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CRITICAL REVIEW

Glutathione-coordinated metal complexes as substrates for cellular transporters

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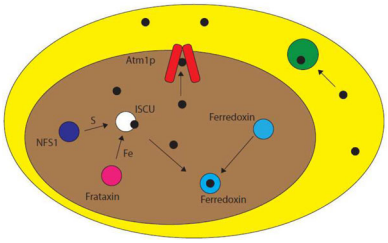
Abstract

Glutathione is the major thiol-containing species in both prokaryotes and eukaryotes and plays a wide variety of roles, including detoxification of metals by sequestration, reduction, and efflux. ABC transporters such as MRP1 and MRP2 detoxify the cell from certain metals by exporting the cations as a metal–glutathione complex. The ability of the bacterial Atm1 protein to efflux metal–glutathione complexes appears to have evolved over time to become the ABCB7 transporter in mammals, located in the inner mitochondrial membrane. No longer needed for the role of cellular detoxification, ABCB7 appears to be used to transport glutathione-coordinated iron–sulfur clusters from mitochondria to the cytosol.

Graphical abstract



Glutathione has long been implicated in the trafficking and export of cellular metal species. Recent evidence suggests a connection to Fe-S cluster assembly and trafficking. In eukaryotes, Fe–S cluster biosynthesis is mediated through the mitochondrial scaffold protein, ISCU. Clusters are then transferred to apo targets within the mitochondria or exported to the cytosol via an ABCB7-type protein, such as Atm1p (yeast). This transport protein is thought to have evolved from heavy metal transporters via endosymbiotic evolution from the *alphaproteobacteria* bacterial family.

Significance to Metallomics

Glutathione adducts of metal ions and complex metallocofactors serve as substrates for cellular detoxification or mobilization by membrane-spanning transporters. Eukaryotic mitochondrial ABC-type exporters appear to be based on bacterial heavy metal detoxification transporters, consistent with the endosymbiotic hypothesis. In particular, bacterial heavy metal transporters display striking homologies and glutathione-binding chemistry to human ABCB7, localized to the inner mitochondrial membrane, which is suggested to export glutathione-coordinated iron–sulfur clusters from mitochondria for use in the cytosol.

Background

Glutathione (GSH; (2S)-2-amino-4-{[(1R)-1-[(carboxymethyl) carbamoyl]-2-sulfanyl ethyl]carbamoyl}butanoic acid) is the major thiol-containing species in both prokaryotic and eukaryotic cells, with levels reaching ~10 mM.[1,2] It is biosynthesized from three amino acids, glutamate, glycine, and cysteine, in reactions that are catalyzed by two enzymes, namely γ-glutamylcysteine synthetase and GSH synthetase. The former catalyzes the dehydration reaction between the γ-carboxyl group of glutamate and the amino group of cysteine. After the dipeptide is formed through the γ-linkage, glutathione synthetase adds glycine to the carboxyl group of cysteine, forming glutathione. Reduced glutathione exists in equilibrium with its oxidized dimer (GSSG), with the GSH:GSSG ratio varying depending on the organism (~100-fold greater GSH in mammalian cells and 50 000 times greater in *Saccharomyces cerevisiae*) and cellular compartment.[3,4]

Glutathione performs many cellular roles, including reduction of proteins, neutralization of reactive oxygen species and free radicals, regulation of the nitric cycle, DNA replication and repair, protein production and activation, amino acid transport,

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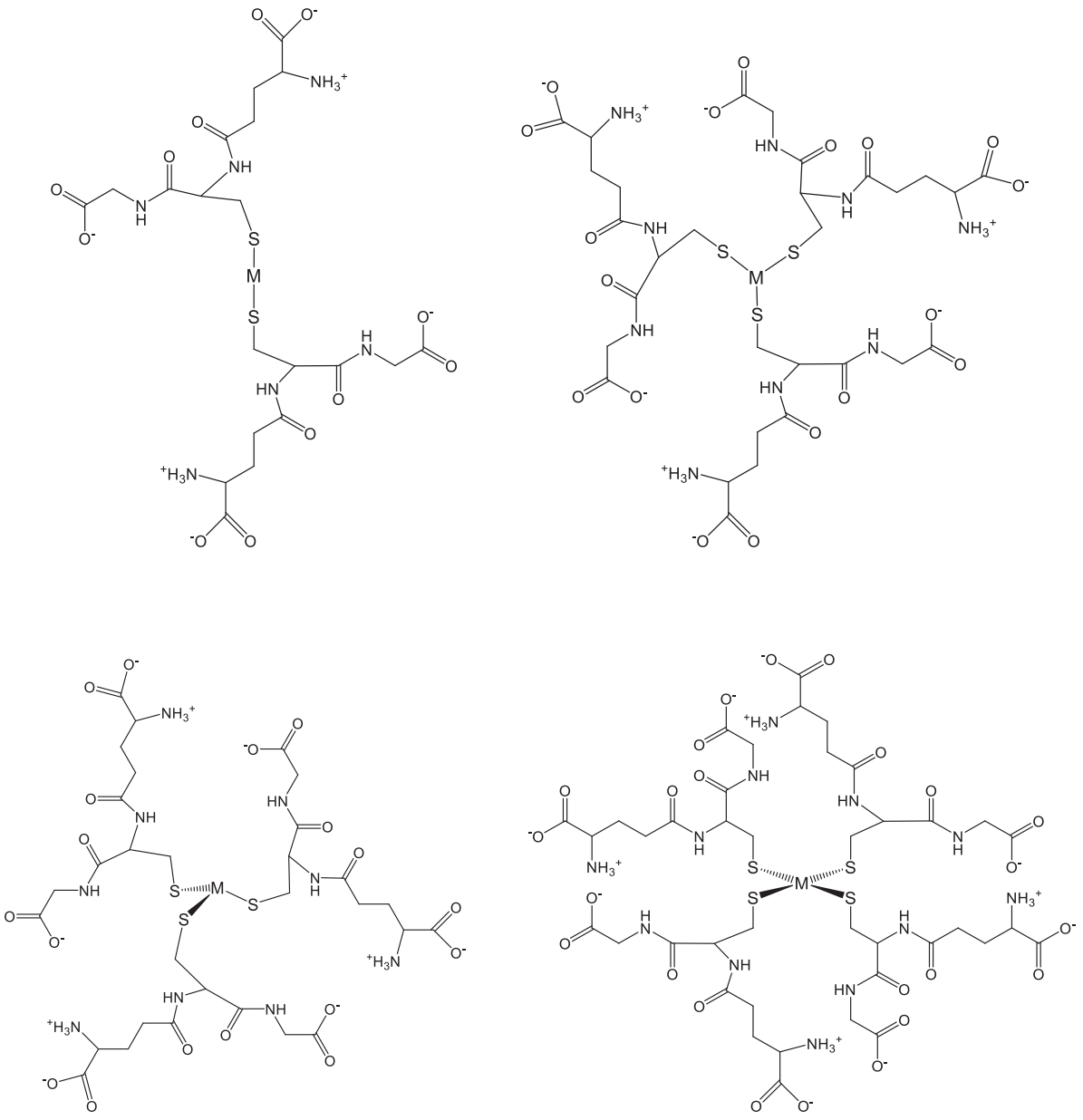

Fig. 1 Metal–glutathione complex structures. Structures of different metal–glutathione complexes described in the text. The metal in each structure is indicated by "M". Top left: linear; top right: trigonal planar; bottom left: trigonal pyramidal; bottom right: tetrahedral.

iron metabolism, and cell differentiation and death.[5–11] In animals, glutathione has been found to play a role in preventing and treating acetaminophen overdose, and detoxification of hydrophobic compounds in the liver and methylglyoxal in the glyoxalase system.[12–16] In plants, glutathione takes part in the glutathione–ascorbate cycle, is a phytochelatin precursor, helps with defense against pathogens, and plays a role in flower development.[10,17–20]

A principal reason for the ability of glutathione to serve the cell in such a large variety of roles stems from the redox activity of the sulfhydryl group and its ability to both bind to metals and react with other thiol groups. The structure of various metal–glutathione complexes is shown in Fig. 1. The sulfur of reduced glutathione has a high affinity for softer metal cations and complexes, such as mercury, lead, arsenic, and cisplatin.[21] Glutathione has also been shown to coordinate iron–sulfur (Fe–S) clusters (Fig. 2).[22] The high affinity for metals and metal complexes allows for glutathione to be an efficient molecule for heavy metal detoxification by one of three methods, namely sequestration, reduction, or efflux.[23] A summary of metals able to form such complexes is shown in Fig. 3 and transporter proteins identified for a variety of metal–glutathione complexes are detailed in> Table 1.

Metal–glutathione complex transport

Mercury

Methyl mercury rapidly combines with GSH to form a stable tripeptide complex that circumvents heavy metal poisoning.[24] The first evidence that glutathione may play a role in the secretion of metals into the bile was obtained from animal studies with

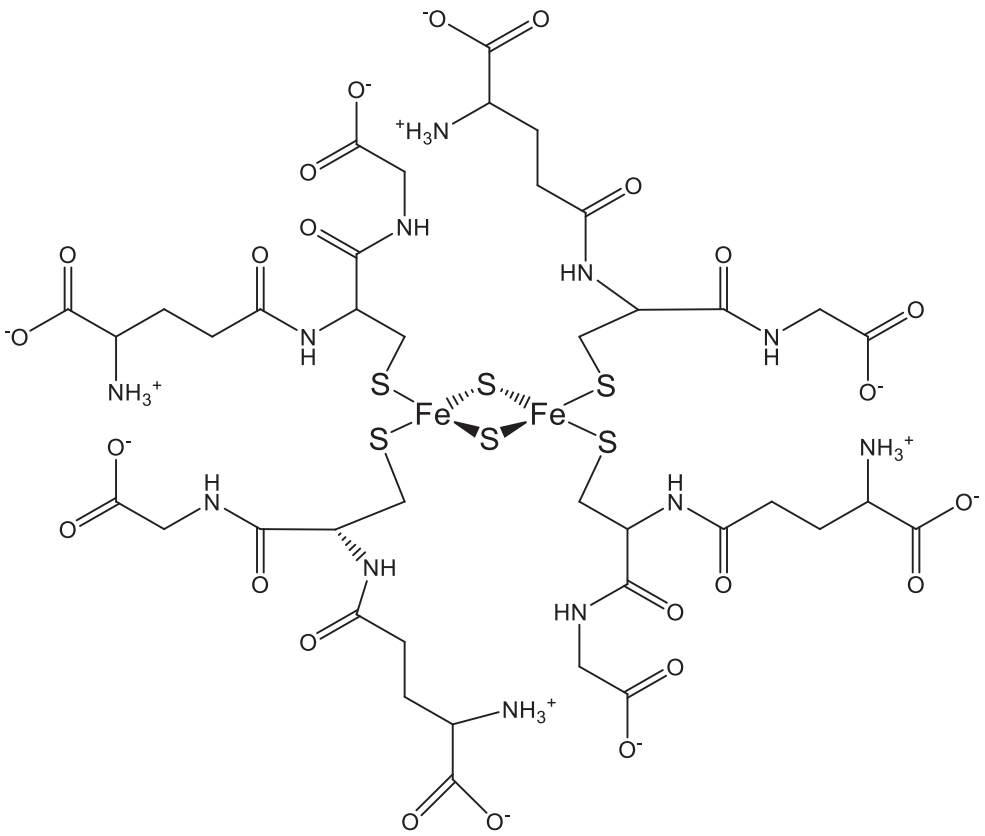

Fig. 2 Structure of glutathione-coordinated iron–sulfur cluster. A 2D representation of the "macrocyclic-like" tetrameric glutathione network in $[2Fe\text{-}2S](GS)_4$, stabilized by intramolecular salt bridges between the amine and carboxylate groups of neighboring glutathiones.[109]

rats, where mercury was found to be complexed by glutathione following administration of methyl mercury. Glutathione levels have also been correlated to the amount of both methyl mercury and inorganic mercury transported into rat bile. Organic anions such as sulfobromophthalein (BSP), indocyanine green (ICG), and phenol-3,6-dibromphthalein disulfonate (DBSP) were observed to inhibit both GSH and methyl mercury biliary secretion without affecting the normal flow of bile or levels of Hg and GSH in the liver. Moreover, the rate of biliary excretion of methyl mercury is independent of methyl mercury concentration but is dependent on GSH, consistent with the importance of GSH in protection against mercury poisoning.[24]

The mechanism by which mercury is exported from the cell is likely to involve an ABC-type transport protein and mercury as a GSH complex.[21,25–27] Hg-GSH conjugates were found to stimulate the ATPase activity of MRP1 and MRP2, with GSH alone having no additional effect on ATPase activity. Mercuric chloride resulted in upregulation of GSH levels, and normal levels of GSH efflux were diminished in the presence of mercury, while $HgCl_2$ inhibited transport of the substrate calcein acetoxymethylester by both human MRP1 and MRP2 in MDCKII cells, suggesting that the Hg–GSH complex is exported through MRP1 and MRP2.[21] Mercury export by MRP has also been demonstrated in sea urchins[25] and mammals.[28,29]

Drosophila MRP is highly homologous to human MRP1 (50% identity and 67% similarity), making it a good model to study the effect of methyl mercury on organism development. MRP mutant flies exposed to mercury were less likely to develop into an adult. Knockdown studies show that methyl mercury accumulated in the Malpighian tubules, gut, and nervous system, suggesting that MRP1 may help prevent neurotoxicity of methyl mercury in humans.[30]

In *Escherichia coli*, it has long been known that GSH plays a role in resistance to mercury,[31] but it was not until recently that a bacterial ABC transporter with the potential to transport a Hg–GSH complex was identified.[32] The protein identified in *Novosphingobium aromaticivorans*, Atm1, is a homolog to other heavy metal transporters in yeast and humans and conveys resistance to mercury in bacteria. The form of mercury that appears to be the substrate for this protein is $Hg(GS)_2$, which is the same form that has been identified as a substrate in eukaryotes.[32] Additionally, Ycf1 has been shown to detoxify *S. cerevisiae* of mercury by transport of $Hg(GS)_2$ via an ATP-dependent mechanism. Ycf1 also plays a role in detoxification of other heavy metals, as discussed later.[33]

Arsenic

As–GSH complexes have been shown to form at physiological and clinically relevant levels between 0.2 and 0.5 μM but appear to be unstable.[21,34] In canine cells transfected with genes for human MRP1 and MRP2, administration of As_2O_3 resulted in upregulation of MRP2 and to a lesser extent MRP1. These cells lines also had elevated levels of GSH when exposed to arsenic, and As_2O_3 inhibited calcein transport.[21] These data suggest a role for GSH in mediating the export of arsenic from the cell by MRP1 and MRP2, potentially as $As(GS)_3$; however, the identity of the transported species is yet to be confirmed.[21,35] In rats, GSH also mediates the biliary secretion of As(III), while GSH is also involved in the metabolic

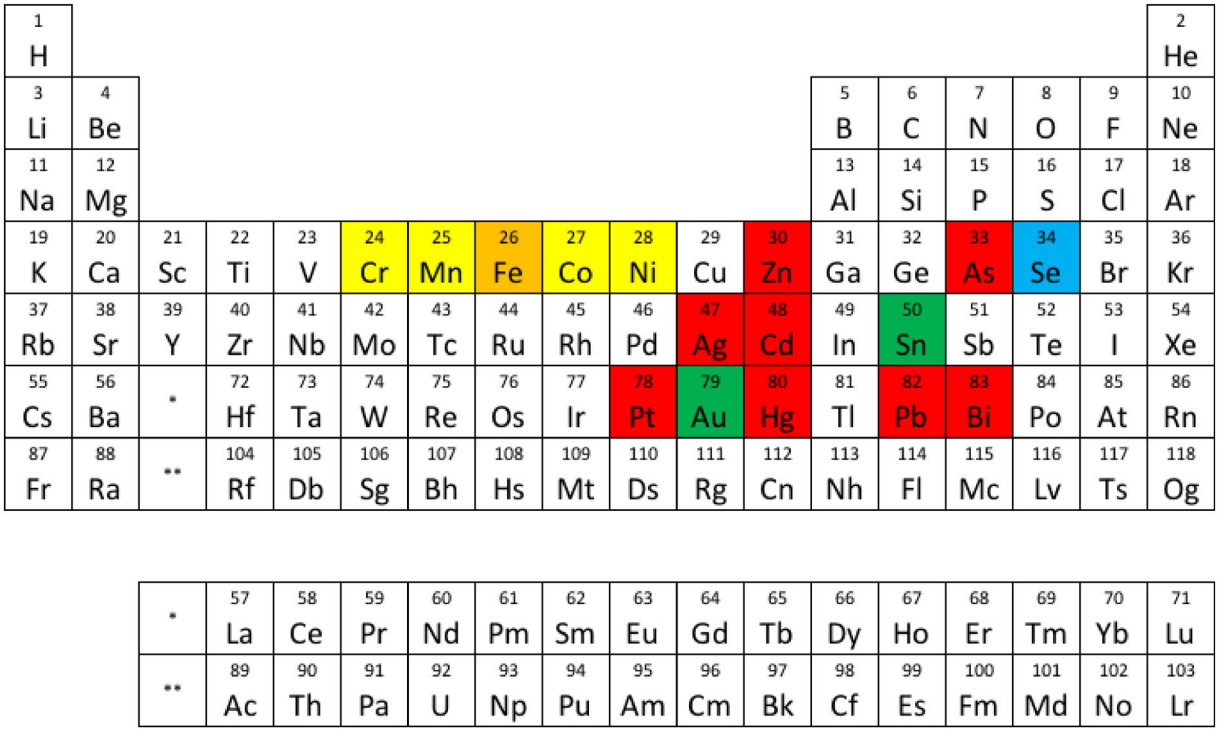

Fig. 3 Metal–glutathione complexes and ABC transporters. Metals in red are known to be transported and/or stimulate ATPase activity as a metal–glutathione complex.[21,25–27,32,50,58,69,72,74,77,110–112] Metals highlighted in yellow have been shown to not be transported as a metal–glutathione complex in known heavy metal transporters.[23,31,81,83,113] Metals highlighted in green most likely bind to phytochelatin in plants.[68] GSH plays a role in tolerance to the metals highlighted in blue, but the mechanism of tolerance is unlikely to be through export via ABC transporters.[83] Metals highlighted in orange have been shown to be transported by ABC transporters as a metal–sulfur cluster complexed by glutathione.[114]

processing of arsenate to arsenite and formation of monomethylated arsenic.[35] The reduction of arsenate to arsenic has also been reported in human erythrocytes and is of physiological relevance as As(V) is the most common form of environmental arsenic.[35,36]

Yeast cells may have multiple ways of dealing with arsenic poisoning, two of which involve export proteins (Acr3 and Ycfl).[37] Acr3 yields higher tolerance as Ycf1 cells were less sensitive to arsenic. However, this is likely concentration dependent, as As efflux through Acr3 saturates quickly. Acute arsenic stress results in arsenic coordination to proteins. However, long-term exposure upregulates GSH and leads to the formation of cellular $As(GS)_3$. In Acr3 knockout cells, Ycf1 becomes upregulated when exposed to arsenic due to the need for Ycf1 to transport more As(III) in the cell. Similar to other metals, As(III) is transported into vacuoles by Ycf1 as a glutathione-coordinated metal complex, and most of the metal is found in vacuoles. Even with the As(III) exporter knocked out, yeast cells can rid themselves (albeit slowly) of arsenic, suggesting a mechanism for arsenic export from the vacuole.[37]

Arsenic export is especially of interest in humans as As_2O_3 is used to induce apoptosis to treat a range of cancers, yet resistance to As_2O_3 has been observed.[38–43] Resistance has been linked to higher expression levels of GSTP1-1, a glutathione transferase, in leukemia, lymphoma, and prostate cancers.[38,39,41,42,44] GSH reduces arsenate to arsenite, while GSTP1-1 is needed to form $As(GS)_3$, which has been established as a MRP1 substrate in cancer cells.[45] $As(GS)_3$ has a very high affinity for MRP1, with a K_M of 0.32 μM, and MRP2 can transport $As(GS)_3$[46] in addition to the mercury–glutathione complexes discussed earlier.

Glutathione also plays a key role in arsenic detoxification in lower eukaryotes. The identity of the ABC transporter for the $As(GS)_3$ complex has been a topic of debate since it was observed that PGPa (phosphatidylglycerophosphatase A) located in the plasma membrane of *Leishmania* did not convey arsenic resistance, but the protein does have the ability to transport the complex.[47,48] In *Leishmania*, the mechanism of arsenic detoxification is similar to that of Ycf1 in yeast, sequestering the $As(GS)_3$ complex in vesicles within the organism.[48] In *C. elegans*, arsenic tolerance has been linked to the expression of MRPs (as seen with mercury) and HMT-1.[49] Based on studies of homologous proteins in other eukaryotes, it is likely that arsenic is transported by these proteins as an $As(GS)_3$ complex.

Lead

When *S. cerevisiae* is exposed to lead, free GSH levels decrease, most likely due to Pb(II) forming a cytosolic complex with glutathione as observed with other heavy metals [Hg(II), As(III), etc.]. The lack of glutathione did not raise the susceptibility of *S. cerevisiae* to lead, indicating that GSH itself does not detoxify the cell.[50] Glutathione transferase deletion in yeast results in increased sensitivity to 0.5–1 mM Pb(II) compared with wild type, suggesting that glutathione transferases are needed to form Pb–GSH complexes. However, as a result of the kinetic lability of the complex, it is likely that the dependence arises through an indirect pathway.[51,52] The mechanism for lead detoxification in *S. cerevisiae* most likely involves compartmentalization of Pb–GSH complexes inside vacuoles, as knockouts of ABC transporters Ycf1, VMR1, YBT1, and BPT1 resulted in increased sensitivity to lead, increasing the number of exporters that can potentially transport glutathione-coordinated metals.[51] Other studies conducted with *S. cerevisiae* show that Pb(II) levels in the cell reach the same

Table 1. Summary of metals transported by ABC transporters as a GSH complex

Protein	Metal transported	Organism of tested protein
MRP1/2	Hg	*S. purpuratus*,[25] *Rattus*,[28,29] *Drosphilia*[30]
	As	*Rattus*,[35] *C. elegans* [49]
	Zn	*Rattus*[69]
	Bi	*H. sapiens*[77]
	Pt	*H. sapiens*[80]
Ycf1	Hg	*S. cerevisiae*[33]
	As	*S. cerevisiae*[37]
	Pb	*S. cerevisiae*[53]
	Cd	*S. cerevisiae*[62]
HMT-1/ABCB6	As	*C. elegans*[49]
	Cd	*H. sapiens*[65]
PGPa (MRPA)	As	*L. tarentolae*[47]
Yor1	Cd	*S. cerevisiae*[62]

levels as the environment and confirmed that Pb(II) tolerance is Ycf1 dependent.[53]

Relative to cadmium and mercury, little is known concerning transport of glutathione-coordinated lead complexes via ABC-type transporters. In rat bile, the form of lead that is transported appears to be a lead–glutathione complex, although the protein through which this occurs has not been identified but could potentially be the same protein involved in the biliary secretion of mercury–glutathione conjugates.[54]

Antimony

Antimony resistance is of interest to the scientific community due to its use in drugs. Leishmaniasis is caused by *Leishmania*, which is prevalent in Nepal and Northern India. Antimonial drugs were used to treat Leishmaniasis until 2005; however, *Leishmania* has developed resistance to antimony-based drugs, as indicated in 60% of Indian patients.[55] Overexpression of two ABC transporters, MRPA (PGPa) and ABCI4, has been linked to antimony resistance in *Leishmania* via sequestration in vesicles and export from the cell, respectively.[55,56] These two transporters, in addition to ABCG2, have been shown to transport metal–thiol complexes.[55] ABCG2 has also been shown to transport glutathione, increasing the likelihood that the thiol species is a component of the transported metal complex.[57]

Cadmium

Cadmium is a toxin that causes mutations and, like many other metals, GSH plays a key role in the natural detoxification pathway. When cells are exposed to cadmium, the primary form that is prevalent in the cell is $Cd(GS)_2$.[58] Similar to lead, it is thought that glutathione transferases play a role in formation of the $Cd(GS)_2$ complex via an indirect pathway.[59–61] ABC transporters Yor1p and Ycf1 play a role in cadmium detoxification in *S. cerevisiae*, although by different mechanisms. Yor1 exports $Cd(GS)_2$ directly from the cell, while Ycf1 transports it into vacuoles. At normal levels, both proteins are required to convey cadmium tolerance in *S. cerevisiae* when exposed to 40 μM Cd(II). However, overexpression of Yor1 was able to restore ΔYcf1p cells to wild-type growth rates.[62]

Multiple $Cd(GS)_2$ efflux proteins have also been found in animal cells. In the case of the cystic fibrosis transmembrane conductance regulator protein (CFTR), a chloride channel, exposure to Cd(II) of CFTR knockout mouse cells prompted necrosis, while normal cells undergo apoptosis. When exposed to 5 μM Cd(II), ABCC7 (CFTR) conductance was stimulated, suggesting cadmium to be exported by CFTR, potentially as a $Cd(GS)_2$ complex.[63,64] ABCC1 (MRP1) is another candidate for $Cd(GS)_2$ efflux.[64] Similar to Ycf1 in yeast, and as observed with arsenic, it is thought that HMT1 (ABCB6) can transport $Cd(GS)_2$ into human vacuoles to sequester the toxic metal.[65]

Similar to mercury, detoxification in plants and bacteria appears to be conveyed by a different mechanism than in yeast and animals.[66] Based on transport studies with proteins proposed to convey cadmium resistance in *E. coli*, CzcP, and CadA, transport of the metal was inhibited at glutathione concentrations of 3 mM, demonstrating that the proteins do not transport a glutathione coordinated metal, and likely transport the metal as the ion itself.[67] In plants, the mode of detoxification is by sequestration through phytochelatins, as seen with other metals.[66,68]

Zinc

MRP2 mutant rats are unable to export endogenous zinc efficiently, while bile in the same mutants contained no zinc even after IV injection of the metal ion.[69] Other studies have suggested that biliary zinc excretion is GSH dependent.[70,71] Mutant rats injected with cadmium ions behaved similarly, suggesting that the biliary excrement of Zn(II) and Cd(II) share a similar mechanism of export through MRP2.[69] It is likely that Zn(II) is excreted as a Zn–GSH complex, in comparison with other divalent metals that are biliary excreted as glutathione complexes through the ABC transporter MRP2.[72]

In drosophila, an ABC transporter with 26% identity to *sc*YCF1 is upregulated in the presence of zinc ions, as well as copper and cadmium.[73] YCF1 transports Cd–GSH complexes into vesicles, sequestering cadmium inside.[62] It is possible that zinc tolerance is conveyed in a similar manner as glutathione S-transferase genes are upregulated in the presence of both cadmium and zinc.[73]

Copper

Cu(I) is exported through another ABC transporter, cCOP; however, this transporter does not transport a Cu–GSH complex. CopB was shown to transport copper without GSH, but it was DTT dependent.[69] *Saccharomyces cerevisiae* proteins of the multidrug resistance associated family, which have been shown to transport glutathione conjugated metals, do not appear to play a role in copper tolerance.[74] However, while copper transporters in humans do not appear to transport copper via a glutathione complex, transport still appears to be GSH dependent.[75,76]

In the bacteria *Pneumococci*, GSH conveys Cu resistance as it was observed that copper tolerance decreased in *gshT* and *gor* mutants. These two genes, encoding an ABC transporter that transports GSH into the cell and a glutathione reductase, are required for normal cellular levels of GSH to be achieved. GSH can convey copper tolerance by complex formation and likely export via copA.[31]

Bismuth

Bismuth has a high affinity for glutathione, readily forming complexes with glutathione in cells where it is transported into vesicles via MRP, as observed in human HK-2 cells.[77] Bismuth upregulates GSH levels, potentially forming a positive feedback of bismuth uptake, as the more the GSH is present in the cells, the more bismuth is absorbed. Similar to other post-transition metals, formation of the bismuth–glutathione complex, $Bi(GS)_3$,

is thermodynamically favorable, but kinetically labile, as the exchange rate between GSH molecules is 1500 s^{-1}.[77]

Platinum

Resistance to cisplatin, a common anti-cancer drug, is glutathione dependent. From cisplatin, $Pt(GS)_2$ can be formed as first observed in leukemia cells. The export of this compound is ATP dependent.[78,79] MRP2 (ABCC2) exports $Pt(GS)_2$ as HEK-293 cells expressing MRP2 were found to be resistant to cisplatin, and this resistance is GSH dependent. Both γ-GCSh and MRP2 are expressed at higher levels when exposed to cisplatin. Increased expression of γ-GCSh, the catalytic domain of γ-GCS, and one of the enzymes required for GSH biosynthesis, results in higher levels of GSH in the cell. However, it is unlikely that higher levels of cellular GSH would lead to increased resistance to cisplatin, as GSH is at millimolar levels in the cell, while platinum concentration would be significantly lower (micromolar levels at most).[80] As stated earlier, MRP2 can transport a variety of metal–glutathione complexes, including glutathione complexes containing mercury and arsenic.[21]

Silver

The ATPase activity of *N. aromaticivorans* was stimulated in the presence of several compounds, including metal–glutathione complexes. Although transport of a silver–glutathione complex was not directly tested, resistance to Ag and Hg was conveyed in *E. coli* through expression of *Na*Atm1 and was consistent with transport of a metal–glutathione complex. The *Km* was 10-fold lower for $Ag(GS)_2$ than for $Hg(GS)_2$, suggesting that the protein can detoxify the cell of silver more effectively than mercury.[32] Other proteins with the ability to transfer glutathione-coordinated silver have not been identified, although phytochelatins have been implicated in silver sequestration.[68]

Other metals

Glutathione is important for the detoxification of several other metals, but the proteins identified to transport glutathione-conjugated metals have not been shown to play a role in tolerance to cobalt, manganese, nickel, or chromium. In *Arabidopsis*, elevated levels of GSH confer nickel tolerance, most likely by protecting against the oxidative stress induced by nickel.[81] Glutathione does not have an effect on chromium resistance in *S. cerevisiae*, but it does in *E. coli*.[23,31] In addition, cancer cells expressing MRP1, which were resistant to arsenic and mercury, showed no resistance to chromate.[82] GSH has also been shown to play a role in selenium tolerance, but this is likely a result of limiting uptake.[83] In plants, phytochelatin synthase is upregulated when exposed to tin and gold, suggesting the ability of phytochelatins to protect plants against the toxicity of these metals.[68]

Iron–sulfur clusters

Biogenesis

There are three classes of iron–sulfur cluster assembly systems used in prokaryotes, namely ISC, NIF, and SUF.[84] The ISC and SUF apparatus are found in both prokaryotes and eukaryotes, further suggesting that the mitochondria of eukaryotes retained the Fe–S cluster biosynthesis pathway following endosymbiosis from prokaryotic precursors.[84,85] In all systems, the same general mechanism of synthesis is at play. An iron donor, sulfur donor, and electron donor all interact with a scaffold protein on which the cluster is built, and then subsequently transferred to a wide variety of apo Fe–S cluster acceptor proteins (Fig. 4).[84]

Atm1p homology

The yeast and human homologs of the same protein that transport glutathione conjugates of mercury and silver have shown the ability to transfer glutathione-coordinated iron–sulfur clusters.[86–88] Although these results have yet to be confirmed *in vivo* (a technically challenging problem), it has been shown that $[2Fe–2S](GS)_4$ can reconstitute a number of apo iron–sulfur cluster proteins,[89–91] and it is known that ABCB7-type proteins are involved in cytosolic Fe–S cluster maturation.[92] Although other chemical species have been shown to bind or be transported by Atm1/ATM3, such as GSSG and glutathione trisulfide (GS-S-SG),[92] these molecules are either already present in the cytosol and/or are not known to be directly involved in Fe–S cluster maturation. Binding by glutathione adducts is consistent with binding sites characterized for oxidized glutathione in *Na*Atm1p, where metal–glutathione adducts were observed to bind, and were likely conserved.[32] The $[2Fe-2S](GS)_4$ complex appears to be a more promising candidate to bridge the mitochondrial and cytosolic cluster maturation pathways as it has been shown to be biosynthetically accessible by glutathione extraction from the scaffold protein ISCU1, and demonstrated to be a molecular substrate that is transported by Atm1/ABCB7.[83,84]

In eukaryotes, the biosynthesis of Fe–S clusters is compartmentalized within the mitochondria, and in *S. cerevisiae*, Atm1p is located in the inner mitochondrial membrane (Fig. 4). As the mitochondria are hypothesized to have originated from a bacterial cell, the iron–sulfur cluster assembly machinery in the mitochondria (ICA) has been highly conserved from bacteria to man.[93] The ability for *Na*Atm1p to transport glutathione-coordinated metals, having two binding sites for oxidized glutathione and mimicking a glutathione-coordinated iron–sulfur cluster, combined with the high homology with *Sc*Atm1p, all support a structural model where the genes for *Na*Atm1p and *Sc*Atm1p diverge from a common ancestral gene.[32]

The common ancestral gene is most likely the one found in the bacteria that originally became mitochondria in eukaryotes. *Saccharomyces cerevisiae* Atm1p shares a high % similarity with *N. aromaticivorans* (*Na*) Atm1p (Fig. 5),[32] a bacterial strain from the *alphaproteobacteria* family that is thought to have developed into mitochondria through the endosymbiotic theory.[94–102] BLAST analysis shows that the *Na*Atm1p gene is very similar to other Atm1p genes in the *alphaproteobacteria* family, including *sphingomondae*.[88,103] The *Na*Atm1p gene was most closely related to this genus of bacteria, which is commonly found in environments containing toxic compounds.[104] Since glutathione is involved in detoxification of several different types of compounds, including metals, this is the likely need for such proteins in these bacteria. In bacteria, there is no need to transport iron–sulfur clusters out of the cell, as they would be retained for use with cytoplasmic proteins.

The top 100 matches for the *Na*Atm1p sequence were all found in *alphaproteobacteria*. When expanded to 500 matched results, the protein sequence also matched to several *betaproteobacteria*, mainly *Bordetella* and *Achromobacter*.[88,103] The bacteria of both of these genera are pathogenic in humans, with *Bordetella* being more aggressive and *Achromobacter* affecting immunocompromised individuals.[105,106] These bacteria are in the same phylum as *alphaproteobacteria*, *proteobacteria*, which also have Atm1p genes. Interestingly, of the 416 organisms that appeared in the

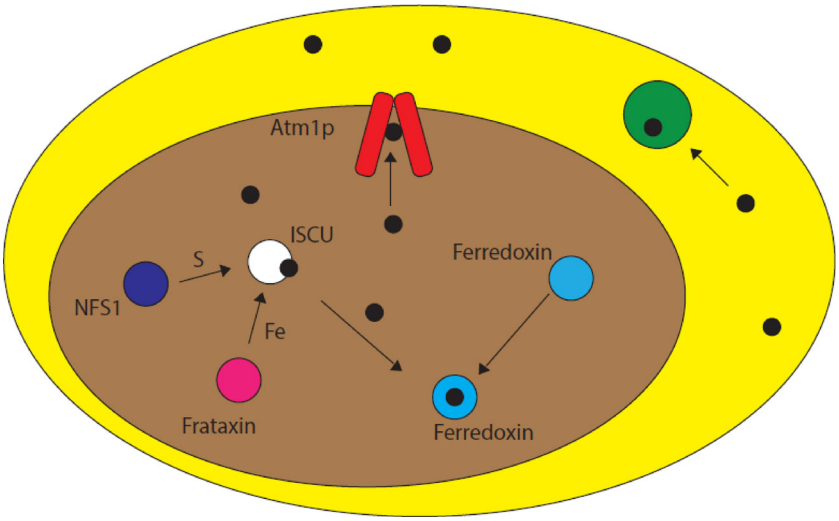

Fig. 4 Eukaryotic Fe–S cluster biogenesis. In eukaryotes, iron–sulfur cluster biogenesis takes place in the mitochondria (brown) on the scaffold protein ISCU (white). Iron is donated to ISCU from frataxin (pink) and sulfur is donated by the cysteine desulfurase NFS1 (purple). Fe–S clusters (black) built on ISCU can then be transferred to apo targets within the mitochondria (e.g. ferredoxin, blue) or exported out of the mitochondria to the cytosol (yellow) via an ABCB7-type protein (red) such as Atm1p (yeast) for use in cytosolic Fe–S cluster proteins (green). Cluster transfer may be facilitated by other proteins.

BLAST results when omitting *proteobacteria*, 383 are eukaryotes, including some of the top matches (*Streptomyces cavourensis* and *Nephila clavipes*).[88] This supports the idea of the Atm1p genes having a common bacterial ancestor that evolved after mitochondria originated.

The BLAST results in the animal kingdom that most closely matched the protein sequence for *Na*Atm1p were those of the arthropods *Ixodes scapularis* (deer tick) and *N. clavipes* (spider). Other arthropods that matched the protein sequence include multiple species of *drosophila* (fly), *Nilaparvata lugens* (Brown planthopper), and non-chordates *Hypsibius dujardini* (tardigrade), *Acanthamoeba castellanii*, (amoeba), and *Acanthaster planci* (starfish) also matched to *Na*Atm1. Higher animals in the chordate phylum also matched to the bacterial gene, including *Python bivittatus* (snake) and multiple species of turtles (*Chrysemys picta bellii* and *Terrapene mexicana triunguis*) and birds (*Cyanistes caeruleus* and *Amazona aestiva*). The highest organism to show up in the results was a koala (*Phascolarctos cinereus*).[88]

Not surprisingly, several different fungi also appeared in the BLAST results. This was expected, as *Sc*Atm1p is a protein known to reside in the inner mitochondrial membrane and has high homology with *Na*Atm1p. Several species of *Aspergillus* contained proteins with similar sequences to *Na*Atm1p. Other fungi that appeared in the BLAST results include *Basidiobolus ranarum*, *Rhizophagus irregularis*, *Cercospora beticola*, *Emergomyces pasteuriana*, and *Paecilomyces variotii*.[88]

Organisms from the plant kingdom were the least represented when conducting a BLAST analysis that omitted all proteobacteria, as only red and green algae and mosses (*Physcomitrella patens* and *Selaginella moellendorffii*) appeared in the results. *Selaginella moellendorffii*, which is considered a primitive moss, is the plant with the highest homology. The lower representation of plants in the eukaryotic group most likely reflects the higher homology that several fungi and animals share with *Na*Atm1p, as fungi and animals are more closely related to each other than to plants. Additional evidence suggests that the Fe–S cluster assembly machinery in plants evolved from cyanobacteria, again based on the endosymbiotic theory.[107,108] The BLAST analysis suggests that plant genes related to Atm1p have diverged from bacteria more so than fungi and animal genes, as the moss *Selaginella moellendorffii* is considered to be a primitive plant.[88,103] This also explains the different roles for glutathione in mediating metal transport in bacteria, animals, plants, and fungi, as summarized earlier. In bacteria, animals, and fungi, glutathione is used by some proteins to transport glutathione-conjugated metals, but in plants, phytochelatins, a glutathione derivative, is the main mechanism by which metals are chelated and trafficked.

Conclusions

In conclusion, there appears to be clear evolutionary development of eukaryotic mitochondrial ABC-type exporters from bacterial heavy metal detoxification transporters, as expected from the endosymbiotic theory. Metal–glutathione complexation arose in bacterial cells as a means for heavy metal detoxification, by sequestration and efflux. In plants, phytochelatins, for which glutathione is a precursor, are used for heavy metal detoxification. The mode of heavy metal detoxification is more conserved in yeast and animals, as complex formation, followed by efflux through an ABC transporter, is similar to that of bacteria. Glutathione plays multiple roles in this process. In addition to binding to metals to reduce toxicity, it also facilitates recognition of the metal–glutathione complex through contacts between glutathione and the transporter. It would be evolutionarily unfavorable for heavy metal exporters to export metal ions themselves, as this would likely require multiple proteins, whereas similar metal–glutathione complexes can be recognized by the same protein. Proteins such as *Na*Atm1 contain multiple sites that can bind glutathione, providing flexibility to recognize several different types of compounds, including multiple metal–glutathione complexes. Following evolution from prokaryotes to eukaryotes, Atm1 was no longer required for the export of heavy metals, as it is located in the mitochondrial membrane. Instead, the binding pocket appears to have evolved to recognize a related molecular complex (glutathione-complexed iron–sulfur clusters) for export of these essential cofactors to the cytosol.

Fig. 5 Alignment of *Sc*Atm1p, *Na*Atm1p, and *Hs*ABCB7. *Sc*Atm1p, *Na*Atm1p, and *Hs*ABCB7 have high sequence identity, with *Na*Atm1p showing 42.74% and 45.39% sequence identity with *Sc*Atm1p and *Hs*ABCB7, respectively.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability

No new data were generated or analyzed in support of this research.

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