

Posttranslational modifications and metal stress tolerance in plants

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21.1 Introduction

On the basis of their density, heavy metals are categorized as metals with relatively high density. These include zinc, cadmium, copper, lead, etc. Heavy metals are placed in the d-block of the periodic table. Rapid industrialization, mining, and land use changes, including urbanization, have contributed to the widespread presence of these elements on the surface (Habitat, 2004). Heavy metals persist in the soil system for long durations due to their long half-life and stability (Jan et al., 2015) have led to heavy metals are now a major environmental concern as well (Ayangbenro and Babalola, 2017). Rai et al. (2019) have reviewed the impact of heavy metal contamination on crop productivity. Heavy metal contamination of soil threatens the food security and is a potential local and global issue (Gill et al., 2012; Tóth et al., 2016, Kaur et al., 2022). Moreover, the climate change scenario further enhances the farming challenges already posed by heavy metals and other stresses (Duchenne-Moutien and Neetoo, 2021). Due to these reasons, heavy metal-contaminated soils are a major focus of pollution assessment studies, which measure different indices such as index of geoaccumulation, enrichment factor, and contamination factor. Using the *Allium cepa* bioassay, arsenic and cobalt have been identified as contaminants of concern in Punjab, India (Kumar et al., 2021). The development of solutions to manage heavy metals in agricultural soils is, therefore, a necessity of the hour.

One option is phytoremediation, that is, the use of plants to reclaim contaminated soils. Oladoye et al. (2022) report that in spite of many potential hyperaccumulator plants being described annually, information on important parameters such as growth rate, biomass production, and metal tolerance remains scanty. Moreover, smallholder farmers of the developing world may not be economically capable of reclaiming their soil by the use of nonmarketable hyperaccumulator plants. Moreover, phyto- or bioremediation technologies would themselves come at a cost to the farmer. Similarly, Lal (2020) highlighted the importance of home

gardening and urban agriculture in the wake of COVID-19, while also citing heavy metal health risk issues. However, the deployment of such systems on a large scale would be an issue. Considering the above issues, it is important to develop resilient crop varieties, which can handle the stress induced by heavy metal exposure.

21.2 Effect of heavy metals on biomolecules

Heavy metals bring changes in the nature of various biomolecules in plants. [Ouariti et al. \(1997\)](#) demonstrated that cadmium and copper stress induces decrease in biomass accumulation and changes in fatty acid desaturation in tomatoes. Exposure to heavy metal alters the total and relative abundance of phospholipids ([Devi and Prasad, 1999](#)). In addition, the amount of unsaturation increases. Due to the metal-catalyzed generation of free radicals, lipid peroxidation happens. [Morsy et al. \(2012\)](#) demonstrated that heavy metal stress altered the composition and content of fatty acids in the plasma membrane of the root of *Zygophyllum album* and *Zygophyllum coccineum*, resulting in increased ratio of saturated to unsaturated lipids in the two species, implicating antioxidant activity and plasma membrane lipid homeostasis as important components to handle heavy metal stress. [Wagatsuma \(2017\)](#) proposed modulation of plasma membrane as a strategy to cope with environmental stress, particularly detrimental ionic conditions. Through the change in the composition of lipids and sterols in the bilayer, retardation of aluminum permeability has been proposed.

In terms of DNA, changes in the physical structure accompanied by cytotoxic and genotoxic effects have been observed for heavy metal stress exposure in plants ([Dutta et al., 2018](#)). The oxidative damage caused by heavy metals leads to an inhibition of DNA replication, gene expression, and cell division. [Das et al. \(2001\)](#) showed that RNA can be degraded by metal ions in vitro. Heavy metals are known to block aquaporin channels, limiting the availability of water in the cell ([Daniels et al., 1994](#); [MacIver et al., 2009](#)). [Yang et al. \(2016\)](#) showed that heavy metals can bind to intrinsic proteins of plasma membrane and replace calcium in the membrane, thereby disrupting normal physiology. On the other hand, [Fatemi et al. \(2020\)](#) demonstrated that the response of Pak Choi (*Brassica rapa*) differs on the basis of concentration of zinc applied, with low dose being beneficial for growth. This indicates that the sensitivity of various proteins toward heavy metals depends on the load of the metal on crop. [Tamás et al. \(2014\)](#) reviewed the effects of heavy metals on protein misfolding and aggregation. Proteins turn out to be the prime targets of heavy metal stress. Heavy metals cause protein function disruption by either complexation with functional side chain groups or displacement of essential metal ions. In addition, they are also known to impair the refolding of misfolded proteins in the cytoplasm. [Fig. 21.1A](#) provides a schematic representation of the effect of heavy metals on different biomolecules, followed by the general plant molecular response ([Fig. 21.1B](#))

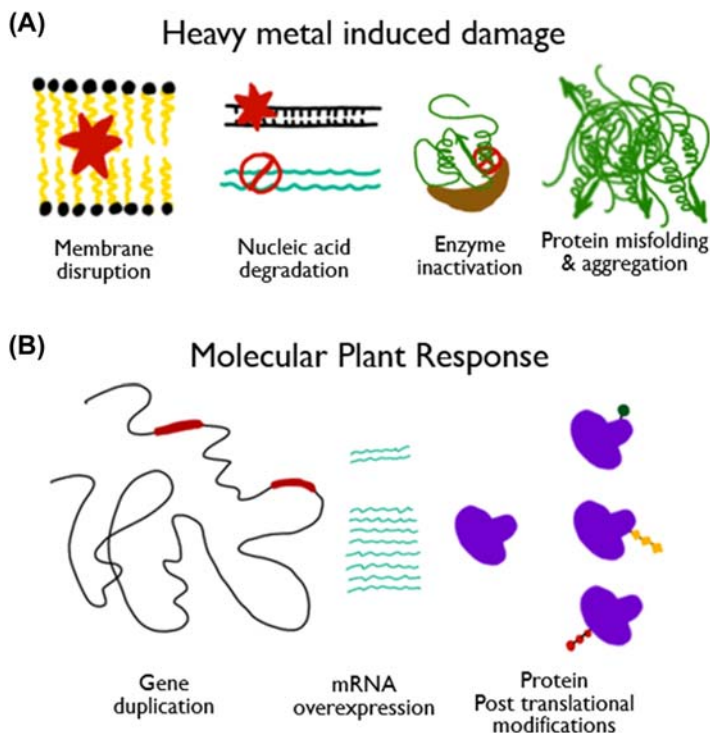


Figure 21.1 Overview of the effect of heavy metal on plant physiology. (A) Effect of heavy metal exposure on biomolecules. (B) General plant molecular response. Posttranslational modifications of proteins allow the generation of functional diversity and hence enhanced capacity to manage environmental stresses.

21.3 Plant molecular response to heavy metal toxicity

To cope with heavy metal stress, plants have devised various strategies. One strategy is chromosomal duplication of genes coding for heavy metal-associated proteins (HMPs). [Li et al. \(2020\)](#) reported gene duplications in rice and *Arabidopsis*. In rice, five pairs of clustered HMP genes are located mostly in chromosomes 1–4, whereas in *Arabidopsis*, six tandem duplications of HMPs distributed more evenly across the genome have been observed. Chromosomal duplications allow more quantity of HMPs in cell to cope with heavy metal stress. [Tian et al. \(2021\)](#) reported colinearity of natural resistance-associated macrophage proteins (NRAMPs) in different food crops, implicating conserved nature of the protein repertoire to deal with heavy metal stress.

[Neri et al. \(2020\)](#) reported the role of overexpression of aquaporin *aqual* mRNA in poplar to mitigate heavy metal stress. The authors demonstrated that *aqual* mRNA overexpression led to enhanced cadmium translocation. In addition, NRAMP3 was also upregulated and is known to have a role in compartmentalization of cadmium. [Gielen et al. \(2012\)](#) reviewed the role of microRNAs in transcriptional modification as a response to heavy metal stress.

21.4 Importance of posttranslational modifications

Posttranslational modification (PTM) is another potent strategy in the armor of plant defense systems against environmental stresses. Han et al. (2022) have reviewed its utilization for managing heat stress in plants. De Vega et al. (2018) have discussed the potential of PTM in priming plants for an enhanced immune response. Similarly, Gough and Sadanandom (2021) have reviewed the exploitation of PTM for disease resistance in plants. Matioli et al. (2022) have reviewed the role of PTM in plant carbon metabolism. Hence, the PTM of proteins is emerging as a new strategy in plant health management.

Fig. 21.2 provides a schematic representation of the metabolic advantages offered by a robust PTM system. Cellular physiology demands newer functionalities, dependent on the existent conditions. In the absence of PTM, old protein components

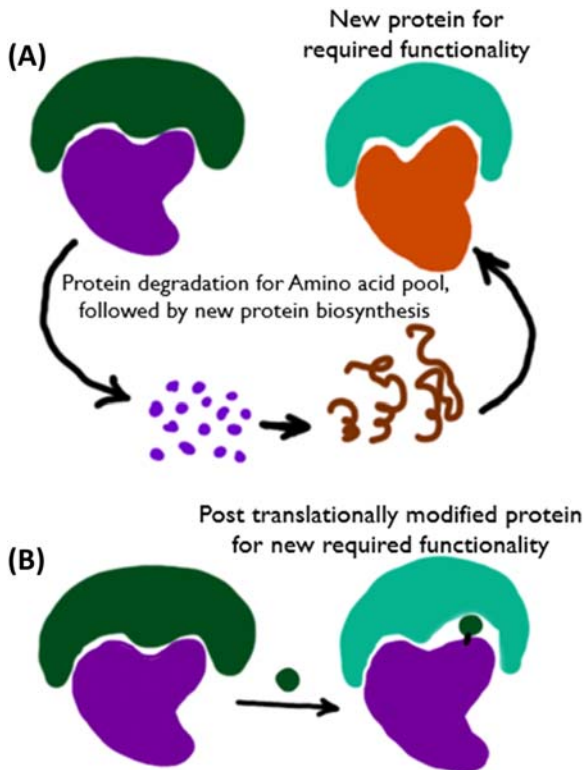


Figure 21.2 Illustrations of functional diversity resulting through posttranslational modifications (PTM). (A) Without PTM, to perform a molecular function dependent on cellular physiology, the old protein(s) would have to be degraded to maintain amino acid pools for the biosynthesis of new protein(s) with required functionality. (B) With PTM, many of the old protein(s) are modified by various functional groups to generate molecular specificities for the required functions.

would have to be degraded to release amino acids for the synthesis of new proteins. This would involve the whole protein biosynthetic machinery. In contrast, in the presence of PTM, much of the new function can be created by PTM of the old protein component, thus saving hugely on metabolic energy, especially important during different types of stresses. Apart from metabolic energy saving, PTMs confer changes of charge and/or structure, allowing new functionality in modified proteins. Different PTMs engage in cross talk with each other. The change induced by PTMs range from local-level stabilization or destabilization of structure to global events like order-disorder transitions.

21.5 Posttranslational modifications of proteins involved in stress response

21.5.1 Phytochelatins

Phytochelatins (PCs) are a family of peptide metal-binding ligands containing repetitions of the γ -Glu-Cys dipeptide followed by a terminal Gly; $(\gamma\text{-Glu-Cys})_n\text{-Gly}$. The length of the dipeptide, n , is generally between 2 and 5 and can go up to 11 in some cases. Apart from plants, some microorganisms have been reported to contain PCs. In the plant cell, low molecular weight PCs get complexed with heavy metals to become high molecular weight PCs, for eventual sequestration inside the vacuoles (Hasan et al., 2017). Glutathione has converted to PCs via enzyme PC synthase (Fig. 21.3). Mendoza-Cózatl et al. (2008) have demonstrated the ability of

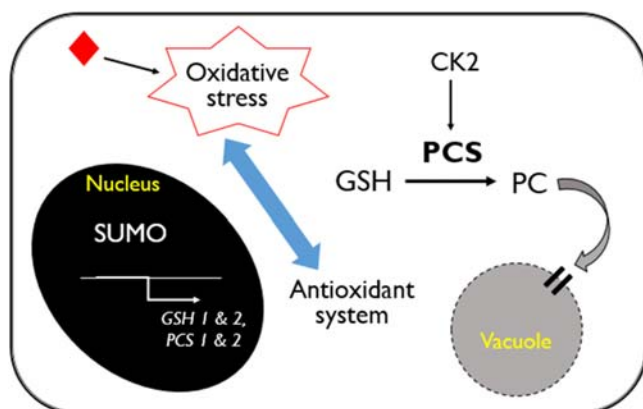


Figure 21.3 Mechanism of phytochelatins (PC) and its posttranslational modification (PTMs) in heavy metal stress. Glutathione (GSH) is converted to PC via phytochelatin synthase (PCS). PCS is regulated by CK2, glutathionylation, and affected by SUMOylation. The levels of GSH depend on the redox state of the cell, initiated by heavy metal oxidative burst and maintained by antioxidant system. Compartmentalization of high molecular weight-heavy metal phytochelatins inside vacuoles is necessary for metal tolerance.

PCs to facilitate long-distance transport of cadmium in *Brassica napus*. Some of the PTMs associated with PCs are:

21.5.1.1 Phosphorylation

In case of *Arabidopsis thaliana*, casein kinase 2 (CK2) catalyzes the phosphorylation of PC synthase, thereby increasing its activity (Wang et al., 2009). The activity of the enzyme decreases upon treatment with alkaline phosphatase. Phosphorylation is believed to assist the enzyme in achieving a functional conformation for enzyme activity.

21.6 S-glutathionylation

PCs are a group of low molecular-weight peptides involved in detoxification of heavy metals (Rodríguez-Ruiz et al., 2019; Bhat et al., 2021). Glutathione (GSH; γ -L-glutamyl-L-cysteinyl-glycine) is a nonprotein thiol compound, which, along with ascorbate, is the one of most abundant antioxidant molecules in plants (Foyer and Noctor, 2011). It is the major molecule involved in redox signaling (Zhang and Forman, 2012). Under oxidative conditions, glutathione gets oxidized to glutathione disulfide. Along with involvement in ascorbate–glutathione cycle and methylglyoxal (MG) detoxification, it is also involved in PC synthesis (Kharbech et al., 2020; Dorion et al., 2021; Bhat et al., 2021). Thus it has been suggested that Calderón et al. (2017) have suggested that glutathionylation/deglutathionylation is associated with changes in redox state during plant development or in response to stress conditions.

21.7 SUMOylation

SUMOylation is a PTM in which SUMO (small ubiquitin-like modifier) gets attached to substrate through various enzymes. In *A. thaliana*, SUMO E3 ligase SIZ1 has been shown to mediate tolerance against cadmium exposure via the glutathione-dependent PC synthesis pathway (Zheng et al., 2022). Augustine and Vierstra (2018) have reviewed the importance of SUMOylation in mediating effective nuclear response during stresses. The AtSIZ1 causes overexpression of GSH1 & 2, and PCS 1&2 to mount an effective plant response against heavy metal exposure.

The level of GSH is kept in balance with the oxidative burst caused by the heavy metal and its control via the antioxidant machinery of the plant cell. It has been shown that excess amounts of PCs do not improve metal tolerance (Furini, 2012). Along with chelating of the heavy metal, its sequestration, and compartmentalization in the appropriate organelles, like vacuoles are important for plant survival. Emamverdian et al. (2015) have opined that PCs and antioxidative machinery of the plant cell may act in synergy to strengthen the plant response against heavy metal stress.

21.8 Heavy metal transporters

Transporter proteins involved in heavy metal transport and cellular detoxification include heavy metal ATPases, ATP-binding cassette (ABC) transporters, ion permeases, NRAMPs, etc (Sharma et al., 2016). The information on PTMs for transporters specifically involved in heavy metal transport is scanty, though some information on plant transporters in other stresses is available. Spetea and Schoefs (2010) have studied solute transporters in thylakoid membranes under photosynthesis and light stress. Willems et al. (2019) have developed a database of plant PTMs. A number of PTMs for proteins involved in heavy metal in model and food crops are available in the database, yet their precise significance in the management of heavy metal stress remains to be elucidated on a large scale. Page and Feller (2015) have reviewed the transport and redistribution of heavy metals in crop plants.

On the other hand, in animals, the transporter systems have been widely studied due to their potential in drug interactions. Czuba et al. (2018) have reviewed PTMS in both solute carrier transporters and ABC transporters. It has been shown that phosphorylation acts as functional regulator and acts in ion gating, glycosylation provides protection from proteases and imparts stability, palmitoylation confers the ability of cross talk with other physiological processes and clustering in lipid rafts, while ubiquitin acts as degradative signal. Other PTMS like acetylation, especially for ABC transporters, are yet unexplored.

21.9 Metallothioneins

Metallothioneins (MTs) are cysteine-rich metal-binding proteins used for sequestration and detoxification of heavy metals in plants (Capdevila and Atrian, 2011). In contrast to the requirement of enzymatic process for the synthesis of PCs, MTs are the direct result of mRNA translation (Verkleij et al., 2003). MTs are known to be involved in metal ion homeostasis, detoxification, and protection against oxidative damage (Hossain et al., 2012). The key features that define MTs are intrinsic protein disorder (Wong et al., 2019) and low kinetic stability with high thermodynamic stability (Maret, 2000; Fig. 21.4).

The precise molecular function of MTs is still a matter of study, though they are associated with a number of physiological processes. It is believed that the ability of MTs to exchange metal ions readily with other proteins allows them to be a part of the signaling regime, ultimately leading to reactive oxygen species (ROS) scavenging (Hassinen et al., 2011). Four types of MTs have been defined as types 1, 2, 3, and 4 for roots, shoots, leaves, and seeds, respectively. There is differential affinity of various MTs toward metal ions.

Since MTs have an intrinsic protein disorder, the addition of chemical groups through various PTMs brings conformational changes in these. Bah and Forman-Kay (2016) have pointed out the diverse effects of different PTMs, ranging from

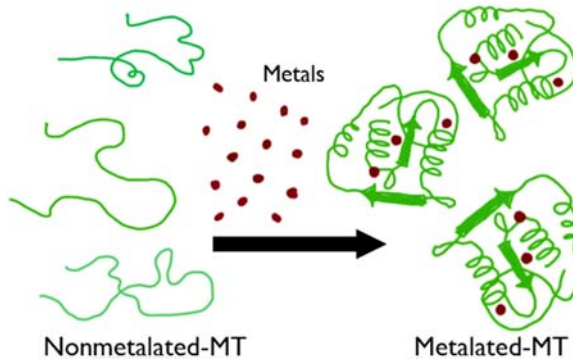


Figure 21.4 Metallothioneins have intrinsic disorder. Upon metal binding, the proteins acquire secondary structure, bind the metal ion tightly, and exchange them readily with other proteins.

stability changes at the secondary structure level to global protein structure-related transitions. PTMs can also guide complexation of proteins, resulting in order-to-disorder and vice versa transitions (Johnson and Lewis, 2001).

21.10 N-Ethylmaleimide modification

N-Ethylmaleimide (NEM) is a small compound that forms covalent thioether bonds with sulfhydryls (e.g., reduced cysteines). These are stable bonds and permanently prevent disulfide bond formation. Irvine and Stillman (2018) studied the effect of NEM modification on apo-MTs through liquid chromatography-mass spectrometry. The apo- and lightly alkylated species were found to be co-eluting, indicative of similar structural properties. With increase in alkylation, the elution differed, implicating structural changes. This study also indicated cooperative alkylation for NEM. The authors were able to achieve clear separation of highly similar MTs, differing by only +125 Da. This indicates the presence of secondary structure in MTs under different metalation states and has implications for metal release, which is vital to its functioning, especially as initiators of ROS scavenging.

21.11 Role of PTM in cellular redox mechanism

Reactive species of an element are highly reactive biomolecules that play an important role in cellular homeostasis. The most important reactive species are ROS, nitrogen, and sulfur species (Gill and Tuteja, 2010; Gruhlke and Slusarenko, 2012; Dai et al., 2015; Choudhury et al., 2017). Their biosynthesis takes place under any physiological condition (with or without stress), although under stress conditions, their production increases, and therefore their ability to initiate different cell signaling events and redox reactions with cellular components also increases.

21.12 Oxidation

Hydrogen peroxide carries the oxidation of cysteine residues in proteins, affecting the stabilization of structure and regulation of the catalytic activity (Bigelow and Squier, 2011). Hydrogen peroxide also oxidizes protein methionine residues (Vogt, 1995). In case the duration of ROS is long and intense, methionine oxidation leads to the generation of sulfoxides, which can be converted back to methionine by methionine sulfoxide reductases (MSRs). MSRs have been shown to play a protective role in plants, including the regulation of seed aging (Rey and Tarrago, 2018). MSRs are also known to preserve the activity of stress-related molecules such as glutathione-S-transferases and chaperones. MSRs are also involved in signaling via Ca^{2+} - and phosphorylation-dependent cascades, thus connecting ROS status to the signaling cascades. Hence, methionine oxidation may be seen as an initiator for the functionality of MSRs. Rinalducci et al. (2005) have implicated tryptophan oxidation as a possible pathway for binding synergy with antioxidant systems.

21.13 Carbonylation

Carbonylation is an irreversible PTM with certain amino acids residues such as threonine, lysine, arginine, or proline (Møller et al., 2011; Friso and van Wijk, 2015). The carbonylated proteins are ultimately degraded by the cellular proteolytic machinery (Polge et al., 2009).

21.14 Nitrosylation

S-nitrosylation is a PTM leading to attachment of nitrite groups to cysteine residues, leading to the formation of nitrothiols (Puyaubert et al., 2014; Feng et al., 2019). This PTM may lead to an increase or decrease in the activity of protein (Feng et al., 2019).

21.15 Nitration

This PTM involves addition of a nitrate group to the tyrosine residues in proteins, leading to the formation of 3-nitrotyrosine (Matamoros Galindo and Becana Ausejo, 2021). Formation of nitrotyrosine leads to loss of function and eventual degradation of the protein.

21.16 Guanylation

Affecting the cysteine residues, this PTM leads to an irreversible change in the state of a protein. Under the conditions of excess light, this PTM is associated with potential acclimation response in plants (Friso and van Wijk, 2015; Petřivalský and Luhová, 2020).

21.17 S-sulfhydration

This PTM is based on the modification of cysteine residues leading to persulfidation (Aroca et al., 2017). Thioredoxins can reverse this modification and this PTM may be an initiator for plant acclimation under stress, as it is known to modify not only the protein structure but its subcellular location as well (Paul and Snyder, 2012; Aroca et al., 2017).

21.18 Role of PTM in ubiquitin-proteasome system

The ubiquitin-proteasome system (UPS) is responsible for degradation of misfolded proteins in the cell to generate amino acid pool for new protein biosynthesis. UPS is a generalized plant response to various kinds of stresses. Here enzymes, namely, E1, E2, and E3 catalyze activation, conjugation, and ligation reactions, respectively, in sequence. This leads to attachment of ubiquitin, especially lysine residues, and marks the ubiquitinated proteins for degradation through the 26S proteasome system. Much work on the deployment of UPS during heat stress has been done (Han et al., 2022). However, the role of PTMs specific to heavy metal stress remains to be done in most crop species. Nevertheless, since UPS is a strategy common in most environmental stresses, it is expected that PTMs regulating the components of UPS in other stresses, including heat stress would share many commonalities with those involved during heavy metal exposure. UPS is known to provide metabolic flexibility and diversity in the response to various plant stresses (Sadanandom et al., 2012).

There is a far greater diversity of the E3 ligases than E1 and E2. Hence, it is at the level of E3 ligases that functional diversity of the physiological response to various conditions occurs (Salas-Lloret and González-Prieto, 2022). While a number of PTMs are known to affect mammalian E1 and E2 enzymes (Stephen et al., 1996; Block et al., 2001), the reports on PTMs of plant E1 and E2 enzymes are very scanty. Reports of the association of kinase activity with E2 enzymes in tomato (Mural et al., 2013) and rice (Chen et al., 2017) indicate that plant proteins are also likewise affected by PTMs as other organisms. Three classes of E3 ligases have been reported, namely, HECT (homologous to the E6-associated protein carboxyl-terminal) domain E3 ligases, RING (Really Interesting New Gene) E3 ligases, and RING-between-RING (RBR) E3 ligases (Rolfe et al., 1995; Deshaies and Joazeiro, 2009; Walden and Rittinger, 2018).

Although ubiquitination is itself a PTM, it also interacts with other PTMs to generate specific cellular response. PTMs have been reported to affect the amount of E2 protein as well as its ability to catalyze conjugation in the cells (Song and Luo, 2019). Similarly, the activity of E3 ligases is influenced by a number of PTMs. Reversible phosphorylation is by far the most prominent PTM. The coordinated phosphorylation of E3 ligase and ubiquitin has been shown to induce mitophagy (removal of damaged mitochondria through autophagy). Acetylation at lysine residues on positions 6 and 48 blocks the synthesis of poly-ubiquitin chains (Ohtake et al., 2015). Phosphorylation and acetylation introduce small-sized chemical moieties and affect the charge of the structure of ubiquitin. On the other hand, deamidation is an irreversible PTM and renders ubiquitin less suitable for polymerization. For example, in bacteria, the cycle inhibiting factor (Cif) deamidase carries this PTM, and the cells infected with pathogenic bacteria and modified by Cif protein cannot be restored and are eventually degraded through apoptosis (Samba-Louaka et al., 2009; Yao et al., 2012). Another PTM is ADP-ribosylation. This is carried by enzymes containing the ADP-ribosyltransferase domain, whereby the ADP-ribose moiety is transferred from NAD^+ to substrate proteins. This PTM allows the coupling of the cellular response to the status of NAD^+ in the cells (Yang et al., 2017).

21.19 Heavy metal-associated isoprenylation

While ROS and UPS are general plant responses to a variety of stresses, a physiological phenomenon specifically related to heavy metals and particularly found in vascular plants is heavy metal-associated isoprenylation. About one-third to half of all proteins interact with metal ions (Dupont et al., 2010). The proteins containing both the heavy metal-associated domain and the isoprenylation motif are referred to as heavy metal-associated isoprenylated plant proteins (HIPPs), also referred to as metallochaperones (de Abreu-Neto et al., 2013). Metallochaperones are soluble proteins, which bind metals and can exchange them with other proteins via protein–protein interactions (Robinson and Winge, 2010). It has been shown that HIPPs are also expressed during other stresses, indicating that significant crosstalk with other stressors is possible, though the relative abundance and composition of the molecular stress response may differ. Barth et al. (2009) demonstrated that HIPP29 protein in *A. thaliana* is located in nucleus and interacts with drought stress-related transcription factor ATHB29. Parasyri et al. (2022) showed the isoprenylated protein HvFP1 to be interacting with abscisic acid-based stress responses in barley. Zhao et al. (2013) showed that HIPP27 in *Arabidopsis* interacts with ubiquitin-specific protease 16, indicating crosstalk with other PTMs. In maize, the relative abundance of ZmHIPP modulates response to lead toxicity in soil (Ma et al., 2022). Rono et al. (2019) characterized OsHIPP42 expression in rice under normal and metal-stressed conditions. The authors reported that under normal conditions, the wild-type and mutant oshipp42 show no phenotypic differences and that the survival advantage of OsHIPP42 comes under metal-stressed conditions only. This indicates

that certain HIPPs may be involved exclusively in response to metal stress. [Manara et al. \(2020\)](#) showed that tomato HIPP26 interacts with metalloprotease inhibitor I under cadmium stress. These results implicated a role for HIPPs in metal ion compartmentalization. HIPPs have also been shown to be involved in the interaction of plants with microorganisms. It has been suggestive that the differences in the carboxyl termini of different HIPPs indicate their involvement in diverse processes and varied cellular locations. Metallochaperones transport metal ions through the plant system, along with other metal-binding peptides.

21.20 Hypusination

[Pálfi et al. \(2021\)](#) have reviewed a PTM of eukaryotic Initiation Factor 5A (eIF5A), hypusination, as an important determinant of development and stress-associated responses. Through the action of two enzymes, namely, deoxyhypusine synthase and deoxyhypusine hydroxylase, hypusine, a rare amino acid, is generated that modifies eIF5A. In rice, heavy metal stress is known to induce the expression of eIF5A-1 and eIF5A-2, ultimately leading to stress tolerance ([Chou et al., 2004](#)). In *A. thaliana*, eIF5A-2 is involved in response to cadmium exposure ([Xu et al., 2015](#)). It has also been found to affect the expression of genes coding for transporter proteins, along with detoxification and long-distance transport of heavy metals.

21.21 Other plant response mechanisms

Apart from the processes mentioned above, phytohormonal signaling and long-distance transport of metal ions are other important determinants in plant response to heavy metal exposure. [Sytar et al. \(2019\)](#) have reviewed the role of phytohormone priming in stress response. Phytohormones are involved in the regulation of the ascorbate–glutathione cycle, nitrogen assimilation and metabolism, and other physiological processes such as transpiration and cell division that have an overall impact on the plant metabolic status. Certain phytohormones specifically modulate the generation of ROS, induction of antioxidant mechanism, and photosynthesis ([Parwez et al., 2022](#)). Besides chemical priming, phytohormone priming is being investigated for its effects on the heavy metal stress response. [Peng and Gong \(2014\)](#) have reviewed the role of the vacuole in heavy metal sequestration and long-distance transport. The authors describe vacuolar sequestration capacity, regulated by interactions between the metal chelators in cytoplasm and transporters in the tonoplast, to be involved in long-distance transport of metal ions. This also has implications in effective breeding for the biofortification of crops.

In addition, plant growth-promoting fungi (PGPF) are involved in mitigating the stress associated with heavy metals on plants ([Geetha et al., 2022](#)). The PGPF secretome consists of secondary metabolites, including low molecular weight compounds such as organic compounds like glomalin, hydrophobins are reported to

result in detoxification and induction of systemic defenses. The role of PTMs on the above processes, namely, response to phytohormones, modulation of vacuolar sequestration and its impact on long-distance transport as well as plant interactions with growth-promoting fungi need to be elucidated to utilize them for understanding basic biology and deployment in the field.

21.22 Technologies to unravel posttranslational modifications and the specific stress response

Although a lot of information on the role of PTMs has been generated, the molecular layers of plant response, specifically to heavy metal stress remain to be fully deciphered. With the discovery of new players in the stress response, novel strategies to detect heavy metals, the molecular network that responds to it, including the PTMs, and the specificities of the otherwise general stress-responsive physiological process like the UPS are being devised. In terms of detection, MTs have been used in designing sensitive biosensors (Fig. 21.5).

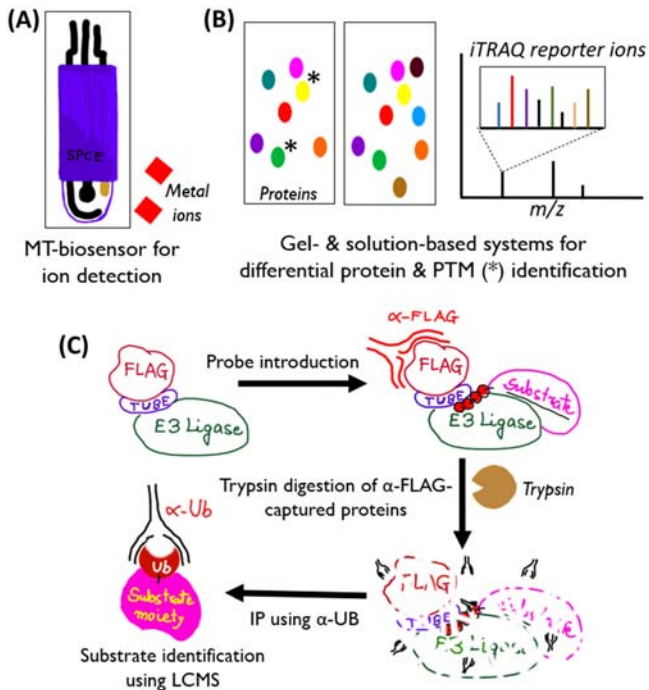


Figure 21.5 Emerging paradigms in research. (A) Utilization of metallothioneins in biosensors for heavy metal detection. (B) Gel- and solution-based electrophoresis for the identification of posttranslational modifications. (C) E3 substrate capture assay to identify UPS response specific to a physiological condition, such as, heavy metal stress.

Their inherent ability to bind metal ions tightly has been used to generate robust electrochemical sensors (Wu and Lin, 2004; Adam et al., 2005; Wu and Lin, 2004). In addition, sensors for the detection of MTs have also been described (Blastik et al., 2006; Dai et al., 2015).

To identify the proteins expressed differentially in response to heavy metal exposure as well as the PTMs, both gel-based and solution-based proteomics can be followed. Use of high-resolution 2D gel electrophoresis in the case of metal-stressed rice has been described (Hajduch et al., 2001). Han and Yang (2016) have described 2D gel electrophoresis for plant phosphoproteomics. 2D electrophoresis, including Difference Gel Electrophoresis and iTRAQ proteomics methodologies, provides high resolution in protein and PTM detection (Meleady, 2018; Lee et al., 2020; Baslam et al., 2020).

While PCs, MTs, and heavy metal-associated isoprenylated proteins have specific roles in relation to heavy metal stress exposure, identification of the key molecules in general stress-responsive physiological processes like UPS and ROS is not straightforward. A number of strategies such as ligase trapping, NEDDylator approach, UBAIT, and TULIP methods are now in place to identify the responsive components of ubiquitin pathway and have been reviewed in Salas-Lloret and González-Prieto (2022), yet their use to characterize plant response in relation to heavy metal exposure in plants remains to be done. Recently, Watanabe et al. (2020) described an elegant substrate capture strategy to identify the targets of E3 ligases (Fig. 21.5C). This involves creation of a probe containing FLAG-TUBE (tandem ubiquitin-binding entity)-E3 ligase and its introduction in the cell. The probe binds to the ubiquitinated substrate proteins. The bound complexes are first captured using anti-FLAG antibody. This is followed by trypsin digestion. The second capture is done using anti-ubiquitin antibody. The captured fragments are characterized using liquid chromatography-mass spectrometry. The use of this methodology would allow capturing of substrate proteins involved in the heavy metal stress response of plants.

21.23 Conclusion

PTMs are an important component in the plant repertoire to manage heavy metal stress. A better understanding of the specific PTMs associated with heavy metal stress tolerance must be obtained using the latest technologies. This should be followed by the selection of germplasm with PTM-enabled metabolic performance. Finally, the favorable alleles should be incorporated into elite varieties for incorporating the trait of heavy metal tolerance along with improved yield and nutritional characteristics. This would result in cost-effective solutions for the timely management of the heavy metal challenge of the global agricultural soils.

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