

The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy

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Abstract This review paper is focused predominantly on the role of the cell wall in the defense response of plants to trace metals. It is generally known that this compartment accumulates toxic divalent and trivalent metal cations both during their uptake by the cell from the environment and at the final stage of their sequestration from the protoplast. However, from results obtained in recent years, our understanding of the role played by the cell wall in plant defense response to toxic metals has markedly altered. It has been shown that this compartment may function not only as a sink for toxic trace metal accumulation, but that it is also actively modified under trace metal stress. These modifications lead to an increase in the capacity of the cell wall to accumulate trace metals and a decrease of its permeability for trace metal migration into the protoplast. One of the most striking alterations is the enhancement of the level of low-methylesterified pectins: the polysaccharides able to bind divalent and trivalent metal ions. This review paper will present the most recent results, especially those that are concerned with polysaccharide level, composition and distribution under trace metal stress, and describe in detail the polysaccharides responsible for metal binding and immobilization in different groups of plants (algae and higher plants). The review also contains information related to the entry pathways of trace metals into the cell wall and their detection methods.

Keywords Heavy metal · Cell wall · Pectin · Pb · Tip growth · Alginate

Abbreviations

CW	Cell wall
ER	Endoplasmic reticulum
GA	Golgi apparatus
HMA	Heavy metal-transporting P-type ATPase
HGA	Homogalacturonan
mAb	Monoclonal antibody
PM	Plasma membrane
PVC	Prevacuolar compartment
TM	Trace metal
TGN	<i>trans</i> -Golgi network.
V	Vacuole
Vs	Vesicle

Introduction

Trace metal (TM) pollution has become an increasingly serious environmental problem over recent decades due to its toxicity and the susceptibility of the environment. It has led to many detrimental effects in living organisms, from plants and animals to humans. TMs are categorized into those that are essential for many physiological processes, e.g., Cu, Zn and Fe, the excess as well as deficiency of which can cause harmful effects in plant cells, and non-essential ones, such as Pb, Cd, Al and Hg, which may be toxic even in relatively low concentrations (for review, e.g., Hall 2002; Patra et al. 2004; Appenroth 2010). Trace metals can enter the plant cell by diffusion through the plasma membrane (PM) (Krzesłowska et al. 2009b),

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via endocytosis (e.g., Malone et al. 1974; Woźny et al. 1982; Książek et al. 1984; Hubner et al. 1985; Krzesłowska and Woźny 1996; Illés et al. 2006; Wierzbicka et al. 2007; Krzesłowska et al. 2009a; Meyers et al. 2009), and due to the activity of special metal transporters that function in the PM. The latter aspect has been the subject of a great deal of attention and many review papers are available (e.g., Colangelo and Guerinet 2006; Krämer et al. 2007; Krzesłowska et al. 2009b; Pilon et al. 2009; Puig and Penarrubia 2009).

The excess of TMs in plant cells can cause a range of toxic alterations, e.g., the generation of reactive oxygen species (ROS) resulting in oxidative stress (Rucińska-Sobkowiak and Pukacki 2006; Przymusiński et al. 2007), which leads to the destruction of cell membranes: the PM, the endomembrane system and membranous organelles, in particular chloroplast, mitochondrion (Hall 2002; Patra et al. 2004) and nucleus (Prasad 2004; Glińska et al. 2007). Furthermore, trace metals, especially non-essential ones, can destroy enzyme functioning by entering in place of proper ions for building their molecules or by binding to their sulfhydryl and carboxyl groups, thereby changing their conformation (Hall 2002; Patra et al. 2004). Moreover, they destroy nucleic acid conformation (Pawlak and Deckert 2007), alter the water balance and disturb many other plant cell functions (Hall 2002; Patra et al. 2004). All these alterations can lead to plant growth inhibition, necrosis and finally to the death of the whole plant (Prasad 2004). It is worth noting, however, that a low dosage of a stress factor can cause a hormesis effect, i.e., the better functioning of the plant organisms under low TMs dosage (Schwarzerová et al. 2002).

Plants cannot avoid stress conditions by escaping from contaminated areas. Therefore, they have developed a range of resistance mechanisms, which enable them to avoid and/or tolerate stress factors and survive even in highly contaminated soils and waters (Levitt 1980). Defense strategies can be divided into avoidance and tolerance types. Avoidance includes all mechanisms, which protect the plant cell from TMs entering the protoplast. Tolerance concerns those mechanisms that enable the plant to: (1) neutralize toxic metals inside the cell, (2) remove them from the protoplasts (e.g., to the apoplast) and (3) neutralize their toxic effects (Levitt 1980). Thus, outside the protoplast TMs are: accumulated by mycorrhizal fungi (for review, e.g., Schützendübel and Polle 2002), chelated within the rhizosphere, bound by the CW compounds and blocked in their migration by the callose layer. Inside the cell, TMs are, e.g., chelated by metallothioneins, phytochelatins, organic acids and other compounds, and sequestered within the vacuole (V). One of the main strategies of plant cells for coping with TMs is to remove them from the cytoplasm by sequestration in

extracytoplasmic compartments such as the CW and the V. It protects the most sensitive sites within the protoplast from TM's toxicity (e.g., Vollenweider et al. 2006; Kopittke et al. 2007, 2008; Poschenrieder et al. 2008; Krzesłowska et al. 2009a, 2010; Meyers et al. 2009).

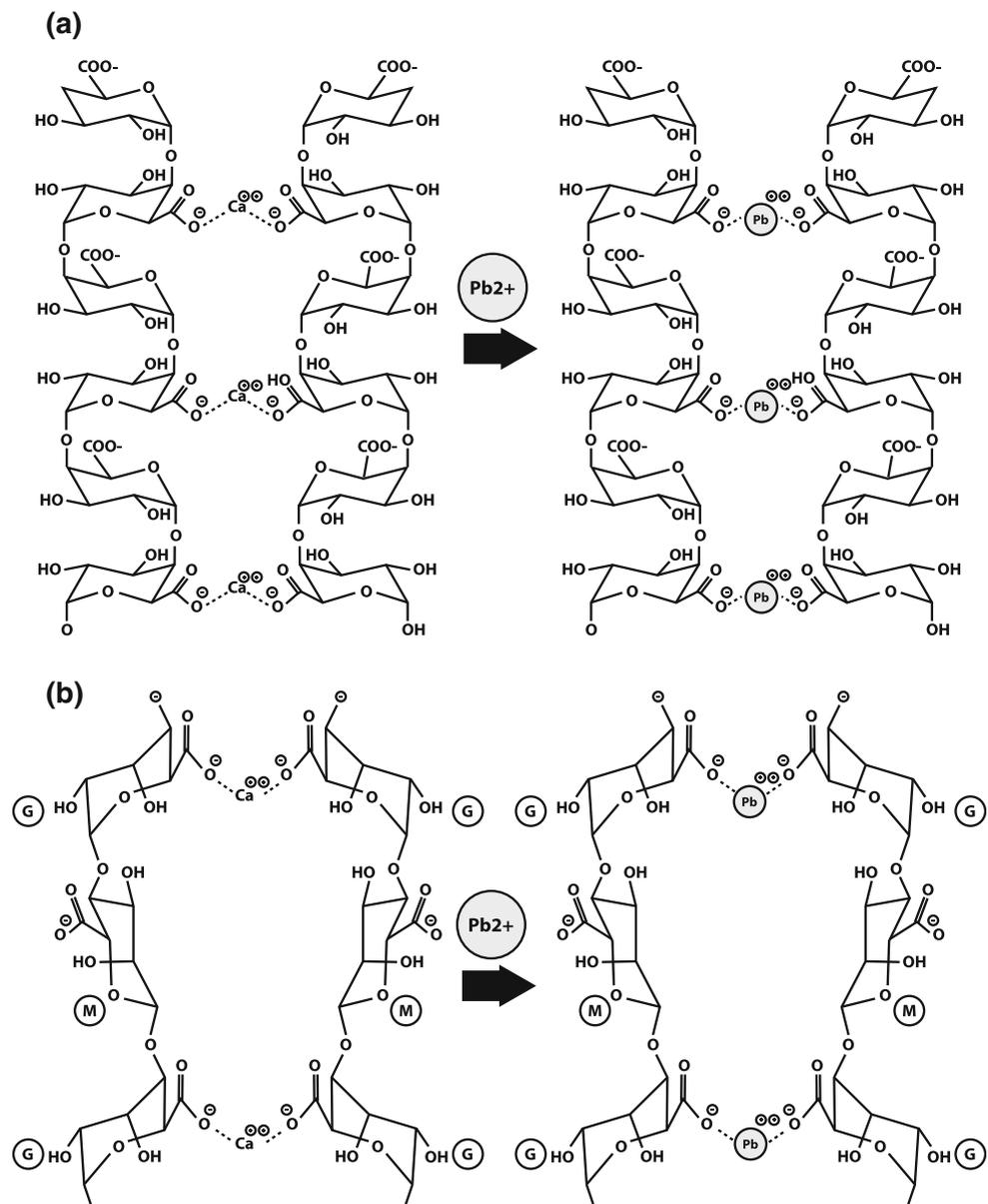
The aim of this review is to show an important role that the CW plays in the defensive response of plants to TM stress. Other resistance mechanisms have been extensively discussed in other review papers and therefore are only mentioned here. The most recent results pertaining to CW remodeling under TM stress, in particular observations involving polysaccharide amount, composition and distribution, and its possible consequences for plant adaptation to enhanced levels of TMs in the soil are described. Moreover, the common features of the CWs that are responsible for metal binding and immobilization of different groups of plants (algae and higher plants) are outlined.

Common features of plant cell walls that enable them to bind trace metals

Plant CWs are rich in compounds, which are able to bind divalent and trivalent metal cations. Polysaccharides play a crucial role in TM binding and accumulation in the CW, although other compounds such as proteins, amino acids and phenolics also take part in this process. The ability to bind divalent metal cations depends on the number of functional groups such as $-\text{COOH}$, $-\text{OH}$ and $-\text{SH}$ occurring in CW compounds (Dronnet et al. 1996; Kartel et al. 1999; Davis et al. 2003; Pelloux et al. 2007). However, the essential capacity of the CW for binding divalent and trivalent metal cations depends mainly on the amount of polysaccharides abundant in carboxyl groups. There are alginates in algae (Davis et al. 2003) and homogalacturonans (HGA) in higher plants, both Bryophytes (Tyler 1990) and vascular plants (Dronnet et al. 1996; Kartel et al. 1999; Fritz 2007; Pelloux et al. 2007; Caffall and Mohnen 2009).

Pectins are very complex and consist of four major polysaccharide domains: HGA, rhamnogalacturonan I (RGI), rhamnogalacturonan II (RGII) and xylogalacturonan (XGA). The domains differ in both the structure of the macromolecule backbone and the presence and diversity of side chains (for review, e.g., Willats et al. 2006; Pelloux et al. 2007; Caffall and Mohnen 2009). Pectins occur in all cells and typically constitute about 30% of type I primary walls occurring in dicotyledons. Secondary walls and type II primary walls, characteristic mainly of grasses, normally have a much lower content of pectins (Pelloux et al. 2007; Caffall and Mohnen 2009). The main pectin domain responsible for binding divalent and trivalent metal cations is HGA (Dronnet et al. 1996). It is synthesized in the Golgi apparatus (GA) and transported by a secretion pathway into the CW (Levy and

Fig. 1 Cross-linking (*egg-box structure*) between polymers **a** of low-methylesterified pectins present in the CW of higher plants (Caffall and Mohnen 2009 modified) and **b** of alginic acid present in the CW of brown algae, in uncontaminated conditions with Ca^{2+} ions and in presence of Pb^{2+} ; *G* α -L glucuronic acid residue, *M* 1,4-linked β -D mannuronic acid residue (Lozanno-Álvarez et al. 2009 modified)



Stahelin 1992; Willats et al. 2006). In the GA, the HGA backbone is substituted by methyl groups (CH_3). This methylesterified fraction of HGA shows at least 80% esterification (Willats et al. 2006). Within the CW, highly methylesterified HGA is mainly modified by two enzymes: pectinmethylesterase (PME) responsible for demethylation of HGA, and pectin acetylase, responsible for modifications of its acetylation degree. Demethylation of HGA leads to the formation of a few fractions with different degrees of methylesterification. These HGA fractions can be recognized by monoclonal antibodies (mAb; for review, e.g., Willats et al. 2006): low-methylesterified HGA (lower than 40%) by JIM5, HGA with a non-blockwise pattern of de-esterification (esterification not higher than 20%) by LM7, and HGA with ca. 30 non-methylesterified GalA residues

(blockwise) by PAM1. Highly methylesterified HGA (esterification not lower than 80%) is recognized by JIM7.

Homogalacturonan fractions, in particular those with a low degree of methylesterification, containing some amounts of free carboxyl groups, are able to bind divalent and trivalent metal ions (Dronnet et al. 1996; Kartel et al. 1999; Fritz 2007).

It has been shown that low-methylesterified HGA can interact through calcium bridging between two free carboxyl groups (Fig. 1; Grant et al. 1973). They then form a so-called egg-box structure (Fig. 1; Grant et al. 1973). This results in calcium gel formation and CW stiffening (for review, e.g., Carpita and Gibeaut 1993; Caffall and Mohnen 2009). Binding studies with low-methylesterified pectins have shown that the affinity of divalent and trivalent

metal cations depends on the origins of the pectin and could be as follows: $\text{Al}^{3+} > \text{Cu}^{2+} > \text{Pb}^{2+} > \text{Zn}^{2+} = \text{Ca}^{2+}$ or $\text{Cu}^{2+} = \text{Pb}^{2+} > \text{Cd}^{2+} = \text{Zn}^{2+} > \text{Ca}^{2+}$ (Dronnet et al. 1996) or $\text{Pb}^{2+} > \text{Cu}^{2+} > \text{Co}^{2+} > \text{Ni}^{2+} > \text{Zn}^{2+} > \text{Cd}^{2+}$ (Ernst et al. 1992; Kartel et al. 1999; Fritz 2007). It clearly shows that among bivalent metal cations, Cu^{2+} and Pb^{2+} are most strongly bound to pectins and Zn^{2+} , Ni^{2+} and Ca^{2+} are much less strongly bound. Thus, calcium ions can be replaced in these connections by trivalent Al^{3+} and also by such divalent TM cations as Pb^{2+} , Cu^{2+} , Cd^{2+} and Zn^{2+} (Fig. 1; Dronnet et al. 1996). The orders explain, moreover, why the amount of Al in the CWs remains uninfluenced by the addition of other TMs (Brunner et al. 2008).

The interaction between divalent or trivalent metal cations and pectin is complex and depends on (1) charge density of the pectin, (2) ionic strength of the solution and (3) strength of the interaction between the cation and pectin (Renard and Jarvis 1999). Methylation, as also acetylation, of pectins decreases their affinity to TMs. However, it is worth noting that acetylated HGA can bind to Pb^{2+} and Cd^{2+} , as shown recently in *Arabidopsis thaliana* (Połec-Pawlak et al. 2007).

Alginate occurs in the CW of all brown algae. The highest amount has been found in *Sargassum* spp. (Davis et al. 2003). It may constitute 10–45% of their dry mass, e.g., in *Sargassum longifolium* about 17% (Chapman 1980), while in *Sargassum fluitans* about 45% (Fourest and Volesky 1996). Alginates are polysaccharides that consist of two kinds of residues: D-mannuronic acid and L-guluronic acid linked via β (1 → 4) glycosidic bonds, forming a non-regular blockwise order along the chain. Trace metals show a higher affinity for alginic acids containing more α -L-guluronic acid (Davis et al. 2003). The rod-like shape of the poly-L-guluronic sections results in an alignment of two chain sections, which enables interaction with divalent cations. Divalent metal ions are lined with the carboxylate and other oxygen atoms of glucuronic acid residues (Fig. 1). This description is known as the “egg-box” structure (Davis et al. 2003). It is similar to the egg-box structure formed by low-methylesterified HGA in the CW of higher plants (Fig. 1; Grant et al. 1973; Pelloux et al. 2007).

To sum up, the binding of divalent and trivalent metal cations both to HGA in CW of higher plants and alginate in brown algae CW is based mainly on the ion exchange mechanism and involves the formation of the egg-box structure, where two alginate chains or HGA interact by a divalent or a trivalent metal cation (Fig. 1). In uncontaminated conditions, the metal that takes part in the interaction between two alginates or HGA macromolecules is calcium. However, in the presence of other divalent metal cations, in particular Pb, Cu, Zn, Cd or trivalent Al, which shows a higher affinity both to alginate and HGA than Ca^{2+} , this cation is replaced in this interaction by these metals (Fig. 1).

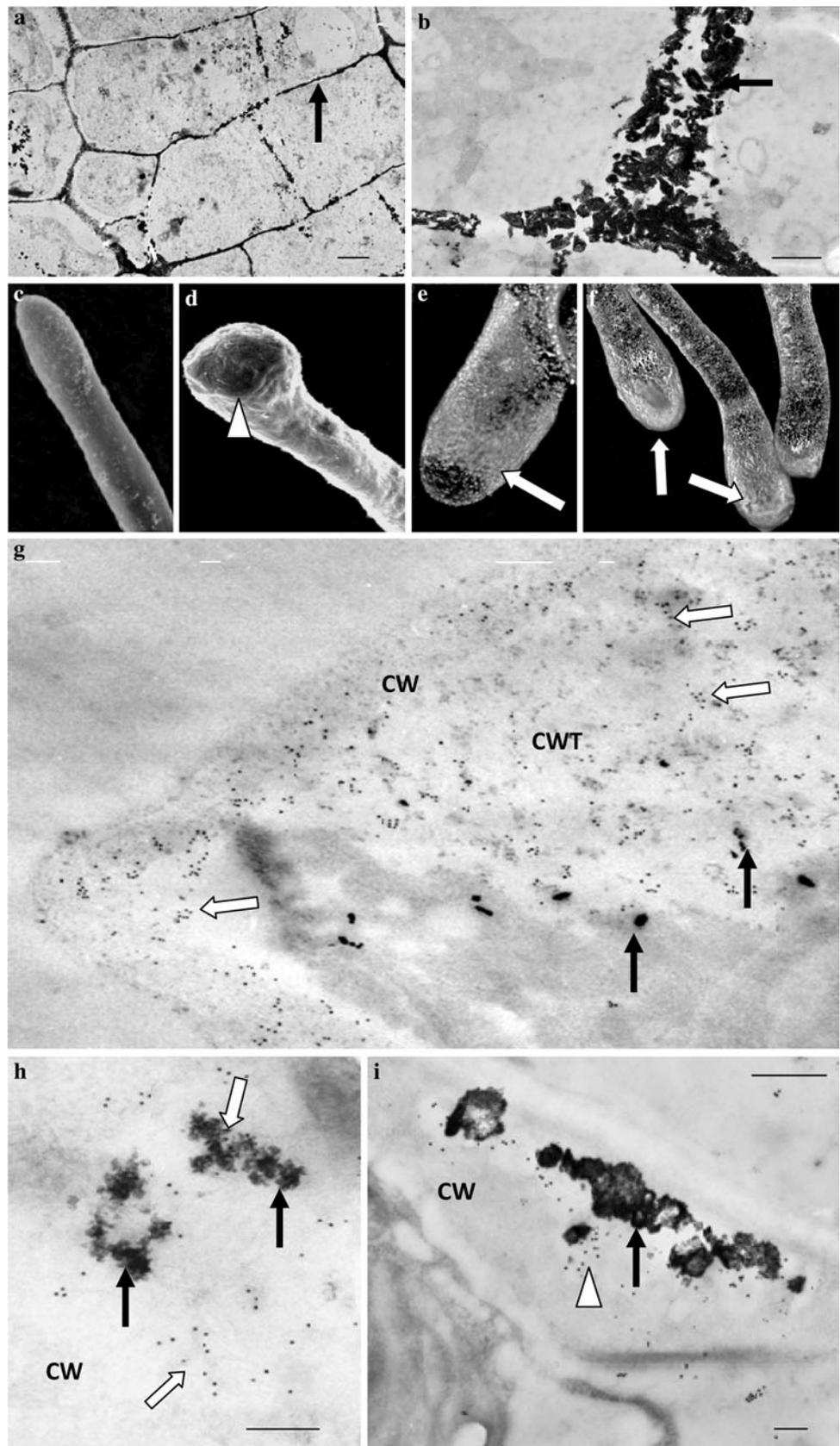
Deposition of TMs in the plant CW and its role in plant cell defense response

Detection of TMs in plant cell wall

The presence of TMs in the CW can be detected by a range of methods. Lead can be identified by optical microscopy, e.g., by rhodizonic acid that forms red complexes with this metal (Tung and Temple 1996). Cadmium may be identified, e.g., by Leadmium reagent (Xiong et al. 2009) in fluorescence and confocal microscopy. Aluminum is often stained with, e.g., morin (unbound, free Al ions; Eticha et al. 2005a) and lumogallion (Silva et al. 2000), the fluorochromes also used for fluorescence and confocal microscopy. Some TMs are clearly recognizable in transmission electron microscopy (TEM) when they form irregular electron-dense deposits. The largest ones are observed for Pb (Fig. 2b, h, i). This metal can form extremely large crystalline-like deposits (Fig. 2b), varying in size and shape. In many cases, such a deposit needs special accommodation space or thickening of the CW at the site (Fig. 2i; Malone et al. 1974; Wierzbicka 1998; Kopittke et al. 2007, 2008; Meyers et al. 2009; Krzesłowska et al. 2010). Lead can also form smaller deposits varying in size and shape and can occur in various amounts. Sometimes, however, Pb deposits can completely saturate the CW (Fig. 2a; Wierzbicka 1998). Other TMs such as Cd and Zn may also form deposits within the CW, but usually smaller than Pb deposits (Carrier et al. 2003; Neumann and zur Nieden 2001).

Moreover, the detection of TMs is possible by X-ray microanalysis (Samardakiewicz and Woźny 2000; Neumann and zur Nieden 2001; Carrier et al. 2003; Kopittke et al. 2008; Meyers et al. 2009; Krzesłowska et al. 2010), energy dispersive transmission electron microscopy (Lütz-Meindl 2007) and micro-proton-induced X-ray emission (Vogel-Mikuš et al. 2008). These methods enable detection of TMs when they do not form deposits and are dissolved, e.g., in cytosol. Moreover, they may provide information regarding the chemical form of TM occurrence. Using X-ray microanalysis, it has been shown that Zn was deposited as silicate in the CW of *Minuartia verna* (Neumann and zur Nieden 2001), Pb was recognized in a non-toxic form of chloropyromorphite ($\text{Pb}_5(\text{PO}_4)_3\text{Cl}$) in the CW of signal grass *Brachiaria decumbens* (Kopittke et al. 2008) and as phosphohedyphane ($\text{Pb}_3\text{Ca}_2(\text{PO}_4)_3\text{Cl}$) in the hyperaccumulating plant *Brassica juncea* (Meyers et al. 2009). Cadmium was deposited in the CW in aggregates with phosphate or calcium (Sela et al. 1988). It was found, moreover, that electron-dense deposits detected in root CW of *Vicia faba* growing on mine tailings contained Pb, Zn, Ca and C (Probst et al. 2009).

Fig. 2 Lead deposits (*black arrows*) and CW modifications in *Funaria hygrometrica* and *Populus tremula x tremuloides* (poplar) root protonema apical cell exposed to lead. **a** The example of CW almost completely saturated with Pb deposits in root cell of poplar (Pb 32 μ M, 24 h), **b** the example of large, crystalline-like Pb deposits in root CW of poplar (Pb 32 μ M, 24 h), **c, d** *F. hygrometrica* protonema apical cells in scanning electron microscopy, **c** control (H_2O , 48 h)—no CW thickening at the cell tip, **d** exposed to Pb (Pb4 μ M, 48 h)—CW thickening (*white arrowhead*) at the swollen tip, **e, f** *F. hygrometrica* protonema apical cells—JIM5-pectins (*white arrows*) immunocytochemical identification in LSM microscopy, **e** control—no JIM5 pectins in the tip CW, it occurred in subapical CW, **f** Pb treated protonemata—CW thickenings rich in JIM5-pectins at the cell tip, **g–i** *Funaria hygrometrica* protonema apical cells exposed to lead in TEM, JIM5-pectins and callose identification by immunogold, **g** Pb 4 μ M, 48 h—CW thickening in TEM rich in JIM5 pectins and Pb deposits, **h** Pb 1000 μ M, 4 h—internalization of Pb deposits together with JIM5-pectins, **i** pulse-chase experiment: Pb 1000 μ M, 4 and 24 h recovering; Large Pb deposits in CW with local thickening. Callose (*white arrowhead*) localized from the PM site separating Pb deposits. Bars **a** 2 μ m, **b** 500 nm, **c–f** 10 μ m, **g–i** 200 nm



The CW, one of the main compartments that accumulates trace metals

The occurrence of TMs within the CW has been reported since the earliest studies of plant cell reactions to TMs (e.g., Malone et al. 1974, Woźny et al. 1982; Wierzbicka 1998; Samardakiewicz and Woźny 2000; Neumann and zur Nieden 2001). Recent papers indicate that the CW is still recognized as one of the main compartments for TM accumulation (e.g., Wójcik et al. 2005; Islam et al. 2007; Małecka et al. 2008; Konno et al. 2010; Meyers et al. 2009) and can contain high amounts of TMs (Kopittke et al. 2007, 2008; Konno et al. 2010; Krzesłowska et al. 2009a, 2010).

The physiological advantage of metal precipitation in the CW is its strong metabolic inactivation (Jiang and Wang 2008). Until recently, it was believed that TMs bound to CW compounds, mainly low-methylesterified pectins, are not chemically active, steadily immobilized and do not enter the protoplast. However, new studies have shown that this may not be the case. Some of the CW compounds, including low-methylesterified pectins, can be internalized by endocytosis, in particular in meristematic, dividing cells (Baluška et al. 2002, 2005). Hence, it seems plausible that at least some amounts of the TMs bound to, e.g., low-methylesterified pectins in the CW can enter the protoplast together with this compound. This hypothesis was tested for the first time and largely verified in *Funaria hygrometrica* protonemata exposed to Pb. It was detected that in Pb-exposed material the endocytic internalization of pectins markedly increased in comparison to the control. Moreover, in apical cells (able to divide), Pb deposits bound to low-methylesterified pectins were detected in many of the PM invaginations (Fig. 2h), vesicles, endosomes and vacuoles. This strongly suggested that during internalization of low-methylesterified pectins from the CW, Pb bound to this compound entered the cell protoplast endomembrane system as a pectin + Pb complex (Krzesłowska et al. 2010). On the other hand, the same protonemata cells were found to possess a mechanism, which could protect their protoplast from the return of sequestered Pb. In pulse-chase experiments, all Pb deposits sequestered within the CW and CW thickenings were separated from the PM by a callose layer: a compound which is known to be impermeable to metal ions (Fig. 2i; Hall 2002; Patra et al. 2004). This could protect the cell from sequestered Pb returning into the protoplast, by endocytosis with CW compounds (Krzesłowska et al. 2010). The results shed a new light on the functioning of the CW in plant cell defense strategy against TMs.

The essential role of plant CW in defense response against TMs has been supported by the results of studies relating to the defense mechanisms functioning in some

hyperaccumulators: plants that are endemic to metalliferous soils and are able to tolerate and accumulate metals to very high concentrations, approximately 100 times more than of a nonaccumulator plant species (Brooks et al. 1977; Milner and Kochian 2008).

It has been found that “copper moss” *Scopelophila cataractae*, a plant that is able to survive under copper-enriched conditions, bound most of the Cu to the pectin in the CW of protonemata cells. Simultaneously, no growth inhibition of this moss was observed. Thus, it may be concluded that binding Cu by pectins within the CW was an effective defense response against this metal and allowed this plant to adapt successfully to Cu-enriched soils (Konno et al. 2010). Furthermore, one of the most common hyperaccumulators, *Thlaspi caerulescens*, accumulated more than 50% of TMs in its CW (Salt et al. 1999). Similar results were obtained for *Lycopodium japonicum*, a fern which grows in Cu-enriched conditions, where 66% of Cu was bound in the CW (Konno et al. 2005). Cell walls were also the preferred compartment for Cu accumulation in spruce and Zn accumulation in poplar (Brunner et al. 2008). Furthermore, in the Ni and Zn hyperaccumulating plants, more than 60% of the TMs were localized in the CWs (Yang et al. 2005). A striking example of TM accumulation in the CW may also be found in *Phragmites australis*, which is considered to be a plant with a high detoxification and phytoremediation potential. Here, Zn concentrations followed a gradient with the sequence: intercellular space < CW < vacuole < cytoplasm, indicating that most of the Zn was accumulated within the apoplast (Jiang and Wang 2008).

As in the case of the essential metals, such as Cu and Zn, the non-essential ones are accumulated in the CW of hyperaccumulating plants. For example, Cd was preferentially accumulated within the CW of *Elodea canadensis*, a water plant known for its ability to accumulate high amounts of TMs. This plant exposed to Cd bound 30–70% of the metal within the CW (Kähkönen et al. 1997; Fritioff and Greger 2003). It was noteworthy that elevated concentrations of Cd in this plant did not cause higher stress in the cells. The resistance mechanism of *E. canadensis* to Cd seems to depend on the removal of metal ions from the protoplast through the PM to the CW where Cd^{2+} is bound by the CW compounds (Nyquist and Greger 2007). In addition, the CW of *E. canadensis* also accumulated other TMs such as Zn and Cu in relatively high amounts: 30 and 50%, respectively (Fritioff and Greger 2003).

In other plants, e.g., signal grass (*Brachiaria decumbens*), non-essential Pb was initially present in the cytoplasm of rhizodermal and cortical cells and was then sequestered in the CWs (Kopittke et al. 2008). Moreover, recent studies of the spatial distribution of Al and Fe within the roots and shoots of *Erica andevalensis* revealed that

these elements were predominantly detected in the CWs of epidermal tissues. The authors concluded that sequestration of Al and Fe in the extracellular matrix of protective tissues, away from the internal parenchyma, constitutes an important detoxification mechanism (Turnau et al. 2007).

All the examples described above clearly show that the CW is one of the preferred and essential compartments for TM accumulation, deposition and sequestration. However, the immobilization of TMs in the plant CW is not as stable as previously believed.

How trace metals can enter plant cell wall

Trace metals may enter the CW during the uptake of water solution from the environment (Malone et al. 1974; Hall 2002; Jiang and Wang 2008) and as a result of TM removal from the protoplast during the sequestration process. The latter may include (1) exclusion of TMs due to the activity of special metal transporters present in the PM (for review, Colangelo and Guerinot 2006; Krämer et al. 2007) and (2) removal via vesicular secretion pathway by exocytosis (Fig. 3.; e.g., Malone et al. 1974; Woźny et al. 1982; Książek et al. 1984; Hubner et al. 1985; Krzesłowska and Woźny 1996; Illéš et al. 2006; Wierzbicka et al. 2007; Meyers et al. 2009; Krzesłowska et al. 2010).

The highest number of metal transporters responsible for metal transport outside the protoplast has been characterized in *A. thaliana*. They are localized in the PM and belong to the group of CPx ATP-ases (ATP-ases containing a conserved intramembranous cysteine–proline/cysteine/histidine/serine sequence that is thought to play a role in translocation and putative metal-binding domains in the amino or carboxyl terminus, which distinguish them from other P-type ATP-ases (Williams and Mills 2005). Metal-transporting P-type ATPases were named heavy metal-transporting P-type ATPase (HMA) (for review, e.g., Colangelo and Guerinot 2006; Krämer et al. 2007). The following HMAs excluding TMs from the protoplast have so far been identified: *AtHMA2* (Eren and Argüello 2004) and *AtHMA4* (Mills et al. 2005), responsible for the transport of Cd^{2+} and Zn^{2+} , *AtHMA5* for Cu^{2+} (Andres-Colas et al. 2006), *AtPDR8* for Cd^{2+} and Pb^{2+} (Kim et al. 2007), and *AtPDR12* for Pb^{2+} (Lee et al. 2005). Two such transporters, *TgMTPt1* and *TgMTPt2*, carrying Co^{2+} , Zn^{2+} , Ni^{2+} and Cd^{2+} were localized in the PM of the metal hyperaccumulating plant, *Thlaspi goesingense* (Kim et al. 2004). Furthermore, it has recently been found that in tobacco (*Nicotiana tabacum* var. *Xanthi*), a heterologous expression of *AtMRP7* (MRP: the multidrug resistance-associated protein, ABC transporter) modifies its cadmium accumulation, distribution and tolerance and could be correlated with Cd removal from cytoplasm and loading to the CW. Hence, metal transporters responsible for

transferring TMs from protoplast to apoplast can modify its accumulation and distribution within plant cells. They may also function as a link in the TM transport pathway from plant root to the shoot, and their activity is closely correlated with plant tolerance strategy to TMs (Colangelo and Guerinot 2006; Krämer et al. 2007; Wojas et al. 2009).

Trace metals, in particular Pb, have been commonly detected within the structures of the endomembrane system such as PM invaginations, vesicles (Vs), GA, *trans*-Golgi network (TGN), the endoplasmic reticulum (ER) and V. Therefore, for many years it has been considered that TMs can enter the protoplast by endocytosis. Hence, TMs, already present within the endomembrane system, can be easily removed from the protoplast by the vesicular secretion pathway and sequestered in the CW (e.g., Malone et al. 1974; Woźny et al. 1982; Książek et al. 1984; Hubner et al. 1985; Krzesłowska and Woźny 1996; Wierzbicka et al. 2007; Meyers et al. 2009). Results of recent studies, concerning the uptake and sequestration of Al in *A. thaliana* (Illéš et al. 2006) and Pb in *F. hygrometrica* (Krzesłowska et al. 2010) have provided more compelling evidence for this phenomenon.

Uptake of Al by endocytosis was identified by using two vital markers: morin, for aluminum (Eggert 1970; Eticha et al. 2005a), and FM4-64, for endosomal/vacuolar membranes (Vida and Emr 1995). It was detected in vivo that Al was internalized into endosomal/vacuolar compartments in the root meristematic cells and in the cells of the distal portion of the transition zone. In the proximal transition zone where endocytosis did not occur, no uptake of Al was detected. Thus, aluminum was not accumulated in the V and was localized only in the CW, binding probably during the uptake from the environment (Illéš et al. 2006). Hence, the presence of Al in the V was strictly dependent on the endocytic transport pathway.

Pb endocytosis was detected in an *F. hygrometrica* protonemata apical cell. As described above, the metal was internalized into the endocytic structures together with low-methylesterified pectins, but it could also enter alone. Due to endocytosis, Pb present in the endomembrane system can be subsequently sequestered in V and the CW by the vesicular secretion pathway (Krzesłowska et al. 2010). It was clearly shown in a pulse-chase experiment where almost all Pb, which had been commonly present earlier in the endomembrane system, was removed and finally deposited in the CW and also in the V. In the CW it formed large, crystalline-like deposits, which often required a CW thickening formation to accommodate it (Fig. 2i). In addition, an especially large amount of Pb was detected in the region of abundance in low-methylesterified pectins at CW thickenings. They commonly appear under Pb stress at the cell tip (Krzesłowska et al. 2009a, 2010) where intensive endo- and exocytosis take place in tip growing cells (Ovečka et al. 2005; Zonia and Munnik 2008).

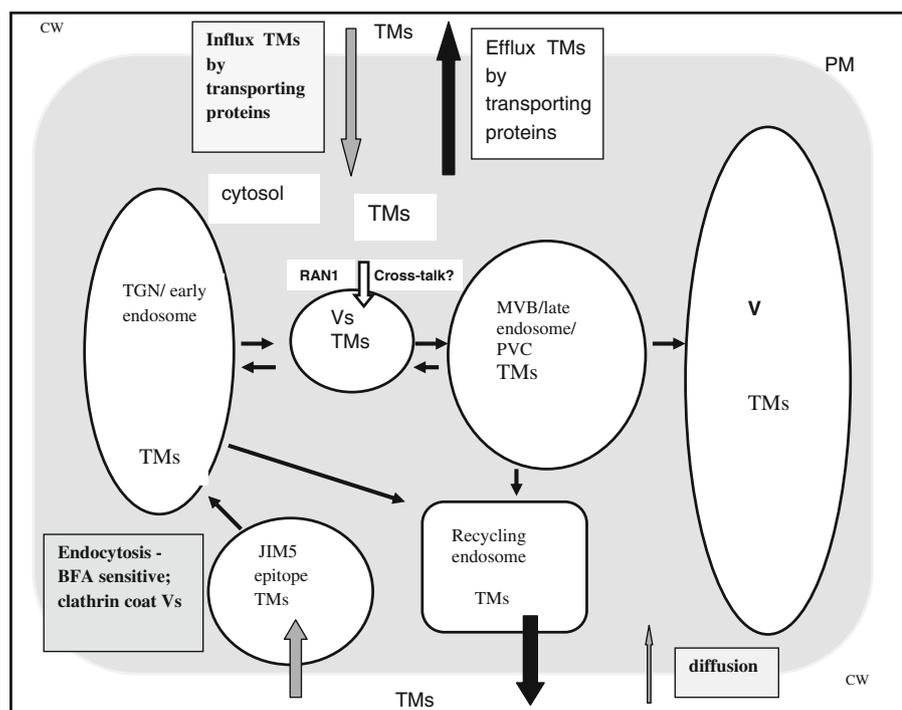
If TMs can be internalized together with low-methyl-esterified pectins (Krzyszowska et al. 2010), then it is possible to hypothesize that they may be transported within the protoplast via the brefeldin A (BFA) sensitive pathway: the same route as detected for the transport of this pectin epitope alone (Šamaj et al. 2004). The BFA-dependent pathway of the endomembrane flow is sensitive to brefeldin A, a fungal toxin that allows endocytosis and inhibits secretion and exocytosis. It is contrary to the BFA-independent pathway, which also exists in plant cells (for review, e.g., Otegui and Spitzer 2008). Taking this hypothesis into account, the route of TMs may include the following stages: (1) internalization from the CW and PM surface into the TGN/early endosome; (2) transport from the TGN back to the PM and sequestration within the apoplast; (3) or transport to a late endosome/multivesicular body (MVB)/prevacuolar compartment (PVC), which also can play a role as a sorting endosome; (4) from MVB transport back to PM by recycling endosome and, as a result of exocytosis, deposited within the CW; (5) or entering and sequestration in the V together with the MVB contents (Fig. 3; Otegui and Spitzer 2008; Krzyszowska et al. 2009b).

The pathway of TM removal from the protoplasts and sequestration in the CW seems to be dependent on its

method of entry into the cell. Trace metals that enter due to the activity of the metal transporters located in the PM or by diffusion, and which occur in the cytosol, are predominantly removed by the activity of the other metal transporters present in the PM. Metals present in the endomembrane system, which have entered by endocytosis, are probably largely removed and sequestered in the CW by the vesicular secretion pathway. However, a cross talk between the two pathways cannot be excluded. For Cu in *A. thaliana* it has been detected that metal present in the cytosol could enter the GA-derived compartments, e.g., Vs. Copper, which enters the cytoplasm by the activity of the copper transporters (COPT1, COPT2) localized in the PM, is subsequently chelated in the cytoplasm by the special chaperone (CCH1). The CCH1 protein brings the copper to a metal transporter RAN1 (responsive to antagonists; Woeste and Kieber 2000; Williams et al. 2000), later called AtHMA7 (Williams and Mills 2005), which may reside in a post-Golgi compartment. RAN1 delivers the copper ion to the ethylene receptors, which then become functional and may be sent ultimately to the PM. Incorporated in the PM, it is responsible for ethylene binding and plays a role in the stress ethylene signaling pathway (Woeste and Kieber 2000).

Moreover, two other transporters, *AtECA3* (Mills et al. 2005) and *MTP11* (Delhaize et al. 2007), which carry

Fig. 3 The possible pathways of TM entering the cell, transport within the protoplasts and exclusion: a summarized scheme (Otegui and Spitzer 2008 modified)



- ← The possible pathway of TMs uptake by endocytosis, transport by endomembrane system and removing by vesicular secretion, BFA sensitive pathway
- ← Possible pathways of TMs influx,
- ← The possible pathways of TMs efflux
- ← TMs entrance from cytosol to Vs, possible site of the cross-talk between two kinds of transporting pathways

metals from the cytoplasm to the endomembrane system have been detected in *A. thaliana* recently. They participate in the transport of Mn and Ca to TGN and prevacuolar compartments (for review Pilon et al. 2009; Puig and Penarrubia 2009).

The discovery of this phenomenon shows that the cross talk of two TM transport pathways in a plant cell is possible, at least for the essential TMs such as Cu. It is still unknown, however, whether such a transport pathway can function in plant cell toxic metal sequestration strategy and for the transport of non-essential TMs.

Remodeling of polysaccharides and plant cell defense strategy to trace metals

An increase in pectin level in response to TMs was first detected in plants exposed to aluminum, e.g., in roots of *Cucurbita maxima* (Le Van et al. 1994), *Triticum aestivum* (Tabuchi and Matsumoto 2001; Hossain et al. 2006), *Zea mays* (Schmohl and Horst 2000; Schmohl et al. 2000) and *Solanum tuberosum* (Schmohl et al. 2000). It has been shown that Al^{3+} causes an increase in the low-methylesterified pectin fraction (Eticha et al. 2005b). It seems likely that this could be the effect of a tolerance mechanism. However, the role of binding Al^{3+} to low-methylesterified pectins in plant defense strategy is still under discussion. It was found that Al-resistant cultivars of *Cynodon dactylon* accumulated 33% more aluminum in CWs than the aluminum-sensitive cultivars (Ramgareeb et al. 2004). On the other hand, most recent studies have shown that an increase in the level of low-methylesterified pectins and binding Al^{3+} within the CW are more characteristic of sensitive plant cultivars than those that are tolerant to this metal (Eticha et al. 2005b; Amenós et al. 2009; Tolrá et al. 2009). The reason for this could be that one of the main plant defense strategies is to release Al^{3+} -binding exudates into the rhizosphere. The exudates contain mainly organic acids (for review, e.g., Poschenrieder et al. 2008) and according to recent papers also pectins (Yang et al. 2008). Both compounds bind trivalent metal ions within the rhizosphere, thereby protecting the plant cells from their entry (Poschenrieder et al. 2008; Yang et al. 2008). Moreover, in response to Al^{3+} , plants increase the amount of phenolics, which neutralize oxidative stress and bind the metal ions within the plant (Tolrá et al. 2009). Thus, binding Al^{3+} within the plant CW and the increase in the low-methylesterified pectin level do not always appear to be essential for plant tolerance to this metal. In this way, plants might probably minimize the inhibition of cell growth because binding TMs within the CW and, in particular, its participation in the formation of interaction between HGA molecules result in CW stiffening, which markedly inhibits cell elongation (Eticha et al. 2005b;

Yang et al. 2008). Besides inhibiting meristematic cell divisions, it is one of the main causes of plant growth inhibition observed under TMs stress (Hall 2002; Patra et al. 2004; Poschenrieder et al. 2008).

Nevertheless, it is worth noting that the presence of TMs in the CW does not always result in CW stiffening and inhibition of cell elongation. It has been found that the presence of Cu and Fe can lead to polysaccharide scission, promoting cell elongation. The mechanism involves generation of OH^{\cdot} in the presence of TMs. Cell wall polysaccharides, such as pectin and xyloglucan, can be broken down by OH^{\cdot} generated in a non-enzymatic Fenton-type reaction, e.g., by the reduction of the O_2 with ascorbate in the presence of Cu ions. Thus, OH^{\cdot} produced in the CW under TM stress could act as a site-specific oxidant targeted to play a role in the CW loosening process supporting cell expansion (Fry 1998; Kärkönen and Fry 2006).

It is not clear when the presence of TMs in plant CW can cause its loosening, and when it causes stiffening. It is possible that the reaction of the CW could depend on the TM dose and, moreover, on the kind of TM. High doses of TMs can result in the oxidative burst of the ROS. The intensive production of ROS in the apoplast under various stresses can drive the oxidative cross-linking of CW components (Wojtaszek 1997; de Cnodder et al. 2005). The cross-linking of structural proteins as well as of polysaccharides, e.g., pectins (Carpita and Gibeaut 1993) in the CW, has been suggested as a possible mechanism for restricting cell growth (Knox 1995). However, it must be noted that plants have developed different defense mechanisms against various trace metals. As mentioned above for Al^{3+} , it is first of all the immobilization of the toxic ions within the rhizosphere which enables the plant to prevent them from binding within the CW and which subsequently leads to growth inhibition (Eticha et al. 2005b; Poschenrieder et al. 2008; Yang et al. 2008). For cadmium ions, binding Cd^{2+} by phytochelatins (small peptides whose synthesis is induced by TM ions; in particular, Cd in the cytosol), transporting and sequestering in the V, and for Cu and Zn chelating by metallothioneins have been well illustrated (for review e.g., Hall 2002; Ernst et al. 2008; Yadav 2009).

Nevertheless, the increase in the amount of pectins, especially low-methylesterified epitopes, could be regarded as a symptom of the defense strategy and plant adaptation to elevated levels of TMs in the soil. Such a point of view lends support to the results of research relating to the defense mechanisms, which function in TM hyperaccumulating plants where the increase in pectin level in response to TMs has been observed. One such example could be the earlier described *Scopelophila cataractae*, which accumulates the highest amount of Cu in its CW pectins. The pectic fraction in CWs of the Cu-treated material showed that the amount of rhamnose, arabinose and xylose were approximately twofold higher than in the

control CWs. Moreover, uronic acid was 1.6-fold higher in Cu-treated CWs than in the control CWs. These data strongly suggested a marked binding of Cu by pectins in *S. cataractae* CW (Konno et al. 2010).

A higher polysaccharide level in CWs has also been detected in *Allium cepa* root cells under Pb stress (Wierzbicka 1998). Simultaneously, large and numerous Pb deposits were observed in the CW of this material. Lead exposition also resulted in an increase in the pectin level in *Funaria hygrometrica* protonemata. The most striking modification in CW polysaccharide composition was a substantial increase in low-methylesterified pectins and the formation of CW thickenings, especially abundant in this pectin epitope (see below). Furthermore, the regions that showed the appearance of low-methylesterified pectin (JIM5-epitope) were related to the distribution and accumulation of Pb deposits (Fig. 2g). In addition, an increase in de-esterified pectins (PAM1-epitope) was observed. It is worth noting that both pectin epitopes, JIM5 and PAM1, are able to bind divalent metal ions (Krzyszowska et al. 2009a).

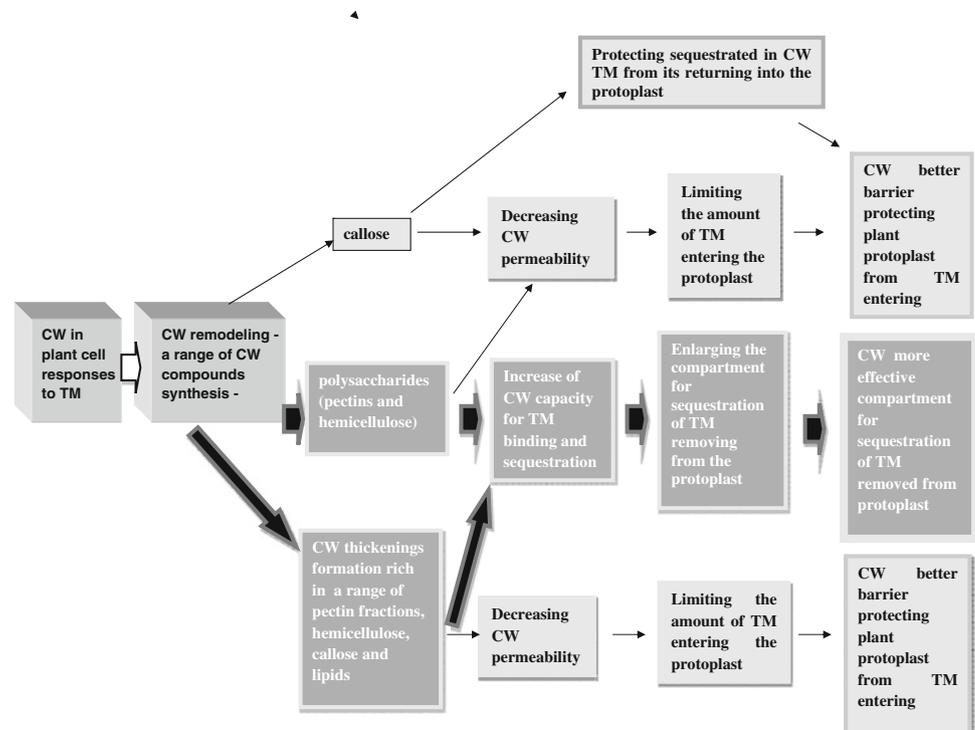
A similar reaction involving an increase in the level of low-methylesterified pectins was detected in *Linum usitatissimum* hypocotyls under Cd stress. Cadmium also caused a reorganization in the distribution of pectin epitopes. It was observed that low-methylesterified pectins (JIM5 epitope) occurred in high amounts within the outer part of the epidermal CW where high methylesterified pectins (JIM7 epitope, generally unable to bind Cd^{2+}) virtually

disappeared. The authors concluded that the outer region of the CW, rich in low-methylesterified pectins, probably bound Cd^{2+} and formed a barrier against its penetration. Additionally, it was noted that the amount of cellulose increased (Douchiche et al. 2007).

Further studies concerning *L. usitatissimum* indicated that Cd also led to an increase in pectin methyltransferase (PME) activity (Paynel et al. 2009). PME, as described above, is the enzyme responsible for the demethylation of high-methylesterified pectins and the formation of low-methylesterified pectin epitopes (Willats et al. 2006; Pelloux et al. 2007). The higher activity of this enzyme in *L. usitatissimum* exposed to Cd resulted in a massive acidification of HGA and the formation of high amounts of low-methylesterified pectin epitopes. Its accumulation was observed in all cell junctions occurring in the cortical tissues, as well as in the most external CW domains of the *L. usitatissimum* hypocotyl epidermis. The levels of two pectin epitopes increased: low-methylesterified pectins (JIM5 epitope) and an epitope that was able to adopt calcium-induced conformations through its polygalacturonic acid domains (2F4 epitope). Simultaneously, the amount of high methylesterified pectins (JIM7 epitope) decreased in those sites. According to the authors, enhanced JIM5 and 2F4 epitopes in the junctions of the inner tissues might oppose cell separation under Cd stress (Douchiche et al. 2010).

Other reorganizations of CW polysaccharides occurred in the outer parts of the epidermal tangential CW of

Fig. 4 Cell wall in plant cell response to TMs: various modifications and remodeling leading to more effective CW functioning in resistant strategy, a summarizes scheme. The essential and most common of the CW remodeling strategies in response to trace metals (*big arrows*)



L. usitatissimum hypocotyls. Here, the amount of pectin with non-blockwise pattern of de-esterification (LM7 epitope) significantly increased, compensating for the marked decrease of JIM7 epitope, while JIM5 epitope remained almost constant and the 2F4 epitope shifted down. These data again pointed to the possible role of a pectin methyl-esterase under Cd stress (Paynel et al. 2009) and concluded that the increase of LM7 labeling in the external tangential wall of the epidermis, together with a decrease of JIM7 labeling, suggested a specific role for randomly partially de-esterified pectins in counteracting the radial swelling stress (Douchiche et al. 2010).

Pectins were also observed to increase in *Salix viminalis* where the main Cd sink was the CW of pectin-rich collenchymas (Vollenweider et al. 2006). The amounts of two CW polysaccharides, pectins and hemicelluloses, increased in *Oryza sativa* exposed to the same metal (Xiong et al. 2009). It must be noted that these effects were triggered by the additional exposition of *O. sativa* to exogenous NO, one of the main stress-signaling molecules (e.g., Wojtaszek 2000; Kopyra and Gwóźdź 2003; Arasimowicz and Floryszak-Wieczorek 2007). Exogenous NO can increase pectin and hemicellulose levels in root CWs, which enhances Cd accumulation in root CWs and subsequently increases rice tolerance to Cd. Furthermore, it was concluded that the CW was a pivotal site of Cd storage in plants (Carrier et al. 2003) and formed the first barrier in blocking the element's penetration of the cells (Xiong et al. 2009). It was demonstrated that about 70–90% of the Cd occurring in the rice cells was located in the CWs, which effectively prevented it from entering the cytoplasm (Nishizono et al. 1987). Thus, deposition of Cd in the CW was considered to be a crucial mechanism for Cd tolerance in this plant species. In *O. sativa* exposed to Cd, contrary to *L. usitatissimum*, a decrease of cellulose content was detected (Xiong et al. 2009). This result showed that cellulose was not a key site for Cd adsorption in rice CW. As the primary structure of cellulose is unbranched, it theoretically should not bind to TMs intensely (Yang et al. 2008).

Modification of CW composition in response to TMs also occur in the plant roots as a result of interaction with mycorrhizal fungi. In general, the higher tolerance of mycorrhizal plants in comparison to non-mycorrhizal ones is a consequence of TM accumulation by fungal hyphae and protection of root cells from TM penetration (Schützendübel and Polle 2002). However, in *O. sativa* inoculated with *Glomus mosseae* under Cu stress, root CWs were modified and indicated higher amounts of pectins than those plants which had not been inoculated. Consequently, the roots of mycorrhizal plants accumulated more Cu in CWs, but much less in the protoplasts compared to non-mycorrhizal ones. This resulted in their higher tolerance to this metal (Zhang et al. 2009).

The results of the studies outlined above have provided evidence that plants, in response to the stress from such metals as Pb, Cd, Cu and Zn, will increase their CW capacity for TM accumulation by elevating the amount of polysaccharides, especially pectins, which bind TM ions. Hence, in the light of present research, the avoidance of metal ion accumulation in the CW as described for Al-tolerant plants and the development of other mechanisms as a defense strategy are more of an exception than a rule.

Another modification of CW polysaccharide composition under TM stress is the appearance of callose (Hall 2002). Callose synthesis is induced by various biotic and abiotic stress factors. It functions as a physical barrier against many stress factors, which may threaten to penetrate the protoplast, e.g., biotic threats such as fungal pathogens (Bolwell et al. 2002), or abiotic threats including wounding (Jacobs et al. 2003) and TM stress (e.g., Hématy et al. 2009). Callose appearance under TM stress has been observed in the response of a range of plant species, e.g., in response to Pb in *Lemna minor* (Samardakiewicz et al. 1996) and *F. hygrometrica* protonemata (Krzesłowska et al. 2009a), and to Al in *Triticum aestivum* (Sivaguru et al. 2000), *A. thaliana* (de Cnodder et al. 2005) and *Zea mays* (Stass et al. 2006). In plants exposed to Al, it has been shown that callose deposition was induced by the appearance of ROS in the CW and alteration of calcium homeostasis, mainly its increase within protoplast caused by the metal (Sivaguru et al. 2000; Jones et al. 2006). Callose bands deposited in the CW protects the PM and protoplast from TM penetration or at least limits the amount that is able to enter the interior of the cell. For example in *F. hygrometrica*, callose appeared in a CW thickening at the cell tip, the main entry way of Pb into the protoplast. It formed a relatively thick layer and undoubtedly blocked metal migration (Krzesłowska et al. 2009a, 2010). Moreover, as described above, callose surrounded large Pb deposits sequestered in the CW after its removal from the protoplast and blocked its potential for entering (Krzesłowska et al. 2010). Hence, it was clear that callose which appeared in *F. hygrometrica* protonemata under TM stress played a protective role for the protoplast.

However, in some other plants where callose appeared under TM stress, the protective role was more elusive. In *L. minor* exposed to Pb, it was found that long bands of callose which could efficiently block TM penetration into the protoplast occurred only in newly formed CWs of roots after cell division and tangential CW. In other CWs callose formed only “patches”, which resulted in relatively high penetration of the cell protoplast by Pb^{2+} . Hence, callose in such cells did not form any efficient barrier for preventing Pb from entering the interior of the cell (Samardakiewicz, personal communication). Moreover, it appeared that under

TM stress callose was accumulated in plasmodesmata. The accumulation of callose within the plasmodesmata was detected in *A. thaliana* under Al stress (Sivaguru et al. 2000; de Cnodder et al. 2005) and in *L. minor* exposed to Pb (Samardakiewicz, personal communication). In general, callose is a structural component of plasmodesmata (PD), where it can regulate the intercellular exchange of ions and molecules including peptides, hormones, proteins and nucleic acids by changing the size exclusion limit (Roberts and Oparka 2003). However, under TM stress, high amounts of callose, which is deposited in plasmodesmata, inhibits cell-to-cell transport. This results in the prevention of a wide incursion of the toxic metal ions, but it simultaneously inhibits the transport of other molecules, e.g., signaling molecules. In Al-treated plants, deposition of callose in the CW, in particular in the root elongation and differentiation zones, and within the plasmodesmata was related to the inhibition of cell elongation (Sivaguru et al. 2000; de Cnodder et al. 2005; Jones et al. 2006). It was shown that a small quantity of callose appeared in Al-tolerant plants, which resulted in low growth inhibition. Synthesis and appearance of callose were more characteristic for sensitive lines (for review, Poschenrieder et al. 2008).

Thus, as in the case of an increase in pectin level, the appearance of callose in plants exposed to Al is not regarded as a tolerance strategy to this metal, but rather as a symptom of its sensitivity that results in plant growth inhibition. However, as mentioned above, plants have developed varying efficient defense strategies to this metal besides its accumulation in the CW.

In response to other TMs callose, whether it occurs as a relatively long band or is deposited in sites where TMs enter the cell, as in *F. hygrometrica* protonemata, it could play a protective role for the protoplast.

Cell wall thickenings

The results of recent studies concerning the response of plant cells to TMs have provided a great deal of information about thickened CW and/or local CW thickening formation. Thickened CWs induced by high metal content have been observed in root cells of *Vicia faba* growing on mine tailing areas. Those thickenings consisted of two layers: a dense outer one containing numerous, electron dense particles of various size including Pb, Zn and C, and an inner less dense layer, containing no electron dense particles. The results suggested that the thickened CW acts as a barrier limiting metal uptake and penetration into the protoplast in plants exposed to Cd (Probst et al. 2009). A similar role of thickened CW has been suggested in earlier studies of *V. faba* exposed to Cd and Cu (Liu et al. 2004). The barrier role of the thickened CW was linked to the effect of an increase in peroxidase activity and CW

lignifications (Liu et al. 2004). It is interesting that these cells of *V. faba*, which contained deposits of TMs only in their thickened CW, were not markedly damaged, while highly damaged cells indicated the presence of metal deposits within the protoplast (Probst et al. 2009). Thus, this observation suggests that an increase of the CW thickness protected plant cells from the toxicity and damaging effects of TMs.

A barrier role of a thickened CW was also found in *F. hygrometrica* protonemata, a tip growing structure (Schnepf 1982) exposed to Pb (Krzyszowska et al. 2009a, 2010). CW thickenings appeared at the tip of apical cells (Fig. 2d, f), one of the main sites of Pb uptake (Krzyszowska and Woźny 1996). It was striking that the nature of the *F. hygrometrica* thickenings markedly differed from that of the CW in unexposed plants. As mentioned above, the most important difference was the appearance of high amounts of low-methylesterified pectins (JIM5 epitope Fig. 2f, g) and de-esterified pectins (PAM1 epitope) able to bind Pb^{2+} . Both of them were absent in the tip CW of unexposed protonemata (Fig. 2e) and were generally absent in apical CW of tip growing cells (Li et al. 1994; Stępką et al. 2000). Thus, their appearance under Pb stress was almost certainly linked with protonema cell defense strategy to this metal. Furthermore, the studies showed that the most extensive part of the CW thickenings was occupied by a granular area absent in the CW of control plants. This region contained both the highest amount of low-methylesterified pectins and the most numerous, large Pb deposits (Fig. 2g). In many cases, gold particles, identifying low-methylesterified pectins, appeared to be bound to Pb deposits. This indicated that this pectin epitope was indeed involved in the immobilisation of high amounts of Pb^{2+} (Krzyszowska et al. 2009a).

The CW thickenings also contained other compounds, which occurred in the tip CW of control cells such as high methylesterified pectins (JIM7-epitope), RGI with an arabinan side chain (LM6-epitope) and xyloglucan. Moreover, they contained marked amount of callose and two kinds of lipid compounds. Thus, CW thickenings localized at the tip of the apical cell, the main region of Pb uptake (Krzyszowska and Woźny, 1996) and rich in low-methylesterified and de-esterified pectins binding Pb^{2+} , callose and lipids, which formed a physical barrier against Pb migration, functioned as an effective blockade, protecting protonema protoplast from the entering of Pb. Indeed, few Pb deposits were detected inside the cell (Krzyszowska et al. 2009a).

The formation of CW thickenings in *F. hygrometrica* protonemata indicating such a different structure and composition to regular CW in the tip region of the cell required significant CW remodeling. This was almost certainly due to the increase of the internalization and transport of low-methylesterified pectins and/or PME

activity and the induction of synthesis of new CW compounds, e.g., callose. Hence, it may be a symptom of a wider defense strategy in plant cells induced by TM stress.

It is worth noting that the addition of Ca^{2+} to Pb solution, which can compete in plant cell uptake with Pb^{2+} , resulted in the appearance of markedly smaller CW thickenings in *F. hygrometrica* protonemata apical cells and the accumulation of Pb deposits was less numerous (Krzyszowska et al. 2004). Thus, it seems to be possible that the formation of CW thickenings was closely related to the amount of Pb entering the cell, and subsequently the level of stress. However, the intercellular signals for CW modifications under TM stress are still elusive. As described above, the intensification of pectins and hemicellulose synthesis could be the effect of the NO signalization pathway in the cell (Xiong et al. 2009). Moreover, recent studies concerning the response of leaves of wheat Yangmai 13 to Cd have shown that CW thickening could be due to the effect of ethylene production (Ge et al. 2009). Ethylene plays an important defense role inducing the expression of various defensive genes in response to Cd stress (O'Donnell et al. 1996). In leaves of wheat Yangmai 13 exposed to Cd, ethylene production resulted in the induction of the UDP-glucose protein transglucosylase (UPTG) enzyme, which plays an important role in synthesizing CW components (Dhugga et al. 1997). According to the authors, it leads to the stimulation of CW thickening formation and increases the CW capacity for Cd immobilization (Ge et al. 2009).

To sum up, thickened CW limits the amount of TMs penetrating the protoplast, chemically by binding TMs to the negatively charged substances and synthesizing callose and lipid compounds to form physical barriers against ion migration (Fig. 4). In this way, CW thickenings function as a compartment, which is able to accumulate TMs before they can enter the protoplast. On the other hand, thickened CW can also function as a compartment for the sequestration of TMs removed from protoplast. Hence, both the increase in the size of the CW by the formation of thickening and the synthesis of polysaccharides, such as pectins, increase the CW capacity for TM immobilization. All of these CW modifications significantly enhance the chance of a plant's survival and growth on soils with elevated levels of TMs (Fig. 4).

Concluding remarks

The results of researches concerning the role of the CW in plant cell response to TMs evidently show that the CW is one of the preferential plant cell compartments, which sequester TMs. In most plant species, sequestration of toxic TMs within the CW is a major tolerance strategy,

which enables them to survive. The CW capacity for binding TMs depends on the amount of negatively charged groups of compounds from which it is built. The presence of carboxyl groups is essential for binding divalent and trivalent TM cations in all plants. Such groups are present both in alginate in the CW of brown algae and in pectins, especially in low-methylesterified fractions, occurring in particularly large amounts in the CW of mosses and also in the CW of ferns and flowering plants. Both compound pectins and alginic acid in normal conditions form an egg-box structure by interacting with Ca^{2+} . When plants grow in places that are contaminated by trace metals, Ca^{2+} is replaced in this interaction by other metal cations such as Al, Pb, Cu, Cd and Zn, which indicates that they have a higher affinity for these compounds. This mechanism is common to all of the groups of plants described above and is crucial for the sequestration of TMs in plant CW. Therefore, it is not surprising that plant cells increase the capacity of the CW by elevating the level of polysaccharides that bind metal cations and form CWT, which is able to accumulate greater amounts of toxic metal ions and separate them from sensitive sites in the protoplast (Fig. 4). This mechanism, lately detected in numerous plant species, appears to suggest a higher level of tolerance strategy, which allows plants to accumulate more TMs in the apoplast while simultaneously protecting the protoplast from their toxicity. Furthermore, plant cells can additionally prevent TMs deposited in the CW from returning to the protoplast, e.g., by internalization and combine with CW compounds, by separating them with the callose layer, as recently detected in moss protonemata (Fig. 2i).

Are plants “wise” or not?

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