Involvement of Plasma Membrane Ca\(^{2+}/H^+\) Antiporter in Cd\(^{2+}\) Tolerance

SHEN Guo-ming\(^1\), DU Qi-zhen\(^1\), WANG Jiang-xin\(^2\)

\(^1\)School of Food Science and Biotechnology, Zhejiang Gongshang University, 149 Jiaogong Road, Hangzhou 310012, China; \(^2\)Center for Biosignature Discovery Automation, Arizona State University, Tempe, AZ 85287, USA

**Abstract:** Cation exchangers (CAXs) belong to the cation/Ca\(^{2+}\) exchanger superfamily which have been extensively investigated in plant tonoplasts over the last decade. Recently, the roles of CAXs involved in heavy metal accumulation and tolerance in plants have been studied for phytoremediation and food security. In this mini review, we summarize the roles of the Ca\(^{2+}/H^+\) antiporter in Ca\(^{2+}\) signal transduction, maintaining ion homeostasis and sequestering heavy metals into the vacuole. Moreover, we present a possible role of the plasma membrane Ca\(^{2+}/H^+\) antiporter in heavy metal detoxification.

**Key words:** Ca\(^{2+}/H^+\) antiporter; Cd\(^{2+}\) detoxification; heavy metal; plasma membrane; rice

The cadmium ion (Cd\(^{2+}\)) is a non-essential trace element harmful to most organisms when released into the environment (Bradl, 2005). It is easily absorbed from the soil by plants, and accumulation in plants poses as a significant health threat to humans because uptake into crops provides a direct route for the heavy metal to enter into the human food chain. Ingestion of Cd\(^{2+}\) is known to damage the liver or kidney (Yazihan et al, 2010), and can even lead to the onset of osteoporosis (Kazantzis, 2004).

In plants, Cd\(^{2+}\) is absorbed by root cell divalent metal carriers or ion channels and combined with phytochelatins (PCs). The metal is then sequestered into vacuoles through the ATP-binding cassette (ABC) transporters (Cobbett, 2000; Clemens, 2006; Kotrba et al, 2009; Redjala et al, 2009; Schwartz et al, 2010; Sylwia et al, 2010). Many investigations have demonstrated that Cd\(^{2+}\) and Cd\(^{2+}\)-PC compounds can be loaded into xylem or phloem and subsequently transported to aerial parts of the plant (Mendoza-Cózatl et al, 2008; Verbruggen et al, 2009; Uraguchi et al, 2009; Liu et al, 2010; Yoneyama et al, 2010). As an organ embedded in the Cd\(^{2+}\) pool, roots accumulate the highest Cd\(^{2+}\) levels in plants (Salviano et al, 2002; Prasad, 2004; Vassilev et al, 2005; Liu et al, 2010; Yoneyama et al, 2010). How crop plants, such as maize (Zea mays L.) and rice (Oryza sativa L.), survive with high Cd\(^{2+}\) concentrations in their surroundings remains unclear (Sterckeman et al, 2004; Wang et al, 2007; Yoneyama et al, 2010).

CAX1 (cation exchanger 1) is an Arabidopsis thaliana Ca\(^{2+}/H^+\) antiporter which was first cloned by suppressing a mutant of Saccharomyces cerevisiae that had a defect in vacuolar Ca\(^{2+}\) accumulation (Hirschi et al, 1996). Arabidopsis CAXs are located in tonoplasts and have been extensively investigated. Yeast Vcx1p and Arabidopsis CAX1, CAX2 and CAX4 function by transporting Cd\(^{2+}\) into their vacuoles (Cheng et al, 2001; Shigaki et al, 2001; Pittman et al, 2004; Korenkov et al, 2007a, b). More recently, an Arabidopsis Ca\(^{2+}/H^+\) antiporter CAX1 variant in petunia (Petunia hybrida L.) was observed to enhance cadmium accumulation in vacuoles (Wu et al, 2010). Based on the investigation, we propose that the Ca\(^{2+}/H^+\) antiporters may play an important role in Cd\(^{2+}\) detoxification.

Previous research confirmed that Escherichia coli, cyanobacteria, lymphocytes, maize and rice root cell plasma membranes contain the Ca\(^{2+}/H^+\) antiporters (Kasai and Muto, 1990; Ivey et al, 1993; Vicente and Vale, 1995; Bevza and Kucherenko, 1998; Waditee et al, 2003; Qi et al, 2005; Shen, 2005). In this short communication, we postulate that Cd\(^{2+}\) is transported from the cytosol to extracellular spaces via the plasma membrane Ca\(^{2+}/H^+\) antiporter in plants.

**Ca\(^{2+}/H^+\) antiporter is involved in Ca\(^{2+}\) signal transduction**

In plant cells, a low cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_{cyt}\)]\) is required for maintaining signal transduction. Here, signal transduction can be triggered by elevating the Ca\(^{2+}\) concentration in response to different stimuli, such as blue and red light, gravity, touch, cold shock, or a fungal elicitor (Fallon et al, 1993; Babourina et al, 2002; Fasano et al, 2002; Kawano and Said, 2005; Hu et al, 2009).

Functionally, Ca\(^{2+}\)-transporters fall into two classes:
(i) the Ca$^{2+}$/H$^+$ influx system (the Ca$^{2+}$ channels), which mobilizes Ca$^{2+}$ from the extracellular space or organelles during developmental stages or abiotic and biotic stress; and (ii) transporters that mediate Ca$^{2+}$ efflux (Ca$^{2+}$-ATPases and Ca$^{2+}$/H$^+$ antiporters) from the cytosol (Sanders et al, 1999; White and Broadley, 2003) and are key in maintaining [Ca$^{2+}$]$_{cyt}$ homeostasis. A homeostatic system maintaining the [Ca$^{2+}$]$_{cyt}$ at a resting level of 100–200 nmol/L was established in plant cells by Ca$^{2+}$-ATPases (Trewavas and Malhó, 1998; Hirschi, 2001). In contrast, the Ca$^{2+}$/H$^+$ antiporters remove Ca$^{2+}$ from the cytosol following signal transduction (Hirschi, 2001). In addition, the Ca$^{2+}$/H$^+$ antiporter plays a vital role in stress resistance by mediating the Ca$^{2+}$ signaling system, thereby ensuring a defense triggered in plants in response to adversity.

**Ca$^{2+}$/H$^+$ antiporter sequesters heavy metals to the vacuole**

In plant cells, the Ca$^{2+}$/H$^+$ antiporters are located in the plasma membranes (Kasai and Muto, 1990; Vicente and Vale, 1995; Qi et al, 2005; Shen, 2005), tonoplasts (Shigaki et al, 2006; Martinoia et al, 2007), chloroplast thylakoid membranes (Ettinger et al, 1999), or the mitochondria inner membrane (Jiang et al, 2009). The transport activities of these antiporters are dependent on one of the components of a proton motive force (White and Broadley, 2003). The roles of tonoplast Ca$^{2+}$/H$^+$ antiporters have been extensively investigated in the last decade.

In *Arabidopsis*, all of the CAXs have the capability of sequestering Cd$^{2+}$ to the vacuole (Wu et al, 2010). The expression of *AtCAX2* in tobacco was shown to enhance Mn$^{2+}$ accumulation in the vacuole. Hirschi et al suggested that *AtCAX2* has a broad substrate range, and it was demonstrated that *AtCAX2* and *AtCAX4* can transport Cd$^{2+}$, Zn$^{2+}$ and Mn$^{2+}$ into the vacuole from the cytosol in transgenic tobacco (Korenkov et al, 2007a, b). Yeast (*Saccharomyces cerevisiae* Meyen ex E.C. Hansen) VCX1 (vacuolar cation exchanger 1) (Del Pozo et al, 1999) and rice tonoplast Ca$^{2+}$/H$^+$ antiporter OsCAX1a (Kamiya et al, 2005) were only tested for Mn$^{2+}$ transport activity, and their roles in sequestering other heavy metals require further investigation.

More recently, CAXs were reported to transport Ni$^{2+}$ and Ba$^{2+}$. AlCAX, located in the tonoplasts, was characterized by Ingle et al (2008) in a Ni$^{2+}$ tolerant plant *Alyssum lesbiacum*. The results showed that the uptake of Ni$^{2+}$ was stored in the vacuole, thereby providing nickel tolerance. PutCAX1, a cation/H$^+$ antiporter from *Puccinellia tenuiflora*, was verified to be localized in the vacuolar membrane and expression of the antiporter in yeast conferred Ba$^{2+}$ tolerance (Liu et al, 2009). It is very likely that the research progress in this field will lead to the discovery of other heavy metal ion/proton antiporters.

Sequestering heavy metals in the plant cell vacuoles represents the main mechanism of heavy metal detoxification in plants. However, heavy metal absorption and storage in the vacuoles is limited to the size of the vacuoles, and therefore as the heavy metals increase in concentration, new vacuoles must be formed. In some cases, plants can survive in environments with high heavy metal concentrations, which raises the question of whether there are heavy metal discharge mechanisms in these particular plants.

**Roles of Ca$^{2+}$/H$^+$ antiporter in plasma membranes**

Biochemical features of the plasma membrane Ca$^{2+}$/H$^+$ antiporter activity have been studied in maize, barley (*Hordeum vulgare* L.) and rice (Kasai and Muto, 1990; Hao and Yu, 1993; Vicente and Vale, 1995; Shen, 2005). Unfortunately, genes encoding the Ca$^{2+}$/H$^+$ antiporters in maize and barley have not been cloned, even though the antiporter activity was found nearly twenty years ago (Kasai and Muto, 1990; Hao and Yu, 1993; Vicente and Vale, 1995).

Recently, genes encoding the Ca$^{2+}$/H$^+$ antiporter in rice have been isolated by two independent laboratories (Del Pozo et al, 1999; Shen, 2005). We cloned the full sequence of *OsCAX* cDNA by rapid amplification of the cDNA ends (GenBank accession No. AY156513), which is similar to the sequence of *OsCAX3* (GenBank accession No. AB112773) subsequently submitted by Maeshima and Kamiya (http://www.ncbi.nlm.nih.gov/nuccore/57157352), and prepared an antibody against this protein. The immunological results showed that the OsCAX3 protein was located at the plasma membrane of rice roots (Fig. 1) (Shen, 2005). Transient expression and subcellular localization analysis of an *OsCAX3*-GFP fusion protein in mesophyll cell protoplasts of *Arabidopsis* also demonstrated that the OsCAX3 protein was localized to the plasma membrane (Fig. 2) (Qi et al, 2005). The transcript of *OsCAX3* was found to be expressed in most detected tissues (Del Pozo et al, 1999), and expression was induced by Na$^+$, Ca$^{2+}$, Mn$^{2+}$ and Cd$^{2+}$ (Fig. 3). Moreover, OsCAX3 was found for Mn$^{2+}$ transport activity (Shen, 2005), and the primary sequence is
A previous study suggested that rice is a relatively Cd²⁺ tolerant plant, because rice was found to survive in the presence of up to 640 μg/g of Cd in the soil (Bingham et al., 1975). We suggest that the Ca²⁺/H⁺ antiporter in the rice plasma membrane may mediate the efflux of Cd²⁺ from the cytosol to an extracellular space, therefore providing Cd²⁺ tolerance.

**PERSPECTIVE**

High cadmium (Cd²⁺) concentrations in the environment lead to Cd²⁺ uptake through leakage of the plasma membrane ion channels and passive absorption through ion carriers. Relative high [Cd²⁺]cyt levels stimulate phytochelatin synthase (PCS) activity (Vatamaniuk et al., 2000). Phytochelatin (PC) synthesis is mediated by PCS using glutathione (GSH) and related thiol tri-peptides as substrates, in which a γ-Glu-Cys unit from one thiol peptide is transferred to another or to pre-existing phytochelatin molecules (Vatamaniuk et al., 2000; Piechalak et al., 2003). Particular PC and Cd²⁺ compounds are sequestered into the vacuole, whereas other PC and Cd²⁺ compounds are transported to the aerial regions of plants through symplastic approaches. When the concentration of Cd²⁺ increases in the cytoplasm, ion homeostasis imbalances occur and the cytoplasm must absorb H⁺ from endoplasts or the extracellular space in an effort to exchange (or sequester) the divalent metal ions. In this process, the transcription of OsCAX may be activated. To maintain cytoplasmic ion homeostasis, the vacuolar membrane OsCAX1a absorbs Cd²⁺ from the cytosol and releases H⁺, whereas the plasma membrane OsCAX3 absorbs H⁺ from the extracellular space and releases Cd²⁺.

Once heavy metal ions enter into the cell, the charge balance is disrupted. The accumulative ability of heavy metals in the vacuole is limited. When the rate of forming new vacuoles does not meet the required demands of heavy metal accumulation, those plants without a Ca²⁺/H⁺ antiporter in the plasma membrane may die. In contrast, plants with Ca²⁺/H⁺ antiporters in the plasma membrane can discharge heavy metal ions, and therefore survive under high concentrations of heavy metal ions. Our hypothesis is based on limited results, and the existence and the roles of plasma membrane Ca²⁺/H⁺ antiporters in heavy metal survival require further characterization. For example, using genome-wide RNAi screens may help to identify plasma membrane Ca²⁺/H⁺ antiporters. Using yeast functional complementation, purification of the plasma membrane vesicles to characterize the Ca²⁺/H⁺ antiporter

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**Fig. 1. Subcellular localization of OsCAX3 in the rice root.**

The immunology results showed that OsCAX3 was located at the plasma membrane of rice roots. TN, Tonoplast; PM, Plasma membrane (Shen, 2005).

**Fig. 2. Transient expression and subcellular localization analysis of the OsCAX3-GFP fusion protein in mesophyll cell protoplasts of Arabidopsis.**

a, A confocal microscopy image of green fluorescence in the protoplasts expressing GFP only; c, An optical sectioning microscopy image of green fluorescence in the protoplasts expressing OsCAX3-GFP; b and d, The same cells of (a) and (c) in the bright field, respectively. Bar = 20 μm (Qi et al., 2005).

**Fig. 3. Expression of OsCAX3 in rice roots.**

OsCAX3 transcripts were examined by RT-PCR of total RNA from rice seedlings treated for 16 h with the following solutions: Water (as a control), 100 mmol/L MgCl₂, 200 mmol/L NaCl, 100 mmol/L CaCl₂, 2 mmol/L MnCl₂ and 5 mmol/L CdCl₂. The band in the top panel represents a 621-bp OsCAX3-specific fragment amplified by RT-PCR. The band in the bottom panel represents a 489-bp beta-tubulin OsTub16 gene fragment amplified by RT-PCR as an internal control.

OsCAX3

OsTub16

H₂O   Mg²⁺   Na⁺   Ca²⁺   Mn²⁺   Cd²⁺

similar to AtCAX2. We therefore postulate that OsCAX3 may be a heavy metal ion/proton antiporter.
activity by an isotope labeled liquid scintillation counting method, or purification of a putative Ca\(^{2+}\)/H\(^+\) antiporter protein reconstructed into artificial liposomes should aid in characterizing the functions of the identified antiporters.

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**REFERENCES**


