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ROLE OF PHYTOCHELATINS AND METALLOTHIONINS AS HEAVY METALS STRESS DEFENCE MECHANISM

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Introduction:

Heavy metals such as Cu and Zn rare essential for normal plant growth, although elevated concentrations of both essential and non-essential metals can result in growth inhibition and toxicity symptoms. Plants possess a range of potential cellular mechanisms that may be involved in the detoxification of heavy metals and thus tolerance to metal stress. These include roles for the following: for chelation of metals in the cytosol by peptides such as phytochelatins; for the repair of stress-damaged proteins; and for the compartmentation of metals in the vacuole by tonoplast-located transporters. Among the heavy metal-binding ligands in plant cells the phytochelatins (PCs) and metallothioneins (MTs) are the best characterized. PCs and MTs are different classes of cysteine-rich, heavy metal-binding protein molecules. PCs are enzymatically synthesized peptides, whereas MTs are gene-encoded polypeptides.

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MTs are cysteine-rich polypeptides encoded by a family of genes. In contrast, PCs are a family of enzymatically synthesized cysteine-rich peptides. In the search for MTs similar to those that had been characterized in animal species, early studies in plants repeatedly identified PCs. Like MTs in animals, PCs in plants are heavy metal-inducible, heavy metal-binding, cysteine-rich polypeptides, and in the absence of evidence for MTs in plants, it was initially suggested that PCs might be functionally analogous to MTs.

Phytochelatins

Chelation of metals in the cytosol by high-affinity ligands is potentially a very important mechanism of heavy-metal detoxification and tolerance. Potential ligands include amino acids and organic acids, and two classes of peptides, the phytochelatins and the metallothioneins The phytochelatins have been the most widely studied in plants, particularly

in relation to Cd tolerance. The phytochelatins (PCs) are a family of metal-complexing peptides that have a general structure (γ-Glu Cys)$_n$-Gly where *n*=2–11, and are rapidly induced in plants by heavy metal treatments. PCs are synthesized non-translationally using glutathione as a substrate by PC synthase an enzyme that is activated in the presence of metal ions. The genes for PC synthase have now been identified in *Arabidopsis* and yeast.

Recently, genes encoding for PC synthases in higher plants and yeast have now been identified, and it has been shown that the *Arabidopsis* gene could confer substantial increases in metal tolerance in yeast. The gene for PC synthase (*CAD1*) has been identified in *Arabidopsis* as well as an homologous gene in *Schizosaccharomyces pombe* ,a mutant of the latter with a targeted deletion of this gene was PC-deficient and Cd-sensitive. To compare the involvement of PCs in metal detoxification, the sensitivity of the *cad 1–3* mutant was tested for sensitivity to a range of heavy metals in both *Arabidopsis* and *S. pombe*. PCs appeared to be important in the detoxification of Cd and arsenate, but played no role in the detoxification of Zn, Ni and selenite ions. In contrast to the *S. pombe* mutant, *cad 1–3* showed slight sensitivity to Cu and Hg. A possible role for PCs in Cu tolerance had also been suggested (Salt *et al*., 1989) from studies on copper-tolerant *Mimulus guttatus*; exposure to Cu in the presence of buthionine sulphoximine (BSO), a potent inhibitor of γ-glutamyl-cysteinyl synthetase, caused a considerable reduction in root growth that was not seen in the presence of inhibitor alone. However, in contrast, when Cu-sensitive and Cu-tolerant ecotypes of *Silene vulgaris* were exposed to concentrations of Cu giving either no or 50% inhibition of growth for each ecotype, they showed equal PC synthesis in the root tips ,it was concluded that differential Cu tolerance in *S. vulgaris* does not rely on differential PC production. Thus the role of PCs in Cu tolerance remains to be resolved. An involvement of PCs in arsenate tolerance has also been proposed.

PC synthase genes were isolated simultaneously by three research groups using different approaches. Two groups used expression of Arabidopsis and wheat cDNA libraries in *S. cerevisiae* to identify genes [*AtPCS1* and *TaPCS1*, respectively] conferring increased Cd resistance. The third group identified *AtPCS1* through the positional cloning of the *CAD1* gene of Arabidopsis . A similar sequence was identified in the genome of *S. pombe*, and targeted deletion mutants of that gene are, like Arabidopsis *cad1* mutants, Cd sensitive and PC deficient, confirming the analogous function of the two genes in the different organisms. Heterologous expression of the *CAD1*/*AtPCS1* and *SpPCS* genes or purification of epitope-tagged derivatives of SpPCS and AtPCS1 was used to demonstrate both were necessary and sufficient for GSH-dependent PC biosynthesis in vitro. This combination of genetic, molecular, and biochemical data was a conclusive demonstration that these genes encode PC synthase.

There is a second PC synthase gene, *AtPCS2*, in Arabidopsis with significant identity to *CAD1*/*AtPCS1* . This was an unexpected finding because PCs were not detected in a *cad1* mutant after prolonged exposure to Cd, suggesting the presence of only a single active PC synthase in wildtype. *AtPCS2* is transcribed, and expression experiments have demonstrated it encodes a functional PC synthase enzyme (C. Cobbett & A. Savage, unpublished data). The physiological function of this gene remains to be determined. In most tissues *AtPCS2* is expressed at a relatively low level compared with *AtPCS1*. However, because AtPCS2 has

been preserved as a functional PC synthase through evolution, it must presumably be the predominant PC synthase in some tissue(s) or environmental conditions, thereby conferring a selective advantage. Full-length or partial cDNA clones encoding presumptive PC synthases have also been isolated from other plant species, including *Brassica juncea* and rice.

Metallothioneins

Higher plants contain two major types of cysteine-rich, metal-binding peptides, the metallothioneins (MTs) and the phytochelatins. MTs are gene-encoded polypeptides that are usually classified into two groups. Class 1 MTs possess cysteine residues that align with a mammalian (equine) renal MT; Class 2 MTs also possess similar cysteine clusters but these cannot be easily aligned with Class 1 MTs. MT genes have now been identified in a range of higher plants including *Arabidopsis* where, in addition to Class 1 and Class 2 MT genes, MT3 and MT4 types have been recognized .Other species are also thought to contain an extensive MT gene family and more than one class of MT gene, while expression studies have revealed tissue-specific patterns. In plants, there is a lack of information concerning the metals likely to be bound by MTs, although Cu, Zn and Cd have been the most widely .Although MTs can be induced by Cu treatments and there is evidence for a role in heavy metal tolerance in fungi and animals, the role of MTs in heavy metal detoxification in plants remains to be established. However, it has been reported that MT2 mRNA was strongly induced in *Arabidopsis* seedlings by Cu, but only slightly by Cd and Zn; when genes for MT1 and MT2 from *Arabidopsis* were expressed in an MT-deficient yeast mutant, both genes complemented the mutation and provided a high level of resistance to Cu. van Vliet *et al*. showed that MT genes can be induced by Cu, and that the expression of MT2 RNA is increased in a Cu-sensitive mutant of *Arabidopsis* that accumulates high concentrations of Cu. 10 ecotypes of *Arabidopsis* were surveyed and a clear correlation between the Cu sensitivity of seedlings and the expression of MT2 RNA was shown (Murphy and Taiz, 1995*a*, *b*). Clearly more evidence is needed to establish a relationship between Cu sensitivity and MT production. By contrast, in a study of the effects of Cd exposure on *Brassica juncea*, it was reported that MT2 expression was delayed relative to PC synthesis and they argued against a role for MT2 in Cd detoxification. Thus the role of MTs remains to be established. They could clearly play a role in metal metabolism, but their precise function is not clear; they may have distinct functions for different metals . Alternatively, they could function as antioxidants, although evidence is lacking, while a role in plasma membrane repair is another possibility.

What are the functions of MT genes in plants?. Reconciling all the available data on plant MTs into a simple model may be impossible and may also be unrealistic given the diverse family of MT genes in plants. However, there is evidence to support the hypothesis that MTs are involved in copper tolerance and homeostasis in plants: Some plant MTs are functional copper-binding proteins; expression of some MT genes is induced by copper; MT gene expression in senescing leaves is coordinated with a set of genes involved in copper homeostasis; the level of expression of a Type 2 MT gene correlated closely with copper tolerance in a group of Arabidopsis ecotypes, expression of a Type 2 MT gene is elevated in a copper-sensitive mutant that accumulates copper, more recently, copper tolerant populations of *S. vulgaris* have been shown to have higher RNA expression and gene copy number of a

Type 2 MT gene. In addition, PCs do not provide tolerance to copper in Arabidopsis, indicating that another mechanism, perhaps involving MTs, must be involved. While supporting a role for MTs in copper tolerance, this evidence is not conclusive.

Summary:

The potential for the use of plants for the detoxification or "phytoremediation" of polluted environments is being increasingly examined. The manipulation of PC expression is one potential mechanism for increasing the capacity of plants for phytoremediation. Understanding the effect of the over expression, possibly in a tissue-specific manner, of the genes of the GSH/PC biosynthetic pathway on metal tolerance and accumulation will soon lead to indications as to their usefulness in this endeavor. Here too, genes controlling other aspects of PC function may be required.

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