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Review Article

Function and Regulation of the Plant COPT Family of High-Affinity Copper Transport Proteins

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Copper (Cu) is an essential micronutrient for all eukaryotes because it participates as a redox active cofactor in multiple biological processes, including mitochondrial respiration, photosynthesis, oxidative stress protection, and iron (Fe) transport. In eukaryotic cells, Cu transport toward the cytoplasm is mediated by the conserved CTR/COPT family of high-affinity Cu transport proteins. This outlook paper reviews the contribution of our research group to the characterization of the function played by the *Arabidopsis thaliana* COPT1–6 family of proteins in plant Cu homeostasis. Our studies indicate that the different tissue specificity, Cu-regulated expression, and subcellular localization dictate COPT-specialized contribution to plant Cu transport and distribution. By characterizing lack-of-function *Arabidopsis* mutant lines, we conclude that COPT1 mediates root Cu acquisition, COPT6 facilitates shoot Cu distribution, and COPT5 mobilizes Cu from storage organelles. Furthermore, our work with *copt2* mutant and COPT-overexpressing plants has also uncovered Cu connections with Fe homeostasis and the circadian clock, respectively. Future studies on the interaction between COPT transporters and other components of the Cu homeostasis network will improve our knowledge of plant Cu acquisition, distribution, regulation, and utilization by Cu-proteins.

1. Introduction

Copper (Cu) functions as a redox active cofactor in a wide variety of plant proteins including plastocyanin, cytochrome *c* oxidase, Cu/Zn-superoxide dismutase (Cu/Zn-SOD), ethylene receptors, laccases, ascorbate and amine oxidases, plantacyanin, and polyphenol oxidases. Consequently, Cu is essential for fundamental biological processes in plants including photosynthesis, mitochondrial respiration, oxidative stress protection, cell wall metabolism, ethylene perception, response to pathogens, and molybdenum cofactor biosynthesis [1–3]. The optimal endogenous Cu levels in plants can substantially range depending on the species and its environmental availability. Adequate Cu levels in vegetative tissues are around 6 μg/g dry weight, with levels below 5 μg/g leading to deficiency symptoms [4]. Cu deficiency defects in plants include a general reduced growth rate, chlorosis, especially in young leaves, curling of leaf margins, damage at the apical meristem, defects in cell wall formation, and lignification, which causes insufficient water transport, defective pollen development and viability, limited fruit formation, and diminished seed production and viability [4]. Plant Cu availability also depends on soil composition, with organic soils being more likely to be Cu-deficient due to higher Cu-binding capacity [4]. In addition to the variable availability of Cu in the environment, plant Cu requirements also change daily given its participation in photosynthesis, during the development of green and reproductive tissues, and in response to other environmental cues.

Higher plants have developed sophisticated mechanisms to efficiently acquire and utilize Cu, especially when it is scarce. Cells from the model plant *Arabidopsis thaliana* respond to Cu deficiency through a dual mechanism that consists in increasing Cu acquisition and optimizing its utilization (reviewed by [1, 2, 5–7]). In response to Cu limitation, *Arabidopsis* master Cu homeostasis regulator SPL7 (SQUAMOSA promoter-binding protein-like 7), similar to *Chlamydomonas reinhardtii* Crr1 transcription factor [8], activates the expression of multiple genes that contain within their promoter repetitive Cu-responsive elements (CuREs)

with a GTAC motif as the essential core sequence [9–11]. Upon Cu limitation, SPL7 activates the expression of Cu^{2+}-reductases (*FRO4* and *FRO5*) and high-affinity Cu transporters (*COPT1*, *COPT2*, and *COPT6*) at the plasma membrane that mediate Cu^{+} transport to the cytoplasm [9, 10, 12, 13](see below). In addition to Cu acquisition, SPL7 triggers the expression of various microRNAs, denoted Cu-microRNAs, which promote the degradation of the transcripts encoding for dispensable Cu-utilizing proteins, including cytosolic Cu/Zn-SOD (*CSD1*), chloroplast stroma Cu/Zn-SOD (*CSD2*), several laccases, and plantacyanin [9, 14–16]. To compensate for reduced Cu/Zn-SOD activity in chloroplasts, the SPL7 transcription factor also enhances the expression of plastid-localized Fe-SOD (*FSD1*) [9, 17, 18]. After entering the cytoplasm, Cu is delivered to specific Cu-containing proteins by specialized Cu chaperones. The major pathway for Cu supply to Cu/Zn-SOD utilizes the CCS1 Cu chaperone [19–22], whereas ATX-like metallochaperones mediate Cu delivery to the Cu-proteins located on the secretory pathway or in plastids by interacting and transferring the cofactor to Cu-transporting P-type ATPases [23, 24]. RAN1 P-type ATPase pumps cytosolic Cu toward the secretory pathway for incorporation into Cu-proteins such as the ethylene receptor [25, 26]. PAA1 ATPase, which is located in the inner chloroplast envelope, mediates Cu transport from the cytoplasm to plastid stroma, and the thylakoid-located PAA2 facilitates final Cu delivery to plastocyanin into the thylakoid lumen [17, 27, 28]. Therefore upon Cu scarcity, cofactor delivery to multiple nonessential or replaceable Cu-consuming enzymes is reduced to prioritize the utilization of Cu in essential Cu-dependent processes such as photosynthetic electron transport [28, 29].

Numerous studies on yeasts, mammals, insects, algae, and plants have revealed that eukaryotes utilize the conserved CTR/COPT family of proteins to facilitate high-affinity (K_m = 1–5 μM) cellular Cu acquisition at the plasma membrane and Cu mobilization from intracellular storage organelles, when Cu bioavailability decreases [30–35]. CTR/COPT proteins are highly specific for Cu^{+} transport (and the isoelectric Ag^{+}), but not for Cu^{2+} [13, 36, 37]. Consequently, they function in coordination with membrane metalloreductases that catalyze Cu^{2+} reduction to Cu^{+} before transport [38, 39]. The conserved features in CTR/COPT proteins include three transmembrane domains (TMDs), an amino-terminal region rich in methionine and/or histidine residues and an essential $Mx_3Mx_{12}Gx_3G$ signature motif embedded within TMD2 and TMD3 (Figure 1(a)). Genetic, biochemical, and structural data suggest that, in the first steps of Cu transport, extracellular methionine/histidine-rich motifs recruit Cu^{+} to the entrance of the pore and facilitate its subsequent translocation to a set of stacked methionine triads that provide a central Cu^{+}-driving path from the external domain of the complex (Figure 1(b)). After passing through the pore, Cu^{+} would bind to the carboxy-terminal cysteine/histidine motifs facing the cytoplasm, which modulate Cu^{+}-transport activity and delivery to membrane-associated metallochaperones for targeted distribution [40–45]. This outlook paper focuses on our contribution to characterizing the function and regulation of the different members of the conserved CTR/COPT family of high-affinity Cu transporters in the model plant *Arabidopsis thaliana*.

2. Contribution of Studies in Yeast to the Identification and Initial Characterization of Plant COPT High-Affinity Copper Transporters

*Saccharomyces cerevisiae ctr1*Δ*ctr3*Δ mutants lack high-affinity Cu acquisition systems at the plasma membrane and, consequently, display defects in Cu delivery to Cu-proteins, including cytochrome *c* oxidase at the mitochondrial respiratory chain and multicopper ferroxidase Fet3 in the plasma membrane high-affinity iron (Fe) uptake system. Thus, yeast *ctr1*Δ*ctr3*Δ mutants display growth defects under both nonfermentable carbon sources (respiratory conditions) and Fe limitation. By functionally complementing the respiratory and low Fe defects exhibited by yeast *ctr1*Δ*ctr3*Δ mutants, we completed the identification of the COPT1–6 family of high-affinity Cu transporters in *A. thaliana* [6, 12, 13, 46–48]. We observed that, with the exception of COPT4, the ectopic expression of all the *Arabidopsis* COPT family members expressed in yeast Cu transport mutants stimulates Cu uptake and accumulation [12, 13, 49]. Regarding yeast growth complementation, *COPT1*, *COPT2,* and *COPT6* fully rescued the *ctr1*Δ*ctr3*Δ defect under nonfermentable and low Fe conditions, whereas the *COPT3* and *COPT5* effect was only partial [6, 12, 13, 48]. We observed that *COPT4* expression, which does not possess the key methionine residues essential for Cu transport, including the MX_3M motif, proves toxic to yeast cells [13]. Thus, the COPT4 potential function, if any, in plant Cu homeostasis remains to be elucidated. All together, these data suggest that, similarly to the CTR/COPT proteins in other organisms, *Arabidopsis* COPT family members are CTR-type proteins that utilize conserved methionine motifs for Cu transport.

3. Subcellular Localization and Copper Regulation of COPT Transporters in *Arabidopsis*

Budding yeast cells possess two Cu transport proteins at the plasma membrane (Ctr1 and Ctr3), whose expression is highly induced upon Cu deficiency in order to facilitate high-affinity Cu acquisition, and an intracellular Cu transporter (Ctr2), which mobilizes Cu from the vacuolar storage compartment when Cu is extremely scarce [50]. Recent studies by our and other research groups have shown that a similar division of functions occurs in *Arabidopsis* COPT proteins. Whereas fusions of COPT1, COPT2, and COPT6 to the green fluorescent protein (GFP) localize at the plasma membrane in plant cells, COPT5 is intracellularly localized [12, 47–49, 51–53] (Figure 2(a)). As indicated above, the Cu-regulated transcription factor SPL7 specifically activates the expression of *COPT1*, *COPT2,* and *COPT6* genes in response to Cu deficiency, whereas no Cu regulation has been observed for *COPT3* and *COPT5* genes [9, 12, 13, 47] (Figure 2(b)).

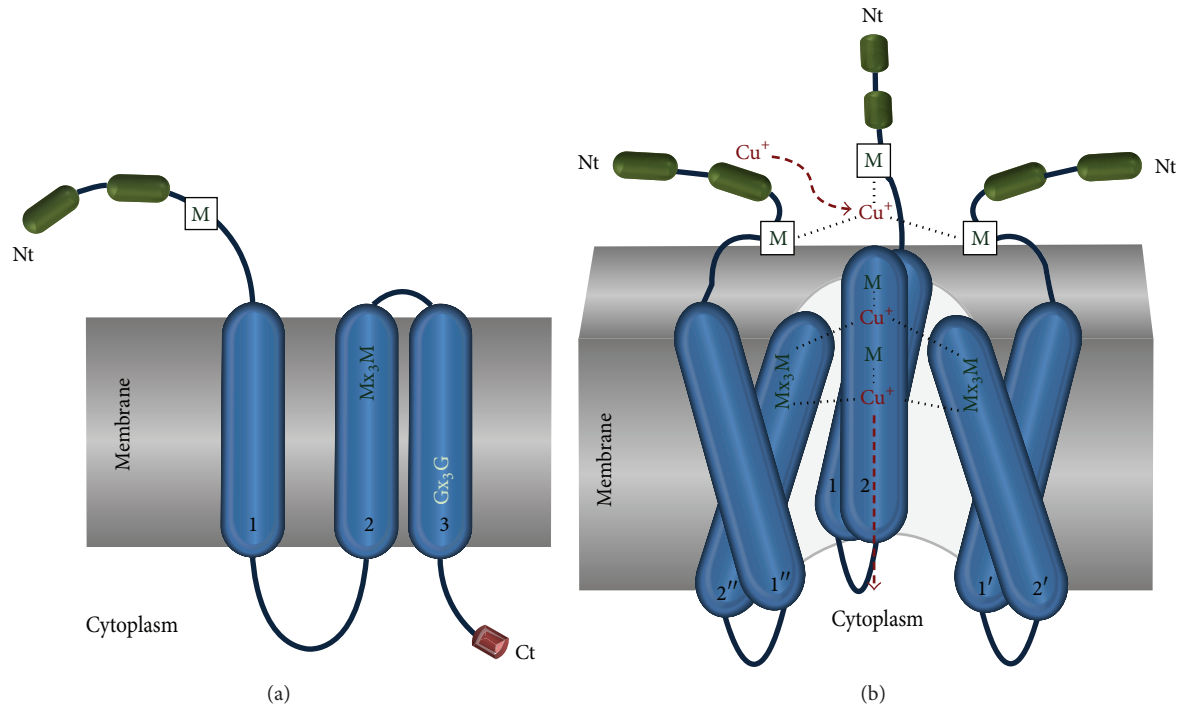

Figure 1: Conserved features in CTR/COPT family proteins. (a) CTR/COPT topology and conserved domains and motifs. The three TMDs are shown in blue. Green boxes represent extracellular methionine/histidine-rich motifs and red boxes indicate cytoplasmic cysteine/histidine motifs. (b) Representation of the assembly of CTR/COPT trimers in the membrane. The model highlights the role of methionines in the mechanism of copper transport. Only TMDs 1 and 2 have been represented for simplicity.

4. COPT1 Protein Constitutes the Major *Arabidopsis* Root Copper Acquisition System

Arabidopsis COPT1 was the first plant COPT family member to be identified [13, 46, 51, 54]. By fusing the *COPT1* promoter region to the *uidA* gene, which encodes β-glucuronidase (GUS), we determined that *COPT1* is expressed mostly in the root apex, cotyledons, stomata, trichomes, pollen grains, and embryos [54] (Figures 2(c) and 2(d)). Consistently with its tissue and Cu-regulated expression, we determined that seedlings with low *COPT1* transcript levels display a 50% decrease in root Cu acquisition, and consequently COPT1-defective plants displayed growth and pollen development defects when Cu availability was limited [54] (Figure 2(e)). It is noteworthy that we reverted these defects by Cu feeding, indicating that the Cu-transporting capacity of the COPT1 protein is crucial for soil Cu acquisition and pollen development when environmental Cu is scarce. By ^{64}Cu uptake and metal competition experiments in yeast cells, we established that COPT1 is highly specific for Cu^+ since its transport is only inhibited considerably by addition of excess Ag^+, whereas only a minor effect is observed for other divalent metals [13]. Our results implied that, as previously described in other eukaryotic organisms, *Arabidopsis* root Cu uptake would also require cell surface Cu^{2+}-reductases to function under Cu-deficient conditions. A recent study by Kramer's group has shown that the mRNA levels of *FRO4* and *FRO5* Fe^{3+}-reductases strongly increase in an SPL7-dependent manner in response to Cu deficiency [10]. More importantly, under low Cu conditions, plants that are defective in *SPL7*, *FRO4,* or *FRO5* expression display markedly diminished root Cu^{2+}-reductase activity, which leads to a drastic drop in Cu uptake at the root tips [10]. All these results strongly suggest that Cu^{2+} is first reduced by Cu^{2+}-reductases FRO4 and FRO5, and then Cu^+ is imported into plant roots by the COPT1 Cu^+ transporter. Little is known about the proteins responsible for the remaining *Arabidopsis* root Cu uptake activity. We postulate that *Arabidopsis* COPT2, which is also highly expressed in roots, may constitute a secondary pathway for root Cu incorporation [47] (see below). In fact, we have observed that *COPT1*-defective plants upregulate *COPT2* transcript levels, which can potentially relieve its defects in root Cu transport partially [54]. Since the Cu accumulation defect of the *copt2* mutant plants is not marked [47] (see below), we seek to combine the effect of both *COPT1* and *COPT2* defects by analyzing Cu uptake in *copt1copt2* double mutants. The sharp drop in Cu uptake at the root tip that the *FRO4*- and *FRO5*-defective plants exhibit does not mean that we can rule out that plant Cu acquisition can eventually occur at other places in the root by different families of metal transporters. For instance, *Arabidopsis* ZIP2 and ZIP4 transporters improve the growth defect in nonfermentable carbon sources of yeast cells that are defective in the high-affinity Cu uptake system at the plasma membrane, and their mRNA levels increase upon Cu limitation [55].

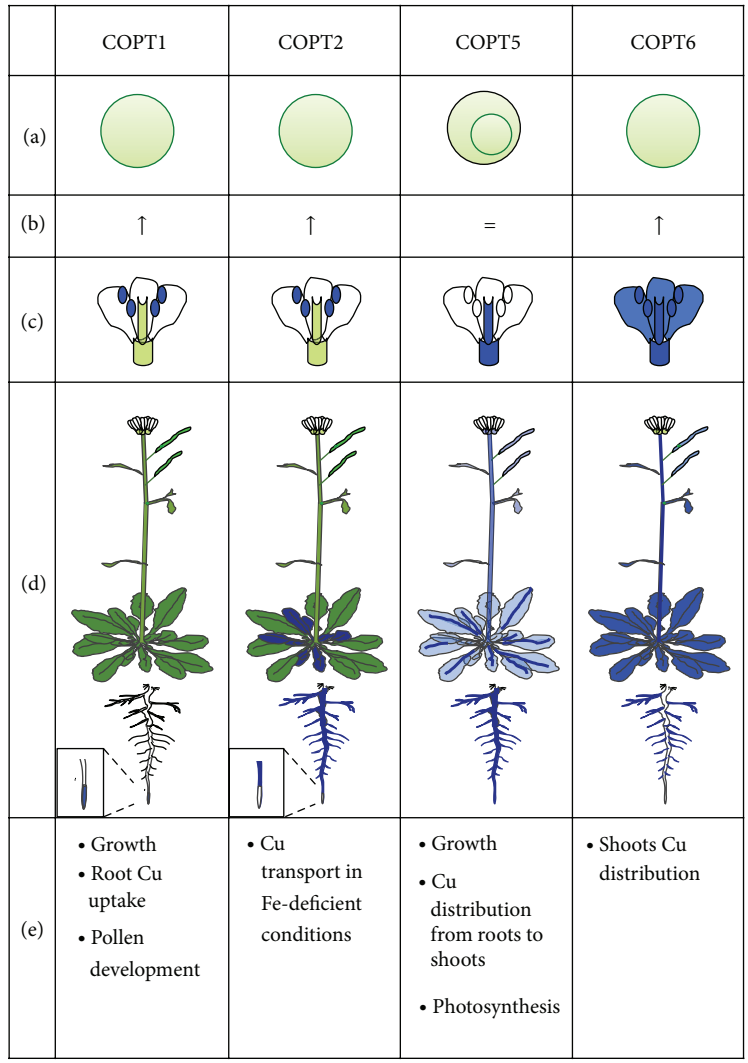

Figure 2: Characterization of *Arabidopsis* COPT family members. (a) Subcellular localization in *Arabidopsis* protoplasts. COPT-GFP protein localization is indicated in green. (b) Transcript regulation in response to copper deficiency. (c) Expression pattern in flowers. (d) Expression pattern in roots and shoots. (e) Parameters affected in *copt* mutant lines upon copper scarcity. COPT3 and COPT4 have not been included due to the small amount of data currently available.

Comparing root Cu uptake and other phenotypes of single mutants to plants that are simultaneously defective in various Cu-regulated transporters will help to decipher their relative contribution to plant Cu acquisition.

5. *Arabidopsis* COPT2 Lies at the Intersection between Copper and Iron Homeostases

Among the COPT genes, *COPT2* mRNA displays the most marked SPL7-dependent increase in response to Cu deficiency [9, 13, 47, 56]. By using transgenic *Arabidopsis* lines that express a fusion between *COPT2* promoter and GUS reporter, we have determined that, under low Cu conditions, *COPT2* is expressed mainly in roots, the vasculature of cotyledons and young leaves, apical meristems, and trichomes [47, 49] (Figure 2(d)). In reproductive tissues, *COPT2* concentrates mostly in stigma, anthers, and pollen [47, 49] (Figure 2(c)). Similar results were obtained by Vatamaniuk's group [47, 49]. Interestingly, our results show that, whereas *COPT1* and *COPT2* transcript expression patterns in shoots are similar, a notable difference is shown in roots. We observe that *COPT1* is present exclusively in primary and secondary root tips, whereas *COPT2* is absent from elongation and meristemic zones but is highly expressed in the root differentiation zone, lateral roots, and hair roots [13, 47] (Figure 2(b)). Despite the *COPT2* expression in roots, its contribution to soil Cu acquisition seems minimum as compared to COPT1 [13, 47].

COPT2 transcript levels also rise in response to Fe deficiency [57, 58], although our results point that its tissue expression pattern differs from that observed under Cu-deficient conditions, and concentrates mostly in cotyledons [47]. Consistently with this predominant aerial expression, root transcription factor FIT1 is only partially responsible for *COPT2* induction by Fe limitation [57]. A potential explanation for *COPT2* mRNA induction by low Fe could

be increasing cofactor availability for Cu-dependent enzymes such as Cu/Zn-SOD, which replaces Fe-SOD when Fe is scarce [17]. In fact, we have observed that wild-type, but not *copt2,* mutant plants increase Cu content in response to Fe deprivation [47, 58, 59]. Furthermore, *ccs1* plants, which are defective in Cu delivery to Cu/Zn-SOD, display increased oxidative damage under Fe deficiency, suggesting a role for Cu in oxidative stress protection [58]. These observations indicate that *Arabidopsis* plants optimize Fe utilization by decreasing its use in Cu-replaceable functions and by prioritizing essential Fe-dependent processes.

Interestingly, we observed that simultaneous Cu and Fe deprivation leads to a further increased *COPT2* mRNA expression, especially in roots [47, 58]. We did not find phenotypic differences between wild-type and *copt2*-defective plants under normal or Cu scarce conditions, whereas *copt2* mutants exhibit better maintenance of the photosynthetic apparatus than wild-type seedlings under simultaneous Cu and Fe deficiencies [47]. Specifically, *Arabidopsis copt2* seedlings display reduced leaf chlorosis, increased chlorophyll, and higher plastocyanin content under simultaneous low Cu and Fe, which leads to improved plant growth and seed production [47]. Although we have not fully elucidated the molecular basis underlying the *copt2* mutant phenotype with both Fe and Cu defects, global gene expression analyses indicate a general effect of COPT2-mediated Cu transport in phosphate starvation signaling, which is highly connected to Fe homeostasis [47, 60].

As previously mentioned, high-affinity Fe uptake at the plasma membrane depends in *S. cerevisiae* on Fet3 ferroxidase, which uses Cu as an indispensable cofactor for its activity. Therefore, Cu deficiency leads to impaired Fe transport in yeast, and consequently to multiple Fe-related symptoms [61]. Likewise in green alga *C. reinhardtii*, multicopper ferroxidase FOX1 participates in cellular Fe acquisition [62]. In humans, inorganic Fe acquisition in the intestine is mediated by divalent metal transport denoted DMT1, which is independent of Cu, but Fe distribution depends directly on multicopper ferroxidases ceruloplasmin and hephaestin (reviewed in [63]). Root Fe acquisition in *Arabidopsis* plants mostly depends on IRT1, a member of the ZIP divalent metal transporter family that does not require Cu for its transport activity [64–67]. Notwithstanding, in addition to ours, various studies have also linked Cu and Fe homeostases in plants. Severe Cu deficiency, achieved by growing *Arabidopsis* seedlings defective in SPL7 transcription factor (*spl7-2* mutants) under low Cu conditions, leads to reduced root-to-shoot Fe translocation that activates various Fe deficiency responses, including an increased root surface Fe^{3+}-reductase activity and higher *IRT1* expression levels, and diminished shoot Fe-dependent enzyme catalase and lower aerial ferritin levels [10]. Interestingly, *spl7-2* mutant plants also display severely reduced root ferroxidase activity when cultivated under Cu deficiency, suggesting that a multicopper ferroxidase may participate in root-to-shoot Fe translocation [10]. Furthermore, the *spl7-2* phenotypic defects under Cu-deficient conditions, including chlorosis, are partially rescued by Fe supplementation [10]. We postulate that COPT2 could also be responsible for Cu delivery to multicopper oxidases LPR1 and LPR2, which are involved in the root growth responses to low phosphate [68]. In agreement with this hypothesis, we observe that the *copt2* mutants display larger roots than wild-type seedlings and phosphate starvation diminishes *COPT2* expression levels [47, 69]. Determining the function of plant Cu-proteins, including multicopper oxidases like ascorbate oxidases and laccases, will help our understanding of the connections of Cu to Fe homeostasis and phosphate metabolism.

6. The COPT6 Protein Facilitates Cu Distribution in Aerial Tissues

Although it was not initially annotated in the *Arabidopsis* genome, our analyses uncovered that At2g26975, denoted COPT6, was a novel member of the COPT family of high-affinity Cu transporters [6]. Unlike *COPT1* and *COPT2*, our studies and those by Vatamaniuk's group indicate that *COPT6* is expressed mostly in shoots, especially in the vasculature of stems and leaves [12, 47] (Figure 2(d)). *COPT6* can also be found in cotyledons, meristems, trichomes, and stomata [12, 47]. In flowers, *COPT6* is present in sepals, petals, pistil, filaments of stamens, pollen, transmitting tissues of siliques, embryos, and seed envelopment [12, 47] (Figure 2(c)). *COPT6* can also be detected in lateral roots, even at low levels, but not in the primary root or at the tip of secondary roots [12] (Figure 2(d)). Whereas aerial *COPT6* transcript upregulation by Cu deficiency is fully dependent on SPL7, its regulation in roots seems to be partially independent of the SPL7 transcription factor [12]. We observed that contrary to *COPT1* and *COPT2*, *COPT6* expression is also present under Cu-sufficient conditions [48]. Our analyses of endogenous Cu levels have shown that the *copt6* knock-out lines do not exhibit a significant defect in total Cu accumulation under either Cu-sufficient or Cu-deficient conditions. However, we observe that *copt6* lines display a Cu distribution defect under low Cu conditions that leads to increased Cu levels in rosette leaves and reduced Cu in seeds [48]. We did not detect Cu distribution differences when the wild-type *COPT6* gene was reintroduced into the *copt6* mutant line or under Cu-sufficient conditions [48]. Thus, we conclude that COPT6 protein functions in Cu redistribution in shoots when Cu becomes limited, facilitating the transit of Cu from green tissues to reproductive organs.

7. The COPT5 Protein Mediates Cu Mobilization from Storage Sites

In addition to the COPT proteins involved in cellular Cu uptake at the plasma membrane, *Arabidopsis* also possesses COPT family members that function in intracellular Cu transport. By using *Arabidopsis* protoplasts, we have localized a functional COPT5-GFP fusion protein to prevacuolar compartments [52, 53]. By using *COPT5* promoter fusion to GUS reporter, we determined that *COPT5* is expressed mostly in the root vasculature, although it is also present

at much lower levels in the apical meristems, trichomes, and vascular tissues of hypocotyls, cotyledons, and leaves [53] (Figure 2(d)). In reproductive organs of adult plants, we found *COPT5* in pistils, ovules, filament of stamens, silique conducts, and embryos [53] (Figure 2(c)). Unlike the plasma membrane COPT proteins, *COPT5* was not present in pollen [53]. Trentmann's group has localized COPT5 to the vacuolar membrane [52, 53], and consistently they showed that plant cells lacking a functional *COPT5* gene accumulate Cu in the vacuole [52]. At the systemic level, their *copt5* knock-out plants do not show any alteration in total Cu levels but are defective in interorgan Cu distribution [52]. Whereas the roots of *copt5* mutant seedlings accumulate Cu, siliques and seeds contain less Cu than wild-type plants [52]. Under severe Cu deficiency, we observed that *copt5* plants exhibit reduced vegetative growth, impaired root elongation, chlorosis, and serious defects in photosynthetic transfer due to reduced plastocyanin accumulation [53] (Figure 2(e)). Despite *COPT5* mRNA levels not being altered by environmental Cu availability, *copt5* knock-out plants display major defects upon severe Cu limitation, suggesting that Cu levels may somehow regulate the COPT5 function in vacuolar Cu export either at a posttranscriptional level or through accessory proteins such as vacuolar Cu-reductase [52, 53]. Therefore, when Cu is abundant, *Arabidopsis* plants accumulate excess Cu in vacuoles, especially in roots. When Cu is scarce, the COPT5 transporter facilitates Cu mobilization from storage sites in roots to photosynthetic and reproductive tissues in shoots.

8. Overexpression of the COPT Proteins Uncovers a Connection between Copper and the *Arabidopsis* Circadian Clock

To further characterize COPT function in plants, we obtained *Arabidopsis* lines that expressed *COPT1* and *COPT3* genes under the control of the CaMV35S promoter. In both cases, *COPT1*- and *COPT3*-overexpressing seedlings accumulated more Cu than wild-type plants and, as indicated by root length assays, they are more sensitive to high Cu concentrations in the growth medium [12, 51]. Although overexpression in both yeast and *Arabidopsis* indicate that the COPT3 protein facilitates Cu transport, we have not yet deciphered its function in plant Cu homeostasis [13, 51]. We observed that when *COPT*-overexpressing plants are grown in soil, their overall size is substantially stunted and they display hyponastic leaves [48, 51]. The root tips of *COPT1*-overexpressing seedlings display membrane damage, increased Ca^{2+} influx and K^{+} efflux, and a drop in the basal peroxide levels, probably due to the Cu-dependent generation of hydroxyl radicals [70]. Interestingly, we showed that the *COPT1*- and *COPT3*-overexpressing lines exhibit phenotypes, such as differential flowering time and hypocotyl length, which are not displayed by those plants grown in high Cu environments but are reminiscent of plants with altered circadian rhythms [51]. We found that the expression of *CCA1* and *LHY*, two MYB transcription factors that participate in the core of the *Arabidopsis* circadian clock, significantly lowers in *COPT1*- and *COPT3*-overexpressing plants, whose survival is compromised in the absence of environmental cycles [51]. Furthermore, addition of Cu to wild-type plants delays the phase and reduces amplitude, but not the period, of *CCA1* and *LHY* gene expression oscillations [51]. Taken together, these observations strongly suggest that Cu influences the *Arabidopsis* circadian clock [51, 71].

9. Conclusions and Future Perspectives

In the last few years, several studies have contributed to our current understanding of the function played by different members of the *Arabidopsis* COPT family of transporters in plant Cu homeostasis. We have performed yeast complementation assays, subcellular localization in plant cells, tissue-specific expression patterns, Cu regulation, and phenotypes associated with *copt* mutant plants concluding that each COPT transporter has developed specialized functions in plant Cu homeostasis, especially when Cu availability is low (Figure 2). For instance, COPT1 mediates root Cu acquisition, COPT6 facilitates Cu redistribution in shoots, COPT5 allows Cu mobilization from storage organelles, and COPT2 functions at the intersection between Cu and Fe homeostases. We have observed complex expression patterns and phenotypes associated with various *copt* mutants indicating that Cu plays a critical role in reproductive organs. We think that analyses of *Arabidopsis* plants simultaneously lacking various *COPT* genes are required to decipher the connection between these transporters and their overall relevance in plant Cu physiology. Furthermore, identifying the Cu-proteins directly responsible for the phenotypes observed and their biological function will prove to be of enormous help to understand plant Cu distribution and utilization. Finally, COPT studies have uncovered fascinating connections between Cu and other processes, including root development, Fe homeostasis, phosphate metabolism, and the circadian clock to be further explored.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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