0A150GM8 KXZ42691.1 (c) |
| Haematococcus lacustris | GFH16011.1 A0A699ZQI7 | GFH07090 (c) GFH14279.1 (c) |
| Helicosporidium sp. | KDD73008.1 (c) A0A59LST8 A0A59LDR7 |
| Micractinium conductrix | A0A2P6V8L3 | PSC71357.1 (c) A0A2P6V9W1 A0A2P6VEW0 A0A2P6VHZ4 PSC73872 A0A2P6VF08 |
| Micromonas commoda | XP_002506217.1 (c) XP_002502856.1 XP_002501646.1 (c) C1EHF7 C1E8J2 C1EGS6 XP_002505982.1 C1E2X1 XP_002504509.1 |
| Species                        | Accession 1          | Accession 2          | Accession 3          | Accession 4          | Accession 5          | Accession 6          |
|-------------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| Micromonas pusilla            | XP_003055300.1       | C1MZU9               | C1MP69               | XP_003057174         | C1N9A6               | XP_003059868.1 (c)   |
| Monoraphidium neglectum       | A0A0D2M031 (c)       | XP_013897551.1       |                     | XP_013898922         | A0A0D2MT62           |                      |
| Ostreococcus lucimarinus      | XP_001415848.1       | A4RUX0               | A4RYZ0               | A4RWG3               | XP_001419576         | A4S9X3               |
| Ostreococcus tauri            | XP_003078141.1       | XP_022838219.1       | Q01C69               | A0A090M6V6           | Q01AH9               | XP_022839639         |
|                               | XP_003081355.2       | 0US42676.1           |                     |                      |                     |                      |
| Pycnococcus provasolii        | GHP08054.1           |                     |                      |                     |                      |                      |
| Raphidocelis subcapitata      | A0A2V0PAY6           | GBF95838.1           | A0A2V0PLS6           | A0A2V0PIR9           | GBF88744             | A0A2V0NV36           |
| Scenedesmus sp. NREL46B-D3    | KAF6244147.1         |                     |                      |                     |                      |                      |
| Scenedesmus sp. PABB004       | KAF8055824.1         |                     |                      |                     |                      |                      |
| Tetrabaena socialis           | PNH03155.1           | A0A2J8AFK7           | A0A2J8A4Z1           |                     | A0A2J8A839           | PNH09606.1           |
| Tetrasdesmus obliquus         | A0A383VZF3           | A0A383V5U1           | A0A383WNF2           | A0A383W6Z2           | A0A383WKP1           | A0A383VDA2           |
| Trebouxia sp. A1–2            | KAA6417420           |                     |                      |                     |                      |                      |
| Volvox carteri f. nagariensis | XP_002951960.1       | D8TNE6               | D8TVN4               | D8TMR1 (c)           | XP_002948275         | D8U1A8               |
|                               |                      |                      |                      |                      |                      | XP_002950872.1 (c)   |

Ref: (c) indicates cytosolic localization, (p) indicates chloroplastic localization, (p,m) indicates dual localization in plastids and mitochondria. All other proteins where no localization is indicated showed a predicted mitochondrial localization.
3.2. Scaffold Proteins

As described above, plant mitochondria use the ISC pathway for the synthesis of Fe-S groups [3,27,31]. After the action of the cysteine desulfurase, the persulfide group is transferred to scaffold proteins such as ISU that participate in the maturation of the cofactor. Alignment of ISU amino acid sequences from plants, human, bacteria and yeast had shown a high conservation of these proteins through evolution [73]. We found that A. thaliana ISU1 (AtISU1) amino acid sequence shares 74.9% sequence identity with EclscU, 75.4% identity with ScISU1, and 78.9% identity with human ISC1 [39,73].

In yeast, it was reported that ISU expression led to a growth restoration of the Saccharomyces cerevisiae Δisu1Δnfu1 thermosensitive mutant [73]. Frazzon et al. (2007) identified the presence of three AtISU proteins in Arabidopsis plants showing mitochondrial localization [66]. Moreover, through the analysis of Arabidopsis lines in which AtISU genes were up or downregulated, it was proposed that the three AtISU proteins contribute to the assembly of (Fe-S) clusters within mitochondria, and this is crucial for normal plant growth and development [66].

Functional interactions have been described among AtISU1 and other proteins form ISC machinery, for instance, with frataxin in humans and yeast [74–78]. More recently, our group reported that AtISU1, together with AthscB chaperon interacts with the Hsp70-type chaperon AtHscA2 and modulate its ATPase activity [79].

As for the other homologous proteins of the Fe-S group synthesis pathway, it has only been described the presence of four ISU genes in Chlamydomonas [80]. Thus, in order to know the existence of possible proteins orthologous genes in other chlorophytes, we performed an amino acid sequence similarity search using the sequence of Arabidopsis AtISU1 as a query in the Uniprot Database [70]. We identified a total of twenty-seven sequences for chlorophytes with 82.1 to 77.8% of identity to AtISU1, all of them not functionally characterized (Table 1). We only found one ISU sequence for most of the analyzed species, except for D. salina, M. commoda and O. tauri, all of them containing two copies of putative ISU proteins.

Twenty-five of these ISU sequences analyzed had between 118 and 174 amino acid residues. All of them present the three conserved cysteines that have been reported to be essential for Fe–S cluster binding in yeast ISU1 and bacterial IscU proteins [80]. The shortest protein of all analyzed, Helicosporidium ISU, had 91 amino acid residues due to a shorter N-terminal region and, as a consequence, lacked one of the conserved cysteines. It has been reported that mutations at the cysteine residues of S. cerevisiae ISU1 resulted in a loss of function of a sof1- deficient mutant [39,81]. The longest ISU homolog analyzed, from M. conductrix, had 615 amino acid residues due to an extra RCC2 like N-terminal domain, proposed to be involved with cell cycle control.

Besides, the LPPVK motif, responsible of interaction with the HscA-like chaperones [73], is rigorously conserved in all analyzed ISU proteins suggesting a similar interaction between these proteins in algae. The bioinformatic analysis in Supplementary Materials of its secondary structure showed five putative alpha helices: (the longest of 27 residues) and four beta sheets. Moreover, most of them would have mitochondrial matrix localization, that is the same location as ISU proteins in Arabidopsis and yeast, with the exception of ISU proteins from A. protothecoides, C. variabilis, D. salina (KAF5839176), M. commoda (XP_002507274.1), Helicosporidium sp. and Micractinium conductrix that would have cytoplasmic localization (Table 1) [72,80].

NifU proteins present in nitrogen-fixing bacteria were reported to contain a C-terminal domain called NFU. Subsequently, NFU proteins were found in humans, yeasts, cyanobacteria, and plants and were shown to be involved in the synthesis of Fe-S clusters, specifically the (4Fe-4S) forms [38,82–84]. Their molecular function is not completely clear. NFU1 is a late-acting factor in the biogenesis of human mitochondrial iron-sulfur proteins. It was proposed that NFU1 is an “assembler” of (4Fe-4S), able to convert two (2Fe-2S) groups bound to transfer proteins of the ISC pathway into a (4Fe-4S) group [85]. More recently, the (4Fe-4S) bound form of ISCU (human homolog of ISU proteins) was reported
to physically interact with NFU1, and to transfer the already formed cubane Fe-S group to the latter [86]. The existence of three NFU proteins in plant chloroplasts (NFU1-3), and two proteins (NFU4 and 5) in mitochondria have been described [38]. NFU proteins were reported to have a highly conserved domain CXXC, containing two cysteines involved in the binding of the Fe-S cluster and thus, essential for their biological function [87].

We performed a bioinformatic search for sequence identity to NFU in Chlorophytes using the Uniprot database [70]. For this, the *A. italiana* NFU sequences were used as a query (Table 1). When AtNFU4 was used as a query, fifteen sequences were obtained in green algae with a percentage identity between 52.9% (B. prasinos) to 67.5% (C. subellipsoidea). Using the AtNFU5 sequence as a query, the results showed a total of fourteen sequences in green algae with a percentage identity between 53.3% (A. protothecoides) to 68.8% (T. obliquus). However, many of the sequences found were the same due to the high percentage of identity between plant proteins. Thus, it was observed that thirteen sequences, from *T. obliquus*, *V. carteri f. nagariensis*, *C. sorokiniana*, *C. eustigma*, *C. reinhardtii*, *T. socialis*, *A. protothecoides*, *M. commoda*, *B. prasinos*, *O. tauri*, *C. variabilis*, *C. subellipsoidea* and *O. lucinarinus* were the same using both, AtNFU4 and AtNFU5 as a query (Table 1). The length of these sequences varied between 206 and 627 amino acid residues.

To analyze the cell location, we use the Depp-Loc1.0 server [72]. The analysis showed that the 13 proteins would have mitochondrial localization and would be found in soluble form.

On the other hand, when AtNFU1 sequence was used as a query, the results showed a total of 33 sequences in green algae with a percentage identity between 34.3% (*M. commoda*) and 57.9% (*C. subellipsoidea*). The length of the proteins found was between 162 to 1391 amino acid residues. Moreover, the analysis of the cellular localization showed that 18 of the 33 proteins found would be located in chloroplasts and of these, 5 would be found associated with the membrane.

### 3.3. Regulatory Proteins

Another protein described to be involved in Fe-S cluster assembly in mitochondria is ISD11. This is a protein that is part of the ISC machinery and it is essential for the formation of iron sulfur clusters. ISD11 is highly conserved in eukaryotes. However, no homologs were found in bacteria [88]. In humans, it was described that Isd11, a member of the LYP protein family, is a small chaperone that binds and stabilizes Nfs1 [88,89]. In yeast, it was shown that it is essential for viability of cells. When Isd11 was depleted, accumulation of iron in mitochondria and reduced protein levels and activities of Fe/S proteins were observed, affecting mitochondrial and also cytoplasmic proteins [88]. The loss of the enzymatic activity was due to the lack of their Fe/S clusters. Thus, it was postulated that Isd11 is required for an early step in this process as it is present in a tight complex with the cysteine desulfurase Nfs1. In the absence of Isd11, Nfs1 tends to aggregate and lose its function. From this data it was proposed that Isd11 would stabilize Nfs1 and mediate the assembly/interaction of Nfs1 with other proteins [90].

We recently reported in *Arabidopsis* that AtNFS1 and AtISD11 regulate the ferrochelatase activity of AtFH in vitro [91]. In addition, we suggested that AtFH, AtNFS1 and AtISD11 form a multiprotein complex in *Arabidopsis* mitochondria. We found that AtFH and AtISD11 modulate the desulfurase activity of AtNFS1, indicating that the complex would have an important role in the early stages of Fe-S cluster synthesis [36]. Moreover, this complex would act in mitigating plant mitochondrial oxidative damage. In AtNFS1 deficient plants, we found a decrease of AtISD11 and AtFH mRNA transcript levels and, when AtNFS1 was overexpressed, the transcript levels of these genes were increased too, indicating the existence of a regulatory link [92].

Regarding the characterization of ISD11 in Chlorophytes, there is no bibliography at present. For this reason, we decided to carry out a bioinformatic search for sequence identity in the Uniprot database [70], using the AtISD11 sequence as a query. We identified a total of six sequences for green algae with a percentage identity between 34.2 and 49.1%
(C. primus, O. lucimarinus, C. reinhardtii, T. socialis, V. carteri f. nagariensis, and C. subellipsoidea), all of them not yet characterized. By refining the search and using the detected Chlamydomonas ISD11 sequence in a second search, we found eight additional homologous proteins (from M. commoda, M. pusilla, O. tauri, R. subcapitata, T. obliquus, C. sorokiniana, H. lacustris, and M. conductrix). Interestingly, all the species show a unique ISD11 protein, except for C. subellipsoidea that contains two isoforms.

The fourteen protein sequences analyzed showed a length of between 84 and 135 amino acid residues, being the T. obliquus protein the longest of all. All of them would have mitochondrial localization, with a transit peptide of approximately 20 residues in length and would be found in a soluble form, not anchored to membranes [72]. Subsequently, we analyzed their secondary structure with bioinformatic methods [93] and we detected three possible alpha helices: the first two of about 15–17 and the third between 25–28 residues. It was also observed that in the T. obliquus ISD11 sequence there would be a beta strand at position 81–84 and a fourth alpha helix between residues 93–102.

When we performed an alignment by Clustal Omega [94] of all sequences of Chlorophytes plus the homologs from Arabidopsis and corn, we detected that three of the critical residues for the interaction with AtNF51 are absolutely conserved (Phe38, Leu61 and Arg66) [36]. The Arg66 residue is homologous to Arg68 in humans and it has been shown that the mutation of this amino acid, although it does not prevent the formation of the NF51-ISD11 complex, causes a severe decrease in NF51 activity [95].

3.4. Chaperones and Co-Chaperones

As mentioned before, other proteins involved in the Fe-S biosynthetic pathway within mitochondria are members of a molecular chaperone system, comprising by Hsp70-type chaperones and a DNA-J-like co-chaperone (J-protein). Both proteins are involved in the maturation of the Fe-S cluster after its insertion into apoproteins [58,96].

E. coli contain two proteins that participate in a specialized chaperone system involved in the Fe–S cluster formation: an Hsp70 (HscA), and a J-protein (HscB), that facilitate the Fe–S cluster delivery mechanism in an ATP-dependent manner [97]. As E. coli, S. cerevisiae contain a similar chaperone system (Ssq1/Jac1), localized in the mitochondrial matrix [97,98]. It was postulated that HscA recognizes and interacts with the conserved LPPVK peptide loop of the scaffold protein IscU, and this interaction is mediated by HscB [2]. It was reported that this chaperone system is conserved in all the eukaryotes [99].

In Arabidopsis, two genes coding for mitochondrial Hsp70-type chaperons were identified: AtHscA1 (At4g37910) and AtHscA2 (At5g09590). It was postulated that these genes arose possibly from a duplication event from a gene encoding a multifunctional Hsp70 protein [100]. Moreover, a Jac1 homolog was also described (AtHSCB, At5g06410) [3]. Xu et al. (2009) reported that AtHSCB and AtISU1 can stimulate the ATPase activity of AtHscA1, suggesting that this protein is involved in the Fe–S cluster synthesis in mitochondria [101]. As mentioned, we demonstrated that AtHscA2 interacts with AtISU1 and AtHSCB, and this interaction modulates the activity of AtHscA2 [79]. On the other hand, we recently demonstrated that AtHSCB would have an additional role to that of participation in the synthesis of Fe-S groups. Using overexpressing and knock-down A. thaliana mutants, we suggest that AtHSCB have a relevant role in plant Fe metabolism because it would be involved in the translocation of Fe from root to shoots [102].

To identify the putative HscA protein in chlorophytes, we searched for homologous in the NCBI Protein Database [103] using Arabidopsis AtHscA1 (At4g37910) protein sequence as a query. We identified at least twenty-one sequences for chlorophytes with 70.2–89.1% of identity (Table 1). While most of the organisms contain only one copy, we identified three copies of putative HscAs in D. salina. It is important to note that the analysis of the amino acid sequence alignment did not allow to differentiate between HscA1 and 2 in the algae sequences.

Regarding HSCB, a bioinformatic search was performed using the NCBI Protein Database [103] and the AtHSCB homolog from A. thaliana (Q8L7K4) as template, limiting the
search to Chlorophytes. As a result, twenty sequences were obtained (Table 1) with only one homolog present in each organism. A second search was performed in the Phytozone Database [22] using the HSCB homolog from C. reinhardtii (A8IK66) as template, obtaining two more HSCB sequences from D. salina and Micromonas sp RCC299. The analyzed sequences presented a percentage of identity between 25.3–43.5% and a length between 144 and 318 amino acid residues, being that from C. reinhardtii the longest one. Most sequences were predicted to have a mitochondrial localization and a soluble form. As exceptions, HSCBs from C. variabilis, H. lacustris and Trebouxia sp. A1–2 were predicted to have a cytoplasmic localization, whereas those HSCBs from C. eustigma and A. protothecoides would have a plastidic localization. Moreover, the homolog from M. neglectum would have a mitochondrial localization but associated with membranes, and finally, the HSCB from G. pectorale would have a dual localization in nucleus and cytoplasm. This potential characteristic of association of some proteins of the ISC pathway with membranes is little known and could indicate a different degree of organization of the Fe-S group synthesis pathway in some of these organisms.

It was shown that the mitochondrial cysteine desulfurase complex from humans and yeast (LYRM4/NFS1 and Isd11/Nfs1, respectively) binds ACP (acyl carrier protein) as an important subunit. This NFS1-ISD11-ACP complex forms the core of the iron-sulfur (Fe-S) assembly complex and associates with ISCU and frataxin to synthesize Fe-S clusters [104]. In higher plants, especially in Arabidopsis thaliana, there are three genes encoding mitochondrial ACPs namely mtACP1 (At2g44620), mtACP2 (At1g65290), and mtACP3 (At5g47630), and five plastidial ACPs, AtACP1 (At3g05020), AtACP2 (At1g54580), AtACP3 (At1g54630), AtACP4 (At4g25050), and AtACP5 (At5g27200) [40].

It was reported that ACP participates in the synthesis of fatty acids and lipoic acid in many organisms. In the case of algae, as well as for plants, the characterization of ACP was also focused on its role in lipid metabolism [105]. For example, Blatti et al. (2012) have demonstrated that the protein-protein interactions between the fatty acid ACP and thiosterase govern fatty acid hydrolysis within the C. reinhardtii chloroplast [106]. In this work, it was reported the presence of a mitochondrial ACP but do not address its participation in the assembly of iron sulfur complexes as in plants.

Following the scheme for the search for homologous proteins that we previously used, we use the three Arabidopsis mtACP sequences as a query. Subsequently, we selected an algae sequence homologous to each mtACP and performed a second search for sequence identity to expand the number of candidates. Thus, we found fourteen sequences homologous to mtACP1, sixteen to mtACP2 and eighteen to mtACP3. However, many of them were the same proteins, due to the high identity between the three plant proteins (55.7% between mtACP1 and 2 and higher than 37% for mtACP3 with the mtACP1 and 2). Therefore, nineteen protein sequences of the following Chlorophytes were selected: C. eustigma, B. prsinos, O. tauri, C. reinhardtii, T. sociales, G. pectorale, V. carteri f. nagariensis, M. neglectum, R. subcapitata, T. obliquus, C. sorokoniana, C. variabilis, Helicosporidium sp., C. subellipsoides, M. comoda, M. pusilla, A. protothecoides, M. conductrix, and O. lucimarinus (Table 1).

The identity percentages with mtACP1 varied between 43.8% for O. tauri and 55.6% for C. subellipsoides; between 48.4% to 60.2% (for V. carteri and M. pusilla, respectively) for mtACP2 and between 30.6% to 41.7% (for O. tauri and M. neglectum, respectively) for mtACP3.

Seventeen of the Chlorophytes protein sequences analyzed showed a length between 88 to 144 amino acid residues, being the M. pusilla protein the smallest. In contrast, two sequences, one belonging to C. sorokoniana and the second from M. conductrix, showed a greater length of about 440 amino acids. Eighteen proteins showed a potential mitochondrial localization, with a transit peptide of approximately 30–40 residues in length and would be found in a soluble form, not anchored to the membrane [72], whereas M. pusilla ACP would have a cytoplasmic localization. However, as it is a predicted sequence and
the shortest one, there could be an annotation error, and the sequence may not be complete.

The ACP residues which interact with ISD11 are conserved in all the algae sequences analyzed, suggesting the possible formation of the same subcomplexes in Chlorophytes. In addition, we detected the absolute conservation of the serine residue, responsible for binding to 4′-phosphopantetheine (4′-PPT, prosthetic group covalently attached) in all algae sequences. Recently, Cory et al. (2017) proposed a possible role of ACP in eukaryotic Fe–S cluster biosynthesis by identifying ACP–lipid interactions that promote the stability of ISD11. This could suggest that the contribution of ACP to the Fe-S assembly complex is a moonlighting function. Due to the finding of homologous sequences and structures in chlorophytes, these functions would be evolutionarily conserved.

3.5. Frataxin

Frataxin is a nuclear-encoded mitochondrial protein highly conserved in prokaryotes and eukaryotes. Its deficiency was initially described as the phenotype of Friedreich’s ataxia, an autosomal recessive disease in humans, commonly resulting from a GAA expansion in the frataxin gene [43]. In yeast, the decreased expression of the frataxin homolog, Yfh1, resulted in respiratory deficiency and mitochondrial iron accumulation [45]. Furthermore, in the absence of Yfh1p, it was observed a decrease in the activity of several Fe-S-containing enzymes such as aconitase and succinate dehydrogenase, indicating a role of frataxin in the formation of Fe-S clusters and Fe-S proteins [45].

It was described that in Arabidopsis plants, there is a single frataxin homolog (AtFH) with dual localization in mitochondria and chloroplasts; while, in maize plants, we described the presence of two isoforms, ZmFH1 and ZmFH2, each protein also located in both plant organelles [41,44,107–111]. In plants, we also showed that frataxin is essential for the activity of Fe-S proteins such as aconitase and succinate dehydrogenase [44]. Furthermore, AtFH has ferrochelatase activity and is involved in the metabolism of the heme group and the synthesis of heme proteins in mitochondria [91,112]. Moreover, we recently demonstrated that AtFH interacts with AtISD11 and AtNFS1, forming a complex that would modulate ferrochelatase activity and cysteine desulfurization by AtNFS1 [36,91].

In order to have a better understanding of frataxin function in algae we performed a bioinformatic search in NCBI using Uniprot database [70] and the amino acid sequence of the frataxin homolog from A. thaliana (NP_192233.2) as a template. As a result of this search ten frataxin homologs with a percentage of identity between 33% to 63% were identified. Only one homolog sequence from each organism was obtained, with the exception of O. tauri, in which two isoforms of frataxin were found (Table 1). By refining the search using the detected frataxin sequence from C. reinhardtii (A0A2K3D5B5) as a template, ten more sequences were obtained in the Phytozone Database [22] with a percentage of identity between 49.2% and 80.1% (V. carteri f. nagariensis, G. pectoral, R. subcapitata, T. socialis, C. sorokiana, C. eustigma, C. subellipsoidae, C. primus, A. protothecoides, M. commoda) all of them not characterized.

The twenty protein sequences analyzed presented a length between 110 and 290 amino acid residues, being that from C. variabilis the longest one. Two types of localization were found: cytoplasmic (for V. carteri, G. pectorale, C. eustigma, C. subellipsoidae, M. pusilla and O. tauri isoform 2) and mitochondrial (C. reinhardtii, R. subcapitata, T. socialis, C. primus, M. commoda, D. salina, O. lucimarinus, O. tauri isoform 1, and E. siliculosus). As an exception, the sequence of C. variabilis presented a nuclear localization. All sequences were predicted to be found in a soluble form [72]. Furthermore, we analyzed their secondary structures with NetSurfP-2.0 [93] and we detected the presence of 2 alpha helices and 6 beta strands, suggesting a structural conservation of these proteins with the human and yeast frataxin, whose 3D structures were determined [113,114]. Moreover, a detailed Conserved Domain (CD) search analysis of the identified sequences was performed with NCBI [115].
Results showed that all the protein sequences contain a frataxin-like domain and a putative iron binding site with significantly high conservation of aminoacidic residues, which confirms the high structural similarity that frataxin homologs contain with each other.

3.6. CIA Pathway

As described above, the ISC machinery drives the formation of Fe-S clusters in mitochondria and is essential for the maturation of Fe-S proteins in this organelle. In contrast, biogenesis of cytosolic Fe-S proteins requires components that are exported from the mitochondria that are later matured in the CIA system (cytosolic iron–sulfur protein assembly machinery) [116,117]. This system is conserved in virtually all eukaryotes and is essential for cell viability. Most of the CIA components were first identified and characterized in yeast and human cells and more recently, in Arabidopsis thaliana. Bernard et al. [118] published in 2013 an interesting work in which they summarize the identified loci, the mutants studied and the phenotypes observed for each component of the CIA pathway from A. thaliana plants. However, as with the ISC pathway, little is known about the occurrence, conservation, and possible proteins involved in the CIA pathway in algae.

Thus, using the same scheme that we used to identify homologous proteins of the ISC pathway, we carried out a search for homologous proteins that would participate in the CIA pathway. When conducting the search based on the CIA proteins described in A. thaliana, we found that, apparently, this pathway would be less conserved than the ISC one, since we could not identify the presence of many CIA homologs in algae. Table 2 shows the homologs found in chlorophytes for NDOR1 and DRE2, which are involved in an electron transfer in early steps of cytosolic Fe-S cluster biogenesis; NBP35, a scaffold protein involved in the formation of (4Fe-4S) groups; NAR1, which may play a role in the transfer of Fe-S clusters into apoproteins; CIA1 and MMS19, involved in the transfer and insertion of Fe-S clusters into target proteins; and AE7, identified as a central member of the CIA pathway [117,118].

We were only able to identify all the components of the CIA pathway in C. reinhardtii, but with some peculiarities. For the NAR1 protein, we found three different homologs in this alga. With respect to AE7, while it has been described three proteins in Arabidopsis (AE7 and two AE7-like proteins), we found only one homolog in C. reinhardtii [118]. Finally, except for DRE2 of T. obliquus, where we found the presence of two homologs, we have not identified the presence of multiple homologs of the CIA pathway in the algae analyzed (Table 2).
Table 2. Orthologs of the cytosol (CIA) pathway in Chlorophytes.

| Function | Identified protein sequences |
|----------|-----------------------------|
| Part of an electron transfer chain functioning in an early step of cytosolic Fe-S biogenesis. | **Organism** |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | **AUXENOCHELLOREA PROTOTHECOIDES** |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | At3g02280 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | At5g18400 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | At5g50960 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | At4g16440 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | At2g26060 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | Atlg68310 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | At5g48120 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | Q6NPS8 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | Q8L7Z3 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | Q8H1Q2-1 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | Q94CL6 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | 080990-1 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | Q9C9G6 |
| Transfers electrons from NADPH to the Fe-S cluster of the DRE2 homolog. | Q0WVF8 |
| May play a role in the transfer of pre-assembled Fe/S clusters to target apoproteins. | **CHLAMYDOMONAS EUSTIGMA** |
| May play a role in the transfer of pre-assembled Fe/S clusters to target apoproteins. | Cre12.g551850.t1.2 |
| May play a role in the transfer of pre-assembled Fe/S clusters to target apoproteins. | Cre11.g475350.t1.3 |
| May play a role in the transfer of pre-assembled Fe/S clusters to target apoproteins. | Cre12.g494400.t1.2 |
| May play a role in the transfer of pre-assembled Fe/S clusters to target apoproteins. | Cre03.g200550.t1.2 |
| May play a role in the transfer of pre-assembled Fe/S clusters to target apoproteins. | Cre03.g199800.t1.1 |
| May play a role in the transfer of pre-assembled Fe/S clusters to target apoproteins. | Cre09.g396600.t1.1 |
| May play a role in the transfer of pre-assembled Fe/S clusters to target apoproteins. | Cre08.g374400.t1.3 |
| May play a role in the transfer of pre-assembled Fe/S clusters to target apoproteins. | A0A2K3D8M0 |
| May play a role in the transfer of pre-assembled Fe/S clusters to target apoproteins. | g14594.t1 |
| Transfer and insertion of Fe-S clusters into target proteins (CIA targeting complex). | **CHLORELLA SOROKINIANA** |
| Transfer and insertion of Fe-S clusters into target proteins (CIA targeting complex). | A0A2P6TP36 |
| Transfer and insertion of Fe-S clusters into target proteins (CIA targeting complex). | A0A2P6TVH6 |
| Transfer and insertion of Fe-S clusters into target proteins (CIA targeting complex). | A0A2P6TVY6 |
| Transfer and insertion of Fe-S clusters into target proteins (CIA targeting complex). | A0A2P6U4B7 |
| Transfer and insertion of Fe-S clusters into target proteins (CIA targeting complex). | PRW58456.1 |
| Transfer and insertion of Fe-S clusters into target proteins (CIA targeting complex). | **CHLOROPICON PRIMUM** |
| Transfer and insertion of Fe-S clusters into target proteins (CIA targeting complex). | A0A5B8MC28 |
| Transfer and insertion of Fe-S clusters into target proteins (CIA targeting complex). | A0A5B8MEG6 |
| Transfer and insertion of Fe-S clusters into target proteins (CIA targeting complex). | A0A5B8MQB3 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | **COCCOMYXA SUBELLIPSOIDEA** |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | I0YJK0 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | I0YZ253 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | I0YLU4 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | I0YK24 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | I0Z017 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | A0A150G73 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | A0A150GJ11 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | A0A150G6L9 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | A0A150G9L7 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | **HELICOSPORIDUM SP. ATCC 50920** |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | A0A059LKT0 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | **MICRACTIMN CONDUCTRIX** |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | A0A2P6VI63 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | A0A2P6V3I3 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | A0A2P6V477 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | A0A2P6V5J4 |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | **MICROMONAS COMMODA** |
| Central member of the cytosolic iron-sulfur (Fe-S) protein assembly (CIA) pathway. | XP_002505452.1 |
| Species            | Accession 1 | Accession 2 | Accession 3 |
|--------------------|-------------|-------------|-------------|
| Micromonas pusilla | C1N4X9      | A0A0D2MN30  | XP_003059724.1 |
| Monoraphidium neglectum | A0A0D2MN30 | A0A0D2JM25  |             |
| Ostreococcus lucimarinus CCE9901 | A4RR54 | A4RRN3 | A4RRB8 | A4SBG2 |
| Ostreococcus tauri | A0A096P7S5  | A0A090M2P3  | Q01GB5      | XP_003083112.2 |
| Raphidocelis subcapitata | A0A2V0PD30 | A0A2V0P7V0 | A0A2V0PR28 | A0A2V0NRG7 |
| Tetrabaena socialis |             |             | A0A2J8A236  |             |
| Tetradesmus obliquus | A0A383VZJ9 | A0A383W531  | A0A383VND8  | A0A383VR66  |
| Volvox cateri f. nagariensis | D8UCR2 | D8U8M9 | D8UE05 | D8UBL6 | D8U2P0 | D8UEN0 |
4. Conclusions

The search in genomic and protein databases showed a high conservation of the genes and proteins involved in the synthesis of Fe-S groups in algal mitochondria. Curiously, despite being unicellular organisms, and that a priori it would be thought that the pathway for the synthesis of Fe-S groups could be of less complexity compared to higher plants, many algae showed the presence of multiple genes and/or homologous proteins that would be members of the ISC pathway. In contrast, in most of the algae analyzed, we observed the presence of a single isoform of the proteins of the CIA pathway, in addition to the absence of many homologs in most of the organisms evaluated, with the exception of C. reinhardtii.

Previously, the presence of some genes that would be in multiple copies in plants has been described, such as ISU1-3, ACPI-3 in Arabidopsis mitochondria. However, the genes encoding AtNFSl, AtISD11, AtHSCB, and AtFH were found in a single copy, although in the case of AtFH, we demonstrate dual localization in mitochondria and chloroplasts. As mentioned above, we also recently identified and characterized the presence of two genes that code for frataxin in corn, ZmFH1, and ZmFH2, both with dual localization in mitochondria and chloroplasts. While much is known about the role of frataxin homologs in mitochondria, their possible roles in chloroplasts and possible interactions with proteins in that compartment are unknown. The potential dual localization and/or the association with membranes, as well as the functions of many of the genes of the ISC pathway in algae is a subject that deserves to be studied. In addition, the presence of multiple genes found in the genomes of many chlorophytes indicates that the ISC pathway would have particular and specific characteristics in each species. This could be related to the needs of both iron and sulfur for each organism, and also with the ability to adapt to growth in different environments.

Supplementary Materials: The following are available online at www.mdpi.com/2223-7747/10/2/200/s1, Bioinformatic Analysis: The sequence similarity searches were carried out using BLASTP against the NCBI nonredundant database (www.ncbi.nlm.nih.gov), UniProtKB database (www.uniprot.org) and Phytozome database (https://phytozome.jgi.doe.gov/pz/portal.html) using A. thaliana and Chlamydomonas Fe-S proteins sequences as query, as described in each case. The selected sequences were aligned with Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo/). Secondary structure prediction was achieved with the servers NetSurfP-2.0 (www.cbs.dtu.dk/services/NetSurfP/) and CD-Search (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Intracellular location was predicted using the Depp-Loc1.0 server (www.cbs.dtu.dk/services/DeepLoc/).

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References

1. Halliwell, B.; Gutteridge, J.M. Biologically relevant metal ion-dependent hydroxyl radical generation. An update. FEBS Lett. 1992, 307, 108–112, doi:10.1016/0014-5793(92)80911-y.
2. Lil, R.; Mühlenhoff, U. Maturation of iron-sulfur proteins in eukaryotes: Mechanisms, connected processes, and diseases. Annu. Rev. Biochem. 2008, 77, 669–700.
30. Zheng, L.; White, R.H.; Cash, V.L.; Jack, R.F.; Dean, D.R. Cysteine desulforase activity indicates a role for NIFS in metallocluster biosynthesis. *Proc. Natl. Acad. Sci. USA* 1993, 90, 2754–2758.
31. Balk, J.; Lobreaux, S. Biogenesis of iron-sulfur proteins in plants. *Trends Plant Sci.* 2005, 10, 324–331, doi:10.1016/j.tplants.2005.05.002.
32. Turowski, V.R.; Busi, M.V.; Gomez-Casati, D.F. Structural and functional studies of the mitochondrial cysteine desulfurase from *Arabidopsis thaliana*. *Mol. Plant* 2012, 5, 1001–1010, doi:10.1093/mp/sss037.
33. Leon, S.; Touraine, B.; Briat, J.F.; Lobreaux, S. The AtNFS2 gene from *Arabidopsis thaliana* encodes a NiFe-like plastidial cysteine desulfurase. *Biochem. J.* 2002, 366, 557–564, doi:10.1042/BJ20020322.
34. Atkinson, A.; Smith, P.; Fox, J.L.; Cui, T.Z.; Khalimonchuk, O.; Winge, D.R. The LYR protein Mzm1 functions in the insertion of the Rieske Fe/S protein in yeast mitochondria. *Mol. Cell. Biol.* 2011, 31, 3988–3996, doi:10.1128/MCB.05673-11.
35. Richards, T.A.; van der Giezen, M. Evolution of the Isd11-IscS complex reveals a single alpha-proteobacterial endosymbiosis for all eukaryotes. *Mol. Biol. Evol.* 2006, 23, 1341–1344, doi:10.1093/molbev/msl001.
36. Armas, A.M.; Balparda, M.; Terenzi, A.; Busi, M.V.; Pagani, M.A.; Gomez-Casati, D.F. Iron-Sulfur Cluster Complex Assembly in the Mitochondria of *Arabidopsis thaliana*. *Plants* 2020, 9, doi:10.3390/plants9011171.
37. Olson, J.W.; Agar, J.N.; Johnson, M.K.; Maier, R.J. Characterization of the NifU and NifS Fe–S cluster formation proteins essential for viability in Helicobacter pylori. *Biochemistry* 2003, 39, 16213–16219.
38. Leon, S.; Touraine, B.; Ribot, C.; Briat, J.F.; Lobreaux, S. Iron-sulphur cluster assembly in plants: Distinct NFU proteins in mitochondria and plastids from *Arabidopsis thaliana*. *Biochem. J.* 2003, 371, 823–830, doi:10.1042/BJ20021946.
39. Tone, Y.; Kawai-Yamada, M.; Uchiyama, H. Characterization and localization of the *Arabidopsis thaliana* ISU1 gene. *Biochim. Et Biophys. Acta* 2004, 1680, 171–175, doi:10.1016/j.bbadis.2004.09.005.
40. Meyer, E.H.; Heazlewood, J.L.; Millar, A.H. Mitochondrial acyl carrier proteins in *Arabidopsis thaliana* are predominantly soluble matrix proteins and none can be confirmed as subunits of respiratory Complex I. *Plant Mol. Biol.* 2007, 64, 319–327, doi:10.1007/s11103-007-9156-9.
41. Busi, M.V.; Gomez-Casati, D.F. Exploring frataxin function. *Jumbi Life* 2012, 64, 56–63, doi:10.1002/jub.577.
42. Castro, I.H.; Pignataro, M.F.; Sewell, K.E.; Espeche, I.D.; Herrera, M.G.; Noguera, M.E.; Dain, L.; Nadra, A.D.; Aran, M.; Smal, C.; et al. Frataxin Structure and Function. *Sub-Cell. Biochem.* 2019, 93, 393–438, doi:10.1007/978-3-030-28151-9_13.
43. Babcock, M.; de Silva, D.; Oaks, R.; Davis-Kaplan, S.; Jiralerspong, S.; Montermini, L.; Pandolfo, M.; Kaplan, J. Regulation of mitochondrial iron accumulation by YhhP, a putative homolog of frataxin. *Science* 1997, 276, 1709–1712, doi:10.1126/science.276.5319.1709.
44. Busi, M.V.; Maliandi, M.V.; Valdez, H.; Clemente, M.; Zabaleta, E.J.; Araya, A.; Gomez-Casati, D.F. Deficiency of *Arabidopsis thaliana* frataxin alters activity of mitochondrial Fe–S proteins and induces oxidative stress. *Plant J. Cell Mol. Biol.* 2006, 48, 873–882, doi:10.1111/j.1365-313X.2006.02923.x.
45. Chen, O.S.; Hemenway, S.; Kaplan, J. Inhibition of Fe-S cluster biosynthesis decreases mitochondrial iron export: Evidence that YhhP affects Fe-S cluster synthesis. *Proc. Natl. Acad. Sci. USA* 2002, 99, 12321–12326, doi:10.1073/pnas.192445999.
46. Gervason, S.; Larkem, D.; Mansour, A.B.; Botzanowski, T.; Muller, C.S.; Pecqueur, L.; Le Pavec, G.; Delaunay-Moisan, A.; Brun, O.; Agrament, J.; et al. Physiologically relevant reconstitution of iron-sulfur cluster biosynthesis uncovers persulfide-processing functions of ferredoxin-2 and frataxin. *Nat. Commun.* 2019, 10, 3566, doi:10.1038/s41467-019-11470-9.
47. Isaya, G.; O’Neill, H.A.; Gakh, O.; Park, S.; Mantcheva, R.; Mooney, S.M. Functional studies of frataxin. *Acta Paediatr.* 2004, 93, 68–71; discussion 72–63.
48. Lesuisse, E.; Santos, R.; Matzanken, B.F.; Knight, S.A.; Camadro, J.M.; Dancis, A. Iron use for haeme synthesis is under control of the yeast frataxin homologue (Yfh1). *Hum. Mol. Genet.* 2002, 12, 879–889.
49. Ristow, M.; Pfister, M.F.; Yee, A.J.; Schubert, M.; Michael, L.; Zhang, C.Y.; Ueki, K.; Michael, M.D.; 2nd; Lowell, B.B.; Kahn, C.R. Frataxin activates mitochondrial energy conversion and oxidative phosphorylation. *Proc. Natl. Acad. Sci. USA* 2000, 97, 12239–12243, doi:10.1073/pnas.220403797.
50. Mihara, H.; Esaki, N. Bacterial cysteine desulfurases: Their function and mechanisms. *Appl. Microbiol. Biotechnol.* 2002, 60, 12–23, doi:10.1007/s00253-002-1107-4.
51. Begley, T.P.; Xi, J.; Kinsland, C.; Taylor, S.; McLafferty, F. The enzymology of sulfur activation during thiamin and biotin biosynthesis. *Curr. Opin. Chem. Biol.* 1999, 3, 623–629, doi:10.s1637-5931(99)00018-6.
52. Van Hoewyk, D.; Abdel-Ghany, E.; Cuhu, C.M.; Herbert, S.K.; Kugrens, P.; Pilon, M.; Pilon-Smits, E.A. Chloroplast iron-sulfur cluster protein maturation requires the essential cysteine desulfurase CpNifs. *Proc. Natl. Acad. Sci. USA* 2007, 104, 5686–5691, doi:10.1073/pnas.0700774104.
53. Marquet, A. Enzymology of carbon-sulfur bond formation. *Curr. Opin. Chem. Biol.* 2001, 5, 541–549, doi:10.1016/s1367-5931(00)00249-0.
54. Mihara, H.; Kurihara, T.; Yoshimura, T.; Soda, K.; Esaki, N. Cysteine Sulfinate Desulfinase, a NIFS-like Protein ofEscherichia coli with Selenocysteine Lyase Cysteine Desulfurase Activities Gene Cloning, Purification, and Characterization of A Novel Pyridoxal Enzyme. *J. Biol. Chem.* 1997, 272, 22417–22424.
55. Kaiser, J.T.; Clausen, T.; Bourenkov, G.P.; Bartunik, H.D.; Steinbacher, S.; Huber, R. Crystal structure of a Nifs-like protein from *Thermotoga maritima* implicating iron sulphur cluster assembly. *J. Mol. Biol.* 2000, 297, 451–464, doi:10.1006/jmbi.2000.3581.
56. Nakamura, R.; Hirota, M.; Ogawa, S.; Takahashi, Y.; Fujishiro, T. Snapshots of PLP-substrate and PLP-product external aldilimes as intermediates in two types of cysteine desulfurase enzymes. *FEBS J.* 2020, 287, 1138–1154, doi:10.1111/FEBS.15081.
57. Blahut, M.; Wise, C.E.; Bruno, M.R.; Dong, G.; Makris, T.M.; Frantom, P.A.; Dunkle, J.A.; Outten, F.W. Direct observation of intermediates in the SufS cysteine desulphurase reaction reveals functional roles of conserved active-site residues. J. Biol. Chem. 2019, 294, 12444–12458, doi:10.1074/jbc.RA119.009471.

58. Cupp-Vickery, J.R.; Urbina, H.; Vickery, L.E. Crystal structure of IscS, a cysteine desulphurase from Escherichia coli. J. Mol. Biol. 2003, 330, 1049–1059, doi:10.1016/s0022-2836(03)0690-9.

59. Zheng, L.; White, R.H.; Cash, V.L.; Dean, D.R. Mechanism for the desulphurization of L-cysteine catalyzed by the nifs gene product. Biochemistry 1994, 33, 4714–4720, doi:10.1021/bi00181a031.

60. Jacobson, M.R.; Cash, V.L.; Weiss, M.C.; Laird, N.F.; Newton, W.E.; Dean, D.R. Biochemical and genetic analysis of the nifUSVWZM cluster from Azotobacter vinelandii. Mol. Gen. Genet. 1999, 261, 49–57, doi:10.1007/ BF00261156.

61. Land, T.; Rouault, T.A. Targeting of a human iron–sulfur cluster assembly enzyme, nifs, to different subcellular compartments is regulated through alternative AUG utilization. Mol. Cell 1998, 2, 807–815.

62. Li, J.; Kogan, M.; Knight, S.A.; Pain, D.; Dancis, A. Yeast mitochondrial proteins, Nfs1p, coordinateely regulates iron–sulfur cluster proteins, cellular iron uptake, and iron distribution. J. Biol. Chem. 1999, 274, 33025–33034.

63. Naamati, A.; Reggev-Rudzki, N.; Galperin, S.; Lill, R.; Pines, O. Dual targeting of Nfs1 and discovery of its novel processing enzyme, icp55. J. Biol. Chem. 2009, 284, 30200–30208.

64. Biederbick, A.; Stehling, O.; Rosser, R.; Niggemeyer, B.; Nakai, Y.; Elsasser, H.P.; Lill, R. Role of human mitochondrial Nfs1 in cytosolic iron–sulfur protein biogenesis and iron regulation. Mol. Cell. Biol. 2006, 26, 5575–5587, doi:10.1128/MCB.00112-06.

65. Heis, M.D.; Ditmer, E.M.; de Oliveira, L.A.; Frazzon, A.P.; Margis, R.; Frazzon, J. Differential expression of cysteine desulfurases in soybean. BMC Plant Biol. 2011, 11, 166, doi:10.1186/1471-2229-11-166.

66. Frazzon, A.P.; Ramirez, M.V.; Warek, U.; Balk, J.; Frazzon, J.; Dean, D.R.; Winkel, B.S. Functional analysis of Arabidopsis genes involved in mitochondrial iron–sulfur cluster assembly. Plant Mol. Biol. 2007, 64, 225–240, doi:10.1007/s11103-007-9147-x.

67. Kushnir, S.; Babiychuk, E.; Storozenko, S.; Davey, M.W.; Papenbrock, J.; De Rycke, R.; England, G.; Stephan, U.W.; Lange, H.; Kispal, G.; et al. A Mutation of the Mitochondrial ABC Transporter Sta1 Leads to Dwarfism and Chlorosis in the Arabidopsis Mutant starik. Plant Cell 2013, 25, 139–149, doi:10.1105/tpc.113.1189.

68. Pilon-Smiths, E.A.; Garifullina, G.F.; Abdel-Ghany, S.; Kato, S.; Mihara, H.; Hale, K.; Burkhead, J.L.; Esaki, N.; Kurihara, T.; Pilon, M. Characterization of a NifS-like chloroplast iron protein from Arabidopsis. Implications for its role in sulfur and selenium metabolism. Plant Physiol. 2002, 130, 1309–1318, doi:10.1104/pp.10.010280.

69. Heidenreich, T.; Wollers, S.; Mendel, R.R.; Bittner, F. Characterization of the NifS-like domain of ABAD3 from Arabidopsis thaliana provides insight into the mechanism of molybdenum cofactor sulfuration. J. Biol. Chem. 2005, 280, 4213–4218, doi:10.1074/jbc.M411195200.

70. UniProtKB. Availabe online: https://www.uniprot.org/ (accessed on 15 November 2020).

71. Black, K.A.; Dos Santos, P.C. Shared-intermediates in the biosynthesis of thio-cofactors: Mechanism and functions of cysteine desulphurases and sulfur acceptors. Biochim. Et Biophys. Acta 2015, 1853, 1470–1480, doi:10.1016/j.bbamcr.2014.10.018.

72. Depp-Loc1.0 Server. Available online: https://www.cbs.dtu.dk/services/DeepLoc/ (accessed on 19 November 2020).

73. Leon, S.; Touraine, B.; Briat, J.F.; Lobreaux, S. Mitochondrial localization of Arabidopsis thaliana Isu Fe-S scaffold proteins. FEBS Lett. 2005, 579, 1930–1934, doi:10.1016/j.febslet.2005.02.038.

74. Gonzalez-Cabo, P.; Vazquez-Manrique, R.P.; Rarey, C.; Galin-Gimen, M.A.; Sanz, P.; Palau, F. Frataxin interacts functionally with mitochondrial electron transport chain proteins. Hum. Mol. Genet. 2005, 14, 2091–2098, doi:10.1093/hmg/ddi214.

75. Ramazzotti, A.; Vanmansart, V.; Fourny, F. Mitochondrial functional interactions between frataxin and Isu1p, the iron-sulfur cluster scaffold protein, in Saccharomyces cerevisiae. FEBS Lett. 2004, 557, 215–220, doi:10.1016/s0014-5793(03)01498-4.

76. Shen, Y.; Cortopassi, G. HSC20 interacts with frataxin and is involved in iron-sulfur cluster biogenesis and iron homeostasis. Hum. Mol. Genet. 2012, 21, 1457–1469, doi:10.1093/hmg/ddr582.

77. Shen, Y.; Napoli, E.; Cortopassi, G. Mitochondrial frataxin interacts with ISD11 of the NFS1/ISCU complex and multiple mitochondrial chaperones. Hum. Mol. Genet. 2007, 16, 929–941.

78. Stemmmler, T.L.; Lesuisse, E.; Pain, D.; Dancis, A. Frataxin and mitochondrial FeS cluster biogenesis. J. Biol. Chem. 2010, 285, 26737–26743, doi:10.1074/jbc.R110.118679.

79. Leaden, L.; Busi, M.V.; Gomez-Casati, D.F. The mitochondrial proteins AtHscB and AtIsu1 involved in Fe-S cluster assembly interact with the Hsp70-type chaperon AtHscA2 and modulate its catalytic activity. Mitochondrion 2014, 19, 375–381, doi:10.1016/j.mito.2014.11.002.

80. Godman, J.; Balk, J. Genome analysis of Chlamydomonas reinhardtii reveals the existence of multiple, compartmentalized iron-sulfur protein assembly machineries of different evolutionary origins. Genetics 2008, 179, 59–68, doi:10.1534/genetics.107.086033.

81. Garland, S.A.; Hoff, K.; Vickery, L.E.; Culotta, V.C. Saccharomyces cerevisiae Isu1 and Isu2: Members of a well-conserved gene family for iron-sulfur cluster assembly. J. Mol. Biol. 1999, 294, 897–907, doi:10.1006/jmbi.1999.3294.

82. Schilke, B.; Voisine, C.; Beinert, H.; Craig, E. Evidence for a conserved system for iron metabolism in the mitochondria of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 1999, 96, 10206–10211, doi:10.1073/pnas.96.18.10206.

83. Tong, W.H.; Jameson, G.N.; Huynh, B.H.; Rouault, T.A. Subcellular compartmentalization of human Nfu, an iron-sulfur cluster scaffold protein, and its ability to assemble a [4Fe-4S] cluster. Proc. Natl. Acad. Sci. USA 2003, 100, 9762–9767, doi:10.1073/pnas.1732541100.
84. Agar, J.N.; Yuwaniyama, P.; Jack, R.F.; Cash, V.L.; Smith, A.D.; Dean, D.R.; Johnson, M.K. Modular organization and identification of a mononuclear iron-binding site within the NifU protein. J. Biol. Inorg. Chem. 2000, 5, 167–177, doi:10.1007/s007750050361.

85. Nasta, V.; Suraci, D.; Gourdoupis, S.; Ciofi-Baffoni, S.; Bacci, L. A pathway for assembling [4Fe-4S](2+) clusters in mitochondrial iron-sulfur protein biogenesis. FEBS J. 2020, 287, 2312–2327, doi:10.1111/FEBS.15140.

86. Cai, K.; Frederick, R.O.; Markley, J.L. ISCU interacts with Nfu1, and ISCU [4Fe-4S] transfers its Fe-S cluster to Nfu1 leading to the production of holo-NFU1. J. Struct. Biol. 2010, 210, 107, doi:10.1016/j.jsb.2010.107491.

87. Azam, T.; Przybyla-Toscano, J.; Vignols, F.; Couturier, J.; Rouhier, N.; Johnson, M.K. [4Fe-4S] cluster trafficking mediated by Arabidopsis mitochondrial ISCA and NFU proteins. J. Biol. Chem. 2020, 2010, doi:10.1074/jbc.RA120.105726, doi:10.1074/jbc.RA120.105726.

88. Adam, A.C.; Bornhovd, C.; Pröksch, H.; Neupert, W.; Hell, K. The Nfs1 interacting protein Isd11 has an essential role in Fe/S cluster biogenesis in mitochondria. Embio J. 2006, 25, 174–183, doi:10.1038/sj.embiol.7600905.

89. Friemel, M.; Marejla, Z.; Li, K.; Leimkuhler, S. The N-Terminus of Iron-Sulfur Cluster Assembly Factor ISD11 Is Crucial for Subcellular Targeting and Interaction with L-Cysteine Desulfurase NFS1. Biochemistry 2017, 56, 1797–1808, doi:10.1021/acs.biochem.6b01239.

90. Gerber, J.; Mühlenhoff, U.; Lill, R. An interaction between frataxin and Isu1/Nfs1 that is crucial for Fe/S cluster synthesis on Isu1. Embo Rep. 2003, 4, 906–911.

91. Armas, A.M.; Balparda, M.; Terenzi, A.; Busi, M.V.; Pagani, M.A.; Gomez-Casati, D.F. Ferrochelatase activity of plant frataxin. Biochimie 2019, 156, 118–122, doi:10.1016/j.biochi.2018.10.009.

92. Armas, A.M.; Balparda, M.; Turowski, V.R.; Busi, M.V.; Pagani, M.A.; Gomez-Casati, D.F. Altered levels of mitochondrial NFS1 affect cellular Fe and S contents in plants. Plant Cell Rep. 2019, 38, 981–990, doi:10.1007/s00299-019-02419-9.

93. NetSurfP-2.0. Available online: http://www.cbs.dtu.dk/ (accessed on 4 December 2020).

94. Clustal Omega https://www.ebi.ac.uk/Tools/msa/clustalo/ (accessed on 5 December 2020).

95. Lim, S.C.; Friemel, M.; Marum, J.E.; Tucker, E.J.; Bruno, D.L.; Riley, L.G.; Christodoulou, J.; Kirk, E.P.; Boneh, A.; DeGennaro, C.M.; et al. Mutations in LMYR4, encoding iron-sulfur cluster biogenesis factor ISD11, cause deficiency of multiple respiratory chain complexes. Hum. Mol. Genet. 2013, 22, 4460–4473, doi:10.1093/hmg/ddt295.

96. Dutkiewicz, R.; Schilke, B.; Knieszner, H.; Walter, W.; Craig, E.A.; Marszalek, J. Ssq1, a mitochondrial Hsp70 involved in iron-sulfur (Fe/S) center biogenesis. Similarities to and differences from its bacterial counterpart. J. Biol. Chem. 2003, 278, 29719–29727, doi:10.1074/jbc.M303527200.

97. Vicker, L.E.; Cupp-Vicker, J.R. Molecular chaperones HscA/Sqsl and HscB/Jac1 and their roles in iron-sulfur protein maturation. Crit. Rev. Biochem. Mol. Biol. 2006, 42, 95–111, doi:10.1080/10409230701322298.

98. Voisine, C.; Cheng, Y.C.; Ohlson, M.; Schilke, B.; Hoff, K.; Beinert, H.; Marszalek, J.; Craig, E.A. Jac1, a mitochondrial J-type chaperone, is involved in the biogenesis of Fe/S clusters in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 2001, 98, 1483–1488, doi:10.1073/pnas.98.4.1483.

99. Lill, R.; Mühlenhoff, U. Iron–sulfur–protein biogenesis in eukaryotes. Trends Biochem. Sci. 2005, 30, 133–141.

100. Huynen, M.A.; Snel, B.; Bork, P.; Gibson, T.J. The phylogenetic distribution of frataxin reveals a role in iron-sulfur cluster protein assembly. Hum. Mol. Genet. 2001, 10, 2463–2468, doi:10.1093/hmg/10.21.2463.

101. Xu, X.M.; Lin, H.; Latijnhouwers, M.; Möller, S.G. Dual localized AtHscB involved in iron sulfur protein biogenesis in Arabidopsis. PLoS ONE 2009, 4, e7662, doi:10.1371/journal.pone.0007662.

102. Leaden, L.; Pagani, M.A.; Balparda, M.; Busi, M.V.; Gomez-Casati, D.F. Altered levels of AtHSCB disrupts iron translocation from roots to shoots. Plant Mol. Biol. 2016, 92, 613–628, doi:10.1007/s11103-016-0537-9.

103. NCBI Protein Database. Available online: https://www.ncbi.nlm.nih.gov/protein/ (accessed on 28 November 2020).

104. Cory, S.A.; Van Vranken, J.G.; Brignole, E.J.; Patra, S.; Winge, D.R.; Drennan, C.L.; Rutter, J.; Barondeau, D.P. Structure of human Fe-S assembly subcomplex reveals unexpected cysteine desulphurase architecture and acyl-ACP-ISD11 interactions. Proc. Natl. Acad. Sci. USA 2017, 114, E5325–E5334, doi:10.1073/pnas.1702849114.

105. Huang, J.; Xue, C.; Wang, H.; Wang, L.; Schmidt, W.; Shen, R.; Lan, P. Genes of ACYL CARRIER PROTEIN Family Show Different Expression Profiles and Overexpression of ACYL CARRIER PROTEIN S Modulates Fatty Acid Composition and Enhances Salt Stress Tolerance in Arabidopsis. Front. Plant Sci. 2017, 8, 987, doi:10.3389/fpls.2017.00987.

106. Blatti, J.L.; Beld, J.; Behnke, C.A.; Mendez, M.; Mayfield, S.P.; Burkart, M.D. Manipulating fatty acid biosynthesis in microalgae for biofuel through protein-protein interactions. PLoS ONE 2012, 7, e42949, doi:10.1371/journal.pone.0042949.

107. Busi, M.V.; Zabaleta, E.J.; Araya, A.; Gomez-Casati, D.F. Functional and molecular characterization of the frataxin homolog from Arabidopsis thaliana. FEBS Lett. 2004, 576, 141–144.

108. Malandi, M.V.; Busi, M.V.; Clemente, M.; Zabaleta, E.J.; Araya, A.; Gomez-Casati, D.F. Expression and one-step purification of recombinant Arabidopsis thaliana frataxin homolog (AtFH). Protein Expr. Purif. 2007, 51, 157–161, doi:10.1016/j.pep.2006.06.007.

109. Turowski, V.R.; Aknin, C.; Malandi, M.V.; Buchensky, C.; Leaden, L.; Peralta, D.A.; Busi, M.V.; Araya, A.; Gomez-Casati, D.F. Fratxin Is Localized to Both the Chloroplast and Mitochondrion and Is Involved in Chloroplast Fe-S Protein Function in Arabidopsis. PLoS ONE 2015, 10, e014443, doi:10.1371/journal.pone.014443.

110. Buchensky, C.; Sanchez, M.; Carrillo, M.; Palacios, O.; Capdevila, M.; Dominguez-Vera, J.M.; Busi, M.V.; Atian, S.; Pagani, M.A.; Gomez-Casati, D.F. Identification of two frataxin isoforms in Zea mays: Structural and functional studies. Biochimie 2017, 140, 34–47, doi:10.1016/j.biochi.2017.06.011.
111. Gomez-Casati, D.F.; Busi, M.V.; Pagani, M.A. Plant Frataxin in Metal Metabolism. *Front. Plant Sci.* **2018**, *9*, 1706, doi:10.3389/fpls.2018.01706.

112. Maliandi, M.V.; Busi, M.V.; Turowski, V.R.; Leaden, L.; Araya, A.; Gomez-Casati, D.F. The mitochondrial protein frataxin is essential for heme biosynthesis in plants. *FEBS J.* **2011**, *278*, 470–481, doi:10.1111/j.1742-4658.2010.07968.x.

113. Dhe-Paganon, S.; Shigeta, R.; Chi, Y.I.; Ristow, M.; Shoelson, S.E. Crystal structure of human frataxin. *J. Biol. Chem.* **2000**, *275*, 30753–30756, doi:10.1074/jbc.C000407200.

114. He, Y.; Alam, S.L.; Proteasa, S.V.; Zhang, Y.; Lesuisse, E.; Dancis, A.; Stemmler, T.L. Yeast frataxin solution structure, iron binding, and ferrochelatase interaction. *Biochemistry* **2004**, *43*, 16254–16262, doi:10.1021/bi0488193.

115. CD-Search. Available online: https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi (accessed on 2 December 2020).

116. Paul, V.D.; Lill, R. Biogenesis of cytosolic and nuclear iron-sulfur proteins and their role in genome stability. *Biochim. Et Biophys. Acta* **2015**, *1853*, 1528–1539, doi:10.1016/j.bbamcr.2014.12.018.

117. Lill, R.; Dutkiewicz, R.; Freibert, S.A.; Heidenreich, T.; Mascarenhas, J.; Netz, D.J.; Paul, V.D.; Pierik, A.J.; Richter, N.; Stumpf, M.; et al. The role of mitochondria and the CIA machinery in the maturation of cytosolic and nuclear iron-sulfur proteins. *Eur. J. Cell Biol.* **2015**, *94*, 280–291, doi:10.1016/j.ejcb.2015.05.002.

118. Bernard, D.G.; Netz, D.J.; Lagny, T.J.; Pierik, A.J.; Balk, J. Requirements of the cytosolic iron-sulfur cluster assembly pathway in Arabidopsis. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **2013**, *368*, 20120259, doi:10.1098/rstb.2012.0259.