A Common Highly Conserved Cadmium Detoxification Mechanism from Bacteria to Humans

HEAVY METAL TOLERANCE CONFERRED BY THE ATP-BINDING CASSETTE (ABC) TRANSPORTER SpHMT1 REQUIRES GLUTATHIONE BUT NOT METAL-CHELATING PHYTOCHELATIN PEPTIDES

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Cadmium poses a significant threat to human health due to its toxicity. In mammals and in bakers’ yeast, cadmium is detoxified by ATP-binding cassette transporters after conjugation to glutathione. In fission yeast, phytochelatins constitute the co-substrate with cadmium for the transporter SpHMT1. In plants, a detoxification mechanism similar to the one in fission yeast is supposed, but the molecular nature of the transporter is still lacking. To investigate further the relationship between SpHMT1 and its co-substrate, we overexpressed the transporter in a Schizosaccharomyces pombe strain deleted for the phytochelatin synthase gene and heterologously in Escherichia coli. In all organisms, overexpression of SpHMT1 conferred a markedly enhanced tolerance to cadmium but not to Sb(III), AgNO3, As(III), As(V), CuSO4, or HgCl2. Abolishment of the catalytic activity by expression of SpHMT1K623M mutant suppressed the cadmium tolerance phenotype independently of the presence of phytochelatins. Depletion of the glutathione pool inhibited the SpHMT1 activity but not that of AthMHA4, a P-type ATPase, indicating that GSH is necessary for the SpHMT1-mediated cadmium resistance. In E. coli, SpHMT1 was targeted to the periplasmic membrane and led to an increased amount of cadmium in the periplasm. These results demonstrate that SpHMT1 confers cadmium tolerance in the absence of phytochelatins but depending on the presence of GSH and ATP. Our results challenge the dogma of the two separate cadmium detoxification pathways and demonstrate that a common highly conserved mechanism has been selected during the evolution from bacteria to humans.

Cadmium is a trace element, the presence of which in the environment is essentially due to human activities. It is a highly toxic non-biological heavy metal able to enter living cells via transporters usually used for the uptake of essential cations such as calcium, iron, zinc, and so forth (1). The reactivity of cadmium with thiol groups and its ability to displace essential biological metals result in oxidative stress and eventually cell death (2). To cope with cadmium toxicity, living organisms have developed different strategies.

In animals, as in the bakers’ yeast cytoplasmic cadmium is complexed with the thiol tripeptide glutathione, a general redox regulator (3, 4). Bis(glutathionato)-cadmium complexes (Cd-GS2)4 are then driven from the cytoplasm to lesser sensitive cellular compartments by dedicated transporters. The prototypical transporter of Cd-GS2 is the GS-X pump, ScYCF1, in Saccharomyces cerevisiae (5) and, even if still controversial, to a lesser extent HsMRP1 in humans (6). HsMRP1 probably acts as an efflux pump at the plasma membrane, delivering cadmium in the extracellular medium, whereas ScYCF1 allows sequestration of cadmium into the vacuole (5). A study of a deficient Scycf1 strain has shown that it was extremely cadmium-sensitive, pointing to a major role of ScYCF1 in cadmium tolerance and detoxification (5). Additionally, ScYCF1 was also found...
involved in arsenic, antimony, mercury, and lead detoxification (references cited within Ref. 7).

A second strategy of cadmium detoxification is found in plants (8), with the exception of mosses, and in the fission yeast Schizosaccharomyces pombe (9). In that case, cytoplasmic cadmium is chelated by enzymatically synthesized thiol peptides, phytochelatins of the structure (γ-Glu-Cys)n-Gly, where n equals 2–11 (10). These peptides are produced upon cadmium exposure by the constitutively expressed phytochelatin synthase enzyme (11), the structure of which has been recently resolved (12). The complex phytochelatin–cadmium (PC-Cd) is then transferred to the vacuole by undetermined transporter(s) in plants and by the ABC half-transporter, SpHMT1, in S. pombe (13, 14). It is interesting to note that in S. pombe, a mutation of the phytochelatin synthase enzyme, SpPCS, or of the SpHMT1 transporter led to similar cadmium-hypersensitive phenotypes (15). This indicates that the transfer of PC-Cd complexes from the cytoplasm to the vacuole is essential in cadmium resistance in this organism.

In mammals, besides GSH, cadmium can be detoxified after association with metallothioneins, a superfamily of ubiquitous cysteine-rich low molecular weight proteins. These biomolecules, discovered as cadmium-containing proteins in horse kidney, have extremely high metal and sulfur contents (up to 10% w/w) (16).

More recently, it appears that cadmium can be taken up, as a free metallic cation, by energized transporters from the P1b-ATPases family. For instance, CadA and AtHMA4 are efficient cadmium transporters, respectively, in Listeria monocytogenes and in Arabidopsis thaliana (17, 18). However, if the detoxification activity of ScYCF1 (or HsMRP1 in humans) is GSH-dependent, cadmium transporters from the P1b-ATPases family are functional in the absence of GSH.

This scheme, discriminating different strategies engaged by plants and animals in cadmium detoxification, has recently been ruled out by the discovery of a functional phytochelatin synthase enzyme in the worm Caenorhabditis elegans that is able to complement the cadmium sensitivity of an S. pombe PCS knock-out strain (19). CePCS1-deficient worms were found markedly more sensitive to cadmium intoxication, leading to the first demonstration of the PC-mediated detoxification of cadmium in an animal (19). In fact, potential phytochelatin synthase orthologs have been found in a large list of eukaryotes outside the plant kingdom (20). The identification of an ortholog of the ABC transporter SpHMT1 in C. elegans, CeHMT-1, has completed the homology between the S. pombe and C. elegans pathways for cadmium detoxification (15, 19). Strikingly, CeHMT-1-deficient worms were found strongly more sensitive to cadmium than CePCS1-deficient worms (19), suggesting that the role of CeHMT-1 was not limited to the transport of PC-Cd in cadmium tolerance.

In the present study, we show that SpHMT1 overexpression is able to confer cadmium tolerance in organisms devoid of phytochelatins, such as S. cerevisiae and Escherichia coli. This function requires the presence of glutathione in the cell and a functional ATP-binding domain in the protein.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains, Bacterial Strains, and Growth Conditions—** The S. cerevisiae strains were provided from the EUROSCARF collection. Y0000 (BY4741; MATa; his3D1; met15D0; ura3D0) and the Scycf1 mutant Y04069 (BY4741; YDR135c::kanMX4) were grown at 30 °C in complete medium (yeast extract peptone dextrose; YPD) or in synthetic medium with dextrose (all amino acids). The transformed yeast were grown in selective medium (synthetic medium with dextrose without uracil) containing 2% of dextrose, raffinose, or galactose.

The S. pombe strains were the previously described the SpHMT1-deficient Δhmt1 strain LK100 and the corresponding S. pombe wild-type strain Sp223 (h−ade6-216, leu1-32, ura4-294) (13) as well as the phytochelatin synthase-deficient Δpcs strain Sp27 and the corresponding S. pombe wild-type strain FY254 (h−ade6-M210 leu1-32 ura4-D18 can1-1) (21). Transformation of S. pombe was performed as described previously (22). Cells were routinely grown at 30 °C in complete (YPD medium or Edinburgh’s minimal medium (EMM) supplemented appropriately (i.e. without Leu (EMM–Leu) as described previously (23). Medium containing different concentrations of CdCl2 was prepared immediately prior to the growth experiment. Plates used for the cadmium spot test were prepared by adding the indicated concentration of CdCl2 to the minimal plate medium.

Plasmid constructions and gene expression were performed in the E. coli strains Top10 (Invitrogen). Bacteria cells were grown at 37 °C in liquid Luria-Bertani (LB) medium supplemented with appropriate antibiotics.

**Plasmid Constructs—** Plasmid pART-SpHMT1 contains the complete hmt1 open reading frame (13). Plasmids pART-SpHMT1 and pEGFP-N2 (BD Biosciences) were used to generate a SpHMT1-EGFP-N2 fusion by the “splicing by overlap extension” technique as already described (7). To generate a plasmid expressing SpHMT1-EGFP in E. coli, the pUC-SpHMT1-GFP was constructed in two steps. First, two primers (SpHMT1–7, 5′-CTCTAGAGAAAGGAGATATACATAGGTCTACGTTACAACAGCC-3′; SpHMT1–8, 5′-GTAGGCGCTTTAGTATGGGAATC-3′) were designed to amplify by PCR (annealing temperature of 50 °C, elongation time of 1 min) a 400-bp fragment containing the first 379 bp of hmt1, the appropriate 5′-ribosomal-binding site (RBS) sequence upstream of hmt1 open reading frame start codon, and an Xbal restriction site in 5′ position. PCR product was sequenced, digested by XbaI, and cloned into the XbaI-Smal digested plasmid pUC19 (New England Biolabs) to obtain the pUC-PCR-RBS-SpHMT1. Finally, a 2.8-kb Stul-Sacl fragment from pART-SpHMT1GFP was subcloned into the same sites of pUC-PCR-RBS-SpHMT1 to obtain pUC-SpHMT1-GFP. All plasmids were confirmed by sequencing.

Plasmids pYES2 expressing EGFP-N2, YCF1, or YCF1-EGFP-N2 were previously described (7). The plasmid pYES HMT1-EGFP-N2 was generated by HindIII-Sacl digestions of pUC-HMT1-GFP and pYES2. The plasmid pUC-SpHMT1K623M-GFP was the result of a directed mutagenesis using the Stratagene QuikChange II XL site-directed mutagen-
es kit with two primers: SpHMT1-F-K623M, 5′-TCAGG-TGTTGGGATGTCAATTATG-3′, and SpHMT1-R-K623M, 5′-CATATAATTGTAAGATCCACACCACCTGA-3′. This plasmid was used to create pART SpHMT1\textsubscript{K623M}-GFP by BglII- Scal digestion in the pART SpHMT1-GFP and pYES2 HMT1\textsubscript{K623M}-EGFP-N2 after ligation of HindIII-Sacl fragment with the pYES2 digested by the same enzymes. Plasmid pYES2 HMA4-EGFP-N2 was previously described (18).

**Growth Inhibition by Cadmium and Determination of Metal Content**—To examine growth in liquid medium, yeast grown to saturation in EMM—Leu medium were subcultured at a starting A600 of 0.1 into the same medium containing different concentrations of CdCl\textsubscript{2}, and the extent of growth after 48 h was determined by measuring the A600. The growth rate of each culture was also determined by taking A600 readings at multiple intervals throughout the entire 48-h period. To examine growth on plates, cells were grown overnight to saturation in EMM—Leu or S-URA 2% raffinose medium. This overnight culture was diluted in EMM—Leu or S-URA 2% galactose medium to an A600 of 0.4, which in turn was diluted in 10-fold increments. Two microliters of each 10-fold dilution was spotted onto EMM—Leu or S-URA 2% galactose plates containing different concentrations of CdCl\textsubscript{2} and incubated for 7 or 3 days at 30 °C. In the case of E. coli, single colonies of bacteria transformed with pUC19 or pUC-SpHMT1-GFP were grown to saturation in LB medium (37 °C, 180 rpm) and were subcultured at a starting A600 of 0.05 into the same medium containing different concentrations of CdCl\textsubscript{2}. The growth rate of each culture was determined by taking A600 readings at multiple intervals throughout the entire 6-h period. Determination of cadmium content was realized by induced coupled plasma experiments. The metal content was determined using ICP (ICP-OES Vista-MPX, Varian). Metal resistance assays in the presence of BSO were carried out as described previously (7).

**Localization of SpHMT1**—The localization of the SpHMT1-EGFP-N2 fusion protein was examined in LK100 S. pombe and in the Y04069 S. cerevisiae transformed strains. After overnight culture, yeast cells (0.8 OD) were resuspended in 1 ml of selective medium with galactose containing 8 \( \mu \)M FM4-64 (Red Synaptar 3-2 Interchim FP-41109A) After 15 min at 30 °C, cells were centrifuged and washed for 2 h at 30 °C under agitation with 1 ml of selective medium containing galactose. Yeast cells were washed two times in phosphate-buffered saline and resuspended in 1 ml of water before observation on a glass slide. The observations were done with a confocal laser scanning microscope (Leica TCS SP2 AOBS) fitted with a krypton/argon laser at \( \times 100 \) magnification. Excitations were performed at 488 or 568 nm for EGFP-N2 (excitation peak at 488 nm and emission peak at 507 nm) and FM4-64 (excitation peak at 515 nm, emission peak at 640 nm), respectively. The fluorescence was collected through 510–570 and 660–800 nm for EGFP and FM4-64, respectively. The localization of SpHMT1 in bacteria was conducted with log-phase cells expressing GFP-tagged SpHMT1. Cells were examined at 100-fold magnification on a poly-lysine-coated slide by using a Nikon Optiphot-2 fluorescence microscope. The fluorescence was collected through a band-pass filter at 510–570 nm (EGFP-N2 excitation peak at 488 nm, emission peak at 507 nm). Images were captured with a Zeiss AxioCam camera and its dedicated software.

**Electron Microscopy**—Bacteria transformed with pUC19 or pUC-SpHMT1-GFP were fixed overnight in 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate-buffered saline and extensively washed in phosphate-buffered saline. Cell pellets were infiltrated in a 2% agar gel and embedded in Unicyrl. Eighty nm-thick sections were cut, collected on EM grids, preincubated in 20 mM Tris buffer (pH 7.5) containing 0.1% bovine serum albumin, 0.1% fish gelatin, 0.05% Tween 20 (buffer-T), and followed by a 90-min incubation in the same buffer—T containing a 1:90 dilution of anti-GFP polyclonal antibodies (AbCam antibody ab290). Sections were washed three times in buffer—T and then incubated in 1:25 dilution of 10-nm gold-conjugated secondary antibodies for 15 min (Amersham Biosciences). After washing in Tris, sections were stained with uranyl acetate and lead citrate and photographed on a FEI CM12 microscope (FEI, Eindhoven, The Netherlands). For statistical analysis, pictures were randomly taken of each of the two samples and of the gold particles located along the plasma membrane, and cytoplasmic background labeling was counted in ~80 different cells from each sample.

**Membrane Extraction and Western Blot Analysis**—Transformed bacteria were grown in 5 ml of LB ampicillin and subcultured in 1 liter of LB for 4 h at 37 °C. The culture was stopped when the A600 reached 1.5. Bacteria were collected by low speed centrifugation (4000 \( \times \) g, 15 min at 4 °C). All following steps were carried at 4 °C. Pellets were resuspended in the buffer 50 mM Tris—HCl, 5 mM MgCl\textsubscript{2}, 1 mM dithiothreitol, 5 \( \mu \)M leupeptin, 5 \( \mu \)M pepstatin A, and 1 \( \mu \)M phenylmethylsulfonyl fluoride. Bacteria were lysed by two successive passages through a French press (18,000 p.s.i.). EDTA was then added at 10 mM. Unbroken bacteria and membrane residues were removed by a 30-min centrifugation at 15,000 \( \times \) g. Membranes were collected by centrifugation at 100,000 \( \times \) g and washed in 15 ml of the buffer 50 mM Tris—HCl, pH 8, 5 \( \mu \)M leupeptin, 5 \( \mu \)M pepstatin A, and 1 \( \mu \)M phenylmethylsulfonyl fluoride and centrifuged again at 100,000 \( \times \) g. The pellet was finally suspended in 50 mM Tris—MES, pH 8, 300 mM sucrose. Membrane extracts were denatured at room temperature and subjected to SDS-PAGE electrophoresis. After transfer onto polyvinylidene difluoride membrane, proteins were detected with an anti-GFP antibody (monoclonal antibody JL-8, BD Biosciences; dilution 1:5000) as already described (7).

**Periplasmic Extraction**—The periplasmic extraction was done as already described (24). Briefly, bacteria transformed with pUC19 or pUC-SpHMT1-GFP grown to saturation in LB medium supplemented with 100 \( \mu \)M ampicillin were subcultured at a starting A600 of 0.1 into 50 ml of LB with 50 \( \mu \)M cadmium and grown overnight. The culture was stopped when the A600 reached 2.6. Bacteria were collected by low speed centrifugation (4000 \( \times \) g, 15 min at 4 °C). Pellets were resuspended in 1.5 ml of Tris—HCl, pH 8, by briefly vortexing, and 600 \( \mu \)l of CHCl\textsubscript{3} was added. After brief vortexing, the tubes were maintained at room temperature for 15 min, and then 5 ml of 10 mM Tris—HCl, pH 8, was added. Intact cells were separated by centrifugation (6000 \( \times \) g, 20 min). The supernatant containing the
periplasm was collected, and 1 ml was used to determine the cadmium periplasm content by ICP.

RESULTS AND DISCUSSION

Overexpression of SpHMT1 in S. pombe Rescues Cadmium Tolerance in a Sphmt1-deleted Strain—As already reported by Ortiz et al. (13, 14), an S. pombe mutant having a defect in the Sphmt1 gene and unable to accumulate PC-Cd complexes in the vacuole was found to be cadmium-sensitive. This defect was rescued by expression of the wild-type SpHMT1 protein in the mutant (Fig. 1A). Three independent clones of S. pombe strains expressing a fusion of SpHMT1 with the green fluorescent protein also exhibited an enhanced cadmium tolerance when compared with the Sphmt1 deletion strain (Fig. 1A). Thus, the C-terminal fusion of GFP did not impair the protein function as already observed for other ABC transporters such as HsMRP1 or ScYCF1 (7, 25). The fusion protein co-localized with the vacuolar marker FM4-64 (Fig. 1B), in accordance with the location of the transporter at the vacuolar membrane previously deduced from membrane fractionation (13). A similar vacuolar location has been observed for CeHMT-1 in S. pombe using a similar GFP fusion strategy (19). The protein was detected in Western blots using anti-GFP antibodies at the 112 kDa expected molecular mass (Fig. 1C). We recently reported that ScYCF1 confers a tolerance to other metal(loid)s besides cadmium (7). Surprisingly, among the metal(loid)s tested, SpHMT1 was able to confer a tolerance to cadmium but not to Sb(III), AgNO3, As(III), As(V), CuSO4, or HgCl2 (supplemental Fig. 1).

Overexpression of SpHMT1 Complements Cadmium Tolerance of an S. pombe Strain Devoid of Phytochelatins—To determine whether SpHMT1 is an exclusive PC-Cd transporter, we used the Sp254 S. pombe strain deleted for the PCS gene, Sp27. This strain is unable to synthesize phytochelatins from glutathione and is highly sensitive to cadmium (21). In this genetic background, overexpression of SpHMT1 was able to completely restore cell tolerance to cadmium in the drop test as well as in liquid media experiments (Fig. 1, D and E). The cadmium resistance was not restored when the /H9004 pcs strain was transformed with the empty vector pART (Fig. 1E). These results indicate that the lack of phytochelatins can be compensated by SpHMT1 overexpression and strongly suggest that SpHMT1 can contribute to cadmium tolerance in S. pombe independently of any phytochelatin synthesis. To confirm this hypothesis, expression of SpHMT1 was investigated in organisms naturally devoid of PC.

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mainly by the formation of bis(glutathionato)-cadmium complexes in the cytoplasm followed by their transfer into the vacuole by the full-sized ABC transporter ScYCF1 (5). In accordance, overexpression of ScYCF1 in the S. cerevisiae wild-type context allowed an increase in cadmium tolerance (Fig. 2A). Remarkably, overexpression of SpHMT1 was even more efficient, allowing a yeast cadmium tolerance up to 200 μM. The ability of SpHMT1 to confer cadmium tolerance was even more pronounced in the cadmium-hypersensitive Scycf1 mutant context (Fig. 2A). In this strain, overexpression of SpHMT1 was able to induce a cadmium resistance greater than the one observed after overexpression of the homologous transporter, ScYCF1. A Western blot analysis revealed that the fusion protein SpHMT1-GFP was reproducibly and largely more detected than ScYCF1-GFP based on a similar quantity of proteins loaded on gel (Fig. 2B). This result might argue with the great efficiency of SpHMT1 in cadmium detoxification. In contrast with its location at the vacular membrane in S. pombe, in S. cerevisiae, SpHMT1-GFP was located in vesicles surrounding the main vacuole (Fig. 2C). Experiments conducted here, with the two major yeast models that diverged ~400 million years ago (26), S. cerevisiae using GSH to complex cadmium and S. pombe using PC, confirm that SpHMT1 activity in cadmium tolerance is independent of PC. Altogether these results demonstrate that SpHMT1 can use another substrate than PC for the cadmium resistance activity. To investigate further possible substrates coming from the thiol pathway, a S. cerevisiae yeast strain depleted of GSH was used.

Cadmium Tolerance Conferred by SpHMT1 Depends on Glutathione Synthesis—The comparison of the action mechanism of ScYCF1, the activity of which is strictly coupled to the presence of GSH and of SpHMT1, depending on the presence of PC, lead us to evaluate the hypothesis that GSH might be a candidate as a co-substrate substitute for PC in cadmium transport by SpHMT1. This hypothesis was tested by a direct application in the culture medium of 1 mM-BSO, an inhibitor of γ-glutamyl cysteine synthetase (27). In S. cerevisiae, after application of 5 mM BSO, the GSH pool was reduced to 32% of its initial content after 6 h and to 50% after 24 h (7). In the absence of metal, application of 5 mM BSO had no effect on growth of the different yeast strains studied (Fig. 2D). In the presence of 5 mM BSO combined with 50 or 100 μM cadmium, the growth of wild-type strain was diminished by about 30 or 50%, respectively, as already reported (7). Similarly, in the presence of BSO, the cadmium tolerance of Scycf1 strains expressing ScYCF1-GFP or SpHMT1-GFP was diminished. In contrast, cadmium tolerance resulting from an overexpression of the P1B-ATPase AtHMA4, (the plasma membrane Arabidopsis Cd-ATPase) was unaffected in the presence of BSO (Fig. 2D) (18). Thus, it is
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likely that SpHMT1, similar to ScYCF1, is able to use glutathione as a co-substrate in cadmium transport.

SpHMT1 Detoxification Activity Depends on ATP Hydrolysis and Multimerization—The transport activity of ABC transporters is linked to the binding of ATP and its hydrolysis at the level of nucleotide-binding domains(28). To ensure that cadmium tolerance conferred by SpHMT1 overexpression was due to a transport activity, a SpHMT1K623M variant of the protein, unable to bind ATP, was produced. Overexpression of this variant did not confer cadmium tolerance to Sphmt1 or Scycf1 deletion strains (Fig. 3A), demonstrating that the binding of ATP by the protein is crucial in the cadmium resistance process. Using Western blot, we confirmed that the SpHMT1K623M variant was properly expressed at a level comparable with the wild-type version of SpHMT1 (Fig. 3B). Moreover, it is interesting to note that SpHMT1K623M had a dominant negative effect when overexpressed in the wild-type S. pombe strain (Fig. 3A). ABC transporters are generally multimeric proteins, and the simplest explanation of this dominant negative effect is that this non-functional polypeptide invalidates the transport activity of the multimeric protein. The fact that both S. cerevisiae and S. pombe yeast cells transformed by the SpHMT1K623M variant plasmid were sensitive to cadmium intoxication strongly suggests that the resistance given by the wild-type SpHMT1 is not due to a chelation process but rather due to an active transport mechanism.

SpHMT1 Is Expressed at the Periplasm of E. coli Cells—Because a recent study has shown that a few amounts of PC were synthesized in S. cerevisiae (29), SpHMT1-GFP was also expressed in a prokaryote, E. coli DH10B, fully devoid of PC. Under fluorescence microscopy, only cells transformed with pUC-SpHMT1-GFP were exhibiting a green fluorescent signal (Fig. 4A). Proteins from the soluble and microsomal fractions were extracted, separated by SDS-PAGE, blotted, and immunodetected with an anti-GFP antibody (Fig. 4B). A 112-kDa apparent polypeptide was detected in the SpHMT1-GFP microsomal fraction that is consistent with the predicted molecular mass of SpHMT1-GFP. A putative dimeric complex was detected at 250 kDa as well as bands at around 65 kDa that could be proteolytic fragments from SpHMT1, as already described in the natural host (13). Finally, taking advantage of the C-terminal EGFP tag and 10-nm gold-conjugated secondary antibodies, immunocytolocalization of the transporter was investigated by electronic microscopy in E. coli (Fig. 4C). Although a mean of 1.6 ± 0.4 plasma membrane gold particles per µm² was observed in...
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**FIGURE 5.** Scheme of the known mechanisms of cadmium detoxification by ABC transporters in various organisms. Depending on the organism, cadmium is detoxified either by excretion or by sequestration. The cationic metal can be directly excreted by the ATPase AtHMA4 in the plant *A. thaliana* (18). Most of the time, cadmium is associated to glutathione (GS) or PC. In the first case, Cd-GS complexes might be excreted by the human HsMRP1 (6) or sequestered by the yeast ScYCF1 (5). In the latter case, Cd-PC complexes are probably sequestered in *C. elegans* by *C. elegans* heavy metal tolerance factor 1 (*CeHMT1*) (19) and in *S. pombe* by SpHMT1 (13). Here, we demonstrate that SpHMT1 does not require PC but needs glutathione. The biosynthesis of glutathione and phytochelatins was blocked by the external application of BSO.

High molecular mass complex (7 kDa), the nature of which was not resolved.

**Conclusion**—We have shown that SpHMT1 is able to rescue the cadmium-sensitive phenotype of an *S. pombe* mutant deficient for the PCS gene, suggesting that PC-Cd is not the only substrate of SpHMT1. When heterologously overexpressed in the *Scycf1* deletion mutant of *S. cerevisiae*, which is cadmium-hypersensitive and devoid of PCS, SpHMT1 also allowed recovery of a wild-type phenotype. The complementation of the *Scycf1* strain was found BSO-sensitive, suggesting that cadmium conjugated to glutathione is a substrate of SpHMT1. Overexpression of a SpHMT1<sub>K623M</sub> variant exhibiting a point mutation in the nucleotide-binding domain led to a suppression of the cadmium tolerance, disclosing a possible cadmium chelation by the overexpressed protein. This mutated form had a dominant negative effect, suggesting that SpHMT1 functions as a multimeric protein. Because *S. cerevisiae* has been shown to synthesize a few amounts of phytochelatins (29), SpHMT1 was also overexpressed in *E. coli*. Even in the context of this prokaryote, fully devoid of PC, SpHMT1 was able to confer a strong tolerance to cadmium, accompanied by a different partitioning of cadmium in the bacteria. These results demonstrate that SpHMT1 is not an exclusive PC-Cd transporter but that it can likely take in charge cadmium-glutathione conjugates. This gives an explanation for the stronger phenotype of the CeHMT1-1-deficient mutant when compared with the CEPCS1-deficient mutant under cadmium stress. The absence of phytochelatin would notably affect free cadmium chelation in the cytoplasm, thus conferring cadmium sensitivity. Invalidation of CeHMT1 has a higher impact on cadmium resistance through the invalidation of cadmium sequestration in the vacuole. Thus, invalidation of this transporter results in critical levels of cadmium in the cytoplasm, leading to a strong toxicity.

Altogether these results demonstrate that SpHMT1 is a polyvalent transporter that can take in charge different forms of cadmium complexes, as illustrated on Fig. 5. It is the first study showing that an ABC transporter (HMT1) can accommodate glutathione, as well as phytochelatin-cadmium complexes. This information would help identify the transporter in charge of cadmium detoxification in plants, a protein that has long been sought after by different groups for many years based on its ability to use phytochelatin-cadmium complexes. Our results challenge the dogma of the two separate cadmium detoxification pathways and demonstrate that a common highly conserved cadmium detoxification mechanism has been selected during the evolution from bacteria, including plants and yeast, to humans. Moreover, besides the fact that cadmium is implicated in cancer in humans (in liver and kidney), the nature of the human transporter responsible for cadmium detoxification remains an open question. Our results would lead to building new models of heavy metal detoxification to prevent/cure diseases linked to the exposition of humans to this toxin.

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