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Mitochondria and copper homeostasis in plants

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ABSTRACT

Copper (Cu) and other transition metals are essential for living organisms but also toxic when present in excess. To cope with this apparent paradox, organisms have developed sophisticated mechanisms to acquire, transport and store these metals. Particularly, plant mitochondria require Cu for the assembly and function of cytochrome *c* oxidase (COX), the terminal enzyme of the respiratory chain. COX assembly is a complex process that requires the action of multiple factors, many of them involved in the delivery and insertion of Cu into the enzyme. In this review, we summarize what is known about the processes involved in Cu delivery to mitochondria and how these processes impact in Cu homeostasis at the cellular level. We also discuss evidence indicating that metallochaperones involved in COX assembly play additional roles in signaling pathways related to changes in Cu and redox homeostasis and the response of plants to stress. We propose that cysteine-rich proteins present in the mitochondrial intermembrane space are excellent candidates as sensors of these changes and transducers of signals originated in the organelle to the rest of the cell.

1. Introduction

Considering their role as co-factors, metals are required for many different cell functions, including photosynthesis, respiration, ethylene perception, regulation of the circadian clock and programmed cell death (Helmersson et al., 2008; Hirayama and Alonso, 2000; Perea-García et al., 2010; Puig et al., 2007). However, at high levels metals become toxic for the cell due to interference with functional sites in proteins, displacement of essential elements for enzymatic functions or enhanced reactive oxygen species (ROS) production (Sharma and Dietz, 2009). Redox-active metals such as copper (Cu) and iron (Fe) directly induce ROS production through Fenton and Haber–Weiss reactions (Halliwell, 2006). Due to the highly redox-active nature of the processes that take place in chloroplasts and mitochondria these organelles are preferential sites of metal-induced ROS production (Keunen et al., 2011; Pilon et al., 2009). Although the maintenance of metal homeostasis is essential for the correct functioning of plant mitochondria, how metals are transported and delivered to their correct location and how metal levels are kept within physiological limits inside this organelle are largely unknown processes.

2. Regulation of copper homeostasis in plant cells

As mentioned above, Cu is an essential micronutrient involved in numerous cellular processes. Within plant tissues, Cu ions can be present in two oxidation states: Cu(I) and Cu(II). These redox properties are used by Cu-binding proteins that participate in electron transfer reaction. Based on the same redox properties, Cu is also toxic when present in excess since free ions can directly induce ROS production. Therefore, free Cu levels must be precisely regulated in the cell in order to minimize the damage produced by Cu excess and also to avoid the deleterious effects of Cu deficiency.

Copper is an oxidizing metal that may form coordination complexes with several molecules. In this sense, Cu is associated with several metalloproteins with essential functions both within mitochondria and the rest of the cell. Thus, Cu(II) is often bound to the amino acid histidine, while Cu(I) preferentially interacts with cysteine and methionine (Burkhead et al., 2009). As a part of plastocyanin (PC), essential for photosynthetic electron transport in chloroplasts, and of Complex IV of the mitochondrial respiratory chain, Cu is involved in metabolic pathways that supply energy for cellular processes. Furthermore, Cu is part of the ROS scavenging cell repertory, being a co-factor of superoxide dismutase (SOD) isoforms present in the cytosol, chloroplasts and peroxisomes (Kliebenstein et al., 1998). Copper is also present in plantacyanin, a blue protein involved in fertilization (Dong et al., 2005), and in several oxidases such as laccases (Marusek et al., 2006) and the enzyme ascorbate oxidase (Nakamura and Go, 2005).

Plant cells have evolved several strategies to adapt their metabolism to different metal concentrations and availability. These include the regulation of metal uptake, trafficking and allocation, and also the coordination of the levels of Cu- and Fe-containing proteins in response to changes in metal availability. Thus, in order to prioritize the use of metals in pathways in which they are essential, the first response is a modification in gene expression by transcriptional and/or post-

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transcriptional mechanisms. The second action is the replacement of enzymes that use Cu in their active centers with other proteins with equal functions that use other available metals (Yamasaki et al., 2007, 2009).

In the unicellular green alga *Chlamydomonas reinhardtii*, PC is degraded under low Cu conditions and its function is replaced by the Fe-containing protein cytochrome c_6 (Merchant et al., 2006). Moreover, in the promoter region of the gene that encodes cytochrome c_6 (*CYC6*) the CuRE element (GTAC, Copper Response Element), which acts as transcriptional enhancer under Cu deficit in the cell, was identified (Quinn et al., 2000). GTAC elements are recognized by the SBP (Squamosa Binding Protein) DNA binding domain present in the CRR1 (Copper Response Regulator 1) transcription factor (Eriksson et al., 2004). CRR1 activates *CYC6* gene transcription under Cu deficiency and, when Cu levels reach normality, presumably Cu atoms bind to the transcription factor, thereby inactivating it (Merchant et al., 2006; Sommer et al., 2010).

Unlike *C. reinhardtii*, land plants do not degrade PC under Cu deficiency and use a Cu-dependent Fe uptake system. Under Cu depletion, the expression of *Arabidopsis thaliana* (Arabidopsis) Cu/Zn-SOD isoforms CSD1 and CSD2 is down regulated and their function is compensated by Fe-SOD proteins (Abdel-Ghany and Pilon, 2008). This post-transcriptional regulatory process is accomplished by miR398, a miRNA whose targets are transcripts for several proteins that contain Cu, like CSD1, CSD2, the Cu/Zn-SOD chaperone CCS1 and the COX5b-1 subunit. Additional miRNAs, like miR397, miR408 and miR857, target transcripts for the Cu-containing proteins plantacyanin and laccases. These miRNAs accumulate when Cu is limiting but not under Cu-sufficient conditions (Abdel-Ghany and Pilon, 2008). This regulatory system allows the cell to limit Cu use, which is then preferentially transferred to PC, essential for photosynthesis in higher plants (Yamasaki et al., 2007).

As in *C. reinhardtii*, proteins containing the SBP domain (Birkenbihl et al., 2005), named SPL (*Squamosa Promoter Binding Protein-Like*), are implicated in the regulatory mechanism established under Cu limitation in Arabidopsis. While Arabidopsis contains 16 proteins of the SPL family with different functions, SPL7 seems to be the regulator of Cu homeostasis (Yamasaki et al., 2009). Although the SPL7 mRNA is constitutively detected in several tissues, SPL7 activates transcription only under limiting Cu conditions. This result implies that SPL7 is involved in the primary sensing of Cu concentration. Multiple GTAC motifs are present in the promoter regions of *MIR398*, *MIR397*, *MIR408*, *MIR857*, *COPT1*, *COPT2*, *ZIP2* and *CCH*, which are induced by Cu deficiency, suggesting that these are direct targets of SPL7 action (Yamasaki et al., 2009; Zhang and Li, 2013). How the activity of SPL7 is regulated by Cu levels is not known. Inactivation of SPL7 may be mediated by an unidentified Cu-sensing protein. It is also possible that the stability of SPL7 is regulated by the Cu status. SPL7 protein may be degraded via an unknown mechanism such as ubiquitination under high Cu. Alternatively, Cu may directly inactivate SPL7 by binding to its DNA binding domain, as already shown for CRR1 in *C. reinhardtii* (Sommer et al., 2010).

As a consequence of biotic and abiotic stresses, rapid accumulation of ROS such as superoxide, H_2O_2 and hydroxyl radicals occurs in plants (Bartels and Sunkar, 2005). To counteract oxidative stress, plants evolved several enzymatic barriers represented by SOD, catalases and oxidases, among others. This establishes a connection between Cu homeostasis and stress responses in plants through the action of miR398 on SOD expression (Sunkar et al., 2006). Indeed, the expression of miR398 is up-regulated under low Cu or high sugar levels and down-regulated in response to ozone, salt and biotic stress in Arabidopsis (Abdel-Ghany and Pilon, 2008; Dugas and Bartel, 2008; Jagadeeswaran et al., 2009). Decreased miR398 expression was also observed in Arabidopsis seedlings exposed to excess Cu, while cadmium treatment produced an increase in miR398 levels, suggesting the existence of metal-specific effects in the regulation of miR398 and SOD expression (Cuypers et al., 2011).

3. Copper homeostasis in plant mitochondria

Many plant mitochondrial proteins have been shown to bind metals (Tan et al., 2010). Among them, proteins that form part of the mitochondrial respiratory chain need metals for proper function. The NADH dehydrogenase complex (Complex I), the main entry site of electrons to the mitochondrial respiratory chain, has a membrane arm which contains most of the mitochondrial encoded subunits and a peripheral arm which protrudes into the mitochondrial matrix and contains most Fe–S clusters (Klodmann et al., 2010). The succinate dehydrogenase complex (Complex II), completely encoded in the nuclear genome, is composed of eight subunits forming two major subcomplexes (Millar et al., 2004). The soluble subcomplex covalently binds FAD and has Fe–S clusters (Figueroa et al., 2002). The cytochrome *c* reductase complex (Complex III) transfers electrons from ubiquinol to cytochrome *c* using two polypeptides carrying two different heme configurations and an Fe–S protein (Werhahn and Braun, 2002). Finally, the cytochrome *c* oxidase (COX or Complex IV) accepts electrons from cytochrome *c* to convert oxygen and H^+ into water. Complex IV uses Cu and heme as co-factors, but not Fe–S proteins. Subunit COX1 coordinates a binuclear metal center Cu_B-heme a_3, while COX2 chelates the bivalent Cu_A. At least in yeast, more than 19 factors are required to properly assemble this complex (Herrmann and Funes, 2005). Many of these assembly factors act as metallochaperones that function in the delivery and insertion of metals, particularly Cu, in the right location.

Tan et al. (2010) determined a ratio of 26:8:6 for divalent forms of Fe, zinc and Cu, respectively, in plant mitochondria. They also recovered several subunits of mitochondrial respiratory complexes using immobilized affinity chromatography with different metals (Tan et al., 2010). These results may indicate that these mitochondrial proteins are able to bind different metals, which places them as targets of metal-catalyzed oxidation reactions. This highlights the necessity of a fine tuning of metal homeostasis in mitochondria and the important role of the specificity in complex assembly provided by metallochaperones.

3.1. Copper delivery to plant mitochondria

Copper uptake in plants is mediated by transporters from the COPT and ZIP (ZRT, IRT-like protein) families (Pilon, 2011; Puig and Peñarrubia, 2009). Once inside the cell, Cu has to arrive to different cell compartments. Different transporters are involved in Cu delivery to chloroplasts, vacuoles, the pre-vacuolar compartment, endoplasmic reticulum and the trans-Golgi network (reviewed in Puig and Peñarrubia, 2009). Much less is known about Cu transport to mitochondria and its distribution between mitochondrial compartments. The existence of a Cu pool bound to a low molecular weight anionic compound (known as copper ligand or CuL) in the mitochondrial matrix has been documented in yeast (Cobine et al., 2004), but it is not known how Cu reaches the mitochondrial matrix. The Cu chaperone COX17 has been suggested as a shuttle protein from the cytosol to the intermembrane space (IMS) in yeast and mammals (Oswald et al., 2009). However, the fact that COX17 is functional when attached to the inner mitochondrial membrane in yeast suggests that other processes are involved in Cu acquisition by mitochondria (Maxfield et al., 2004). Also, the Cu chaperone COX19 is localized in mitochondria and the cytosol in a Cu-dependent manner in human cells (Leary et al., 2013). However, this dual location seems to be related to a signaling function of COX19 rather than with a role of this protein in Cu transport to mitochondria. Recently, a member of the mitochondrial carrier family, Pic2, has been implicated in Cu import to the mitochondrial matrix in yeast (Vest et al., 2013). This carrier transports Cu along with CuL and its depletion produces a decrease in the mitochondrial Cu pool. Whether CuL and a homologue of Pic2 exist in plants is not known.

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3.2. The involvement of copper in COX biogenesis

Cytochrome *c* oxidase (COX or Complex IV) is the terminal enzymatic complex of the mitochondrial respiratory chain. Complex IV acts as a dimer and is embedded in the inner mitochondrial membrane facing both the matrix and the IMS. Eukaryotic COX has 11–13 subunits and assembly of the complex requires a tight coordination between nuclear and mitochondrial gene expression since the three largest subunits (COX1, COX2 and COX3) are encoded by mitochondrial DNA while the remaining subunits are encoded in the nuclear genome. Moreover, proper COX function is dependent on five types of co-factors including three Cu atoms, two hemes, and Mg, Zn and Na ions (Brunori et al., 2005; Fontanesi et al., 2008). Copper and hemes form two different catalytic centers in COX1 and COX2. Electrons from the soluble cytochrome *c* enter through the purple binuclear Cu center (Cu_A) coordinated in the solvent-exposed part of COX2. Then, electrons arrive to the COX1 subunit through six-coordinated heme centers to the heteronuclear heme a_3-Cu_B center where oxygen is reduced to water.

Delivery of Cu to COX1 and COX2 is mediated by soluble Cu chaperones housed in the IMS and by transmembrane Cu chaperones embedded in the inner mitochondrial membrane (Horn and Barrientos, 2008; Leary, 2010). Results obtained in *Saccharomyces cerevisiae* suggest that the Cu pool present in the mitochondrial matrix is the source of Cu ions for the COX1 and COX2 subunits (Cobine et al., 2004). Whereas metallochaperones of the IMS bind Cu, how Cu is transported from the cytosol or the matrix to the IMS is poorly understood. In yeast and mammals, the roles of COX17, COX11 and Sco proteins as chaperones involved in Cu delivery to COX are well characterized (for a review see Robinson and Winge, 2010). COX17 is a small soluble protein from the IMS that contains four cysteines arranged in two CX_9C motifs and two additional conserved cysteines involved in Cu binding together with one cysteine of the first CX_9C motif. COX17 is able to bind Cu, undergo redox interconversions and change its oligomerization state, thus adopting different conformations (Fig. 1A; Palumma et al., 2004; Voronova et al., 2007). Sco1 and COX11 are bound to the inner membrane through an N-terminal transmembrane domain and have a globular domain located in the IMS. Both proteins bind Cu through conserved cysteines (and a histidine for Sco1) present in the globular domain (Balatri et al., 2003; Banci et al., 2011; Carr et al., 2002; Horng et al., 2005).

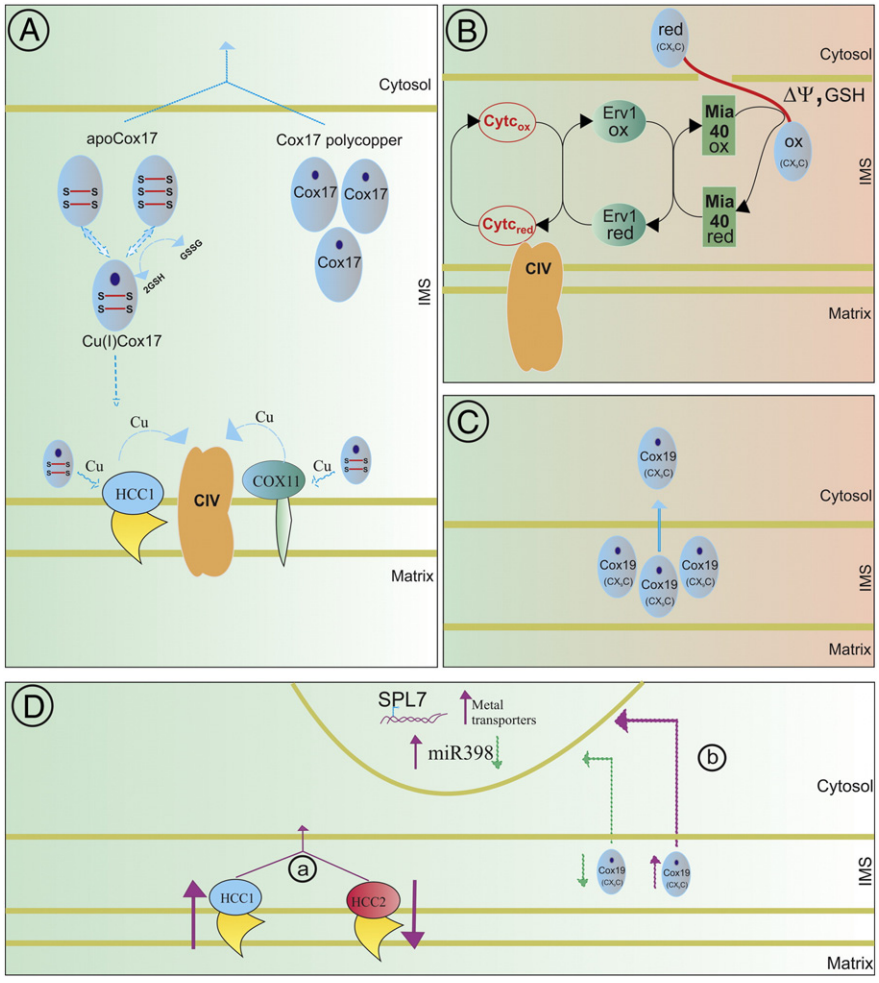

Fig. 1. COX assembly factors and mitochondrial signaling. Current evidence suggests that COX assembly factors involved in Cu delivery also participate in processes related to copper homeostasis and stress responses in different organisms. These functions may be related to the redox-active and/or Cu-binding properties of these proteins. (A) It has been shown that COX17 can undergo redox interconversions and adopt different conformations. Changes in the ratio of oxidized and reduced COX17 or in the amount of Cu-loaded COX17 may act as signals to communicate the redox state inside the IMS to the rest of the cell. (B) Since the import and proper folding of CX_9C proteins is dependent on their oxidation by the Mia40/Erv1 system, which in turn delivers electrons to the respiratory chain via cytochrome *c*, the amount and redox state of CX_9C proteins in the IMS may connect the redox and energetic state of mitochondria with the rest of the cell. (C) As shown in human cells for COX19, CX_9C proteins may change their subcellular location in response to changes in Cu levels. (D) In plants, modifications in the levels of the Sco proteins HCC1 and HCC2 (a) and of the CX_9C protein COX19 (b) produce changes in the expression of low-Cu responsive genes that encode metal transporters and miR398, which are regulated by transcription factor SPL7. Symbols: apoCox17, a form of COX17 without bound Cu; Cox17 polycopper, an oligomeric form of COX17 with multiple bound Cu atoms; GSH and GSSG, reduced and oxidized glutathione, respectively; CIV, Complex IV; Cytc, cytochrome *c*; $\Delta\Psi$, electrochemical gradient across the inner mitochondrial membrane; ox and red, oxidized and reduced forms of the respective proteins; dots in COX17 and COX19 represent bound Cu. References and protein names are listed in the text.

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A simplified model shows that COX17 delivers Cu(I) ions to COX11 and Sco1, which in turn deliver Cu to Cu_B and Cu_A, respectively (Robinson and Winge, 2010). The role of these chaperones is supported by the observation that each protein binds Cu(I) (Carr et al., 2002; Horng et al., 2005; Palumma et al., 2004). Additionally, mutations in each of these proteins induce COX deficiency in mammals and yeast (Carr et al., 2002; Leary et al., 2004; Oswald et al., 2009). The respiratory deficiency of *cox17* mutants in yeast is suppressed by the addition of exogenous Cu(II) and by high copy number expression of Sco1 or Sco2, a similar protein whose deletion does not produce a defect in COX biogenesis (Glerum et al., 1996). Considering that the overexpression of COX17 does not recover the respiratory phenotype of *sco1* mutants in yeast, Sco1 was proposed to act downstream of COX17. Further studies in yeast demonstrated the existence of a specific transfer mechanism of Cu from COX17 to Sco1 (Banci et al., 2008). Resolution of the crystal structure of Sco1, which revealed the presence of a thioredoxin fold, prompted some authors to suggest a different role of Sco proteins in Cu delivery acting as a thiol disulfide isomerase during this process (Williams et al., 2005). Supporting this idea, Abriata et al. (2008) showed that Cu_A insertion in COX2 from *Thermus thermophilus* requires a new metallochaperone (PCu_AC), while Sco1 is required to maintain the proper oxidation state of the acceptor cysteines of the COX2 Cu_A center. In addition, the fact that Sco proteins are present in prokaryotes that do not contain Cu_A-containing oxidases suggests that these proteins have additional roles, not related to COX assembly (Banci et al., 2007). In pathogenic *Neisseria*, for example, it has been shown that Sco is involved in protection against oxidative stress (Seib et al., 2003).

In addition to COX17, the mitochondrial IMS houses other small and soluble proteins with CX_9C twin motifs (Khalimonchuk and Winge, 2008; Longen et al., 2009). This motif forms a helical hairpin stabilized by the presence of hydrophobic amino acids at the helix–helix interface and the formation of disulfide bonds (Abajian et al., 2004; Banci et al., 2009; Longen et al., 2009). In yeast, mutations in most of these proteins produce defects in respiration, suggesting that, as demonstrated for some of them, they are involved in the assembly of components of the respiratory chain. Interestingly, the incorporation of most CX_9C proteins to the IMS takes place through a redox-dependent import machinery, the disulfide relay system, formed by Mia40 and Erv1 (Fig. 1B; Hell, 2008). Mia40 (itself a CX_9C protein) drives membrane translocation coupled to the oxidation of the imported protein. The electrons from this oxidation step are then channeled to the electron transport chain through Erv1, a sulfhydryl oxidase, and cytochrome *c*. Recently, it has been shown that in mammalian cells the kinetics of import through this system are dependent on the glutathione pool and the inner membrane potential (Fischer et al., 2013), thus providing a link between the redox state of the IMS and the amount of imported CX_9C proteins (Fig. 1B).

Proteins similar to COX assembly factors are present in plants. *A. thaliana* has two genes (*AtCOX17-1* and *AtCOX17-2*) that encode putative COX17 homologues able to complement a yeast *cox17* null mutant (Attallah et al., 2007a; Wintz and Vulpe, 2002). Arabidopsis also contains two different genes, *AtCOX19-1* and *AtCOX19-2*, which encode putative homologues of yeast COX19 (Attallah et al., 2007b). *AtCOX19-1* produces two transcripts, which encode proteins with different N-terminal portions, from an alternative splicing event. When imported in vitro, both isoforms are attached to the inner mitochondrial membrane facing the IMS, but only the short form complements a yeast *cox19* null mutant (Attallah et al., 2007b). The protein encoded by *AtCOX19-2* is identical to the short form produced from *AtCOX19-1* (Attallah et al., 2007b).

Sco1 is involved in Cu delivery to COX subunit 2 during COX assembly (Robinson and Winge, 2010). Two proteins with similarity to Sco1, named HCC1 and HCC2, are encoded in the genomes of seed plants (Attallah et al., 2011; Steinebrunner et al., 2011). Notably, HCC2 proteins lack one or both cysteines and the histidine involved in Cu binding in Sco1. The fact that a protein that contains the soluble domain of Arabidopsis HCC1 fused to the transmembrane domain of Sco1 is able to complement a yeast *sco1* mutant has been taken as an indication that HCC1 proteins act as COX assembly factors in plants (Steinebrunner et al., 2011). In agreement with this, knockout of Arabidopsis *HCC1* produces embryo arrest and a decrease in COX activity (Attallah et al., 2011; Steinebrunner et al., 2011). On the contrary, knockout plants in *HCC2* show normal COX activity levels (Steinebrunner et al., unpublished), suggesting that this proteins has a different function. One gene encoding a putative homologue of COX11 is also encoded in the Arabidopsis genome (Welchen and Gonzalez, 2005). Functional studies of this protein have not been reported.

Arabidopsis also contains potential homologues of other twin CX_9C proteins from yeast (Longen et al., 2009). Nothing is known about the role of these proteins except for the case of AtMSM1 (*A. thaliana* Mitochondrial Stress Marker 1; which is similar to yeast Mic17), which has been proposed as a marker of mitochondrial stress (van Aken and Whelan, 2012) and may thus participate in retrograde signaling pathways.

3.3. Copper metallochaperones: more than COX assembly factors?

The analysis of Sco mutants revealed that these proteins participate in the modulation of Cu homeostasis in human cells (Leary et al., 2007). Notably, this function seems to be unrelated to the role of these proteins in COX assembly. Cells with Sco deficiency contain less Cu due to an increase in Cu efflux. The Cu deficiency phenotype is rescued by the knockdown of the Cu-transporting ATPase ATP7A, which promotes Cu efflux during Cu overload (Leary et al., 2013). The authors suggest that the levels and redox state of Sco proteins originate signals that modulate the Cu content of cells acting on the activity of ATP7A. Interestingly, they showed that knockdown of the soluble metallochaperone COX19 also relieves the Cu deficiency of Sco mutant cells and that this chaperone partitions between mitochondria and the cytosol in a Cu-dependent manner (Fig. 1C; Leary et al., 2013). Accordingly, COX19 is a candidate signal transducer of the redox state of Sco proteins. These findings place mitochondrial Cu chaperones as major players in cellular Cu homeostasis in human cells.

In Arabidopsis, plants with a T-DNA insertion in the second exon of the Sco protein gene *HCC2* develop normally and do not show any difference in COX activity with wild-type plants. This suggests that HCC2 function is not directly related to COX assembly. It has been shown that *hcc2* knockout mutants are more sensitive to UV-B radiation (Steinebrunner et al., unpublished), suggesting that this protein may participate in the response of plants to stress. Even though the mechanism for this altered stress response is not completely clear, it can be hypothesized that HCC2 could act to maintain the proper oxidation state of the cysteines in HCC1 during stress situations where H_2O_2 generation is increased (Steinebrunner et al., unpublished). This is based on observations in human cells indicating that Sco1 and Sco2 proteins interact and that Sco2 influences the redox state of Sco1 cysteines (Leary et al., 2009). A role for plant Sco proteins HCC1 and HCC2 in the modulation of Cu homeostasis is also possible, since it has been reported that Arabidopsis plants with altered levels of these proteins show changes in Cu levels and in the expression of metal transporters and of the Cu-regulated miR398 (Fig. 1D; Attallah et al., 2011).

In addition, *AtCOX17* and *AtCOX19* expression is increased after inoculation of leaves with virulent and avirulent strains of the bacterial pathogen *Pseudomonas syringae* and after treatment with compounds that produce an increase in ROS, like 3-aminotriazole and salicylic acid (Attallah et al., 2007a,b; Balandin and Castresana, 2002). This suggests that the proteins encoded by these genes may have a role under stress situations. *AtCOX17* and *AtCOX19* are also induced by Cu and other metals (Attallah et al., 2007a,b). The fact that these metals, at the concentrations used, produce the generation of ROS does not allow to discern if AtCOX19 and AtCOX17 participate in Cu homeostasis or if induction is due to their participation in a general stress response.

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Considering that both genes are induced after different biotic and abiotic stress situations the second option seems more logical. Results from experiments in which metal concentrations vary within optimal and suboptimal levels should clarify if these mitochondrial Cu chaperones play a role in Cu homeostasis in plants. Related to this, it has been observed that changes in AtCOX17 and AtCOX19 expression levels produce alterations in the expression of genes involved in stress responses and Cu homeostasis (García et al., unpublished results). Silencing of *AtCOX19*, for example, results in decreased expression of a reporter gene located under the control of the *MIR398a* promoter, while plants that overexpress *AtCOX19* show the opposite behavior (Fig. 1D). In the case of *AtCOX17* genes, their silencing originates a decreased response of several genes to stress. This suggests that these proteins may act in signaling pathways related to Cu and redox homeostasis in mitochondria and the cell. The fact that AtCOX17 and AtCOX19 are probably able to undergo redox interconversions makes these proteins attractive candidates to act as transducers of redox changes in the IMS. In addition, these metallochaperones may act as sensors to detect cellular Cu levels due to their Cu-binding capacity.

4. Conclusions

Metals are essential for the correct functioning of mitochondrial components. Particularly, Cu is required for the assembly and activity of cytochrome *c* oxidase (COX) or Complex IV of the respiratory chain. Delivery and insertion of Cu into COX is a complex process that involves the action of many proteins, generally known as metallochaperones. These metallochaperones would not only act to deliver Cu to COX, but also to modulate Cu levels and avoid unwanted reactions due to the chemical properties of this metal. It is expected that Cu levels in mitochondria are strictly regulated and that this regulation is coordinated with Cu homeostasis within the whole cell. However, how Cu reaches mitochondria and how metal requirements from the organelle are transmitted to the rest of the cell are poorly understood processes. The presence of several Cu binding factors and related proteins that participate in COX assembly in the mitochondrial IMS suggests that these proteins may act as sensors of metal requirements within the organelle (Fig. 1). The close connection between Cu metabolism and stress responses and the fact that some of these factors respond to stress suggests that these proteins may also mediate signals related to ROS production or redox changes in the mitochondrial IMS.

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