

Commentary

Heavy metals and plants – model systems and hyperaccumulators

Understanding how plants accumulate and store metal ions is relevant to two important applications: metal nutrition – for humans and in agriculture; and metal detoxification – using plants as biological detoxification systems for the ‘phytoremediation’ of metal contamination in the environment. Metal ions, such as Cu, Fe, Zn and Se, are essential nutrients for which deficiencies in animal and human diets cause significant disorders. These, in addition to other non-essential metals, such as Cd, Hg, Pb, Al and As, can cause toxicities when present in excess. Knowing how organisms regulate essential metal ion metabolism will contribute to improvements in essential metal ion nutrition for animals and humans. Similarly, knowledge of the mechanisms by which metals, both essential and nonessential, can be sequestered, stored and detoxified in various organisms may contribute to the optimisation of phytoremediation processes (Clemens *et al.*, 2002). In plants these two aspects of metal metabolism have been described as opposite sides of the same coin (Guerinot & Salt, 2001).

This issue of *New Phytologist* highlights research in the field of metal metabolism and detoxification in plants. It includes a number of articles spanning the breadth of research in the field, from reviews of nonplant model organisms used to inform aspects of plant biology, to the genetic and physiological analysis of metal hyperaccumulation and tolerance, to investigations of ecological aspects of metal accumulation. Often neglected is an understanding, not of the mechanisms of metal tolerance, but of the precise mechanisms of metal toxicity. This is illustrated in a Tansley review by Rengel & Zhang (pp. 295–314), which provides comprehensive coverage of the effects of Al toxicity on the important functions of Ca ions in plants. Other papers explore the potential use of poplars in phytoremediation (see Di Baccio *et al.*, pp. 443–452), important ecological aspects of metal accumulation and toxicity, particularly the influence of metal hyperaccumulation on herbivory (Huitson & Macnair, pp. 453–459, Hanson *et al.*, pp. 461–469) and a rare study of developmental asymmetry as a possible measure of environmental metal toxicity (Batterham *et al.*, 471–477). Here, I will focus on recent developments in the molecular genetic analysis of metal metabolism in plants.

Arabidopsis and other model organisms

Numerous aspects of plant biology, not least metal metabolism, have advanced dramatically with the adoption of *Arabidopsis thaliana* as a model organism for molecular genetic studies, especially over the past 15 years. Genes important in various aspects of metal metabolism have been identified using a range of approaches. Among these the complementation of mutants in yeast or *E. coli* and the identification of genes homologous to those from other organisms known to be involved in metal metabolism have been prominent. With the completion of the *Arabidopsis* genome sequence inventories of various gene families known or expected to be involved in aspects of metal metabolism have been compiled (Maser *et al.*, 2001). One such family, the heavy metal transporting P-type ATPases, well studied in prokaryotes, yeast and mammals, is reviewed in this issue (Cobbett *et al.*, pp. 315–321). Insights into the roles of different members of such gene families can come from studies of differential expression, both tissue-specific and in response to environmental stimuli. This is illustrated here with a study of the expression of members of the metallothionein (MT) gene family in *Arabidopsis* (Guo *et al.*, pp. 369–381). Individual T-DNA or transposable element mutations have been identified in many *Arabidopsis* genes and we are well on the way to having a (nearly) complete set of mutants representing a knock-out of every known gene in the genome. Thus, mutants are (or will soon) be readily available for investigating roles for particular genes in metal metabolism in *Arabidopsis*.

More mutants

There is no question that the identification of *Arabidopsis* genes through sequence comparisons with genes involved in metal metabolism in other organisms or by their capacity functionally to complement particular yeast or *E. coli* mutants has vastly increased our understanding of metal metabolism in plants. As a result, however, many plant genes, and now their corresponding knock-out mutants, have been studied based on what we know of metal metabolism in other organisms. Such approaches may overlook plant-specific functions. This is not entirely true for the functional complementation of mutants where new classes of genes carrying out complementary functions can be identified. However, for the most part, the outcome of such selections is limited by the mutants available in the single-celled host. It is interesting that the foremost strength of *Arabidopsis* as a model organism, the ease with which mutants can be identified solely on the basis of phenotype, has been used relatively little in the analysis of

aspects of metal metabolism and detoxification. There are few published examples where mutants with a phenotype have been isolated, let alone used to identify new genes important in, and possibly specific to, metal metabolism in plants. Strategies for identifying mutants for the analysis of metal metabolism and detoxification have included screens for mutants with increased or decreased resistance or assays for changes in metal content independent of phenotype (Murphy & Taiz, 1995; Delhaize, 1996; Larsen *et al.*, 1996). New approaches for large-scale screens for mutants with altered metal ion content are being developed (Kraemer, 2003). Although these 'forward' genetic approaches have been limited thus far, and few mutants have led to the identification of the genes involved, there have been promising examples where functions not previously identified from another organism have been discovered. In one example, mutants constitutively expressing ferric reductase, identified by direct staining for root-specific enzyme activity, led to the isolation of a member of a family of efflux proteins (Rogers & Guerinot, 2002). In another example, the identification of Cd-sensitive mutants deficient in the biosynthesis of the heavy metal binding peptides, phytochelatins (PCs), led to the isolation of the PC synthase gene (Ha *et al.*, 1999).

Nonplant models inform plant biology

For greater understanding of aspects of metal metabolism and detoxification at the cellular level unicellular model systems are being more extensively developed. In addition to *S. cerevisiae*, which continues to yield data relevant to many other systems, studies with *Schizosaccharomyces pombe* and the unicellular green alga, *Chlamydomonas reinhardtii*, are being used to reinforce those in *Arabidopsis* (Clemens & Sim, pp. 323–330; Hanikenne, pp. 331–340). *S. pombe* is of particular interest because, unlike *S. cerevisiae*, it expresses PCs as a major mechanism of Cd-detoxification. *S. pombe* thus allows the relationships between the roles of PCs and other potential mechanisms of metal detoxification or metabolism to be explored in greater detail at a cellular level. Although PCs are also expressed in *Chlamydomonas*, a more significant advantage of this organism is as a single-celled photosynthetic organism in which the role of essential metal metabolism, particularly Cu and Fe, in photosynthesis can be explored.

Molecular genetics and plant physiology

The molecular genetics of model organisms can lend much to our understanding of the basic components of essential metal metabolism in plants. Already numerous functions in Cu, Zn, and Fe metabolism have been identified. By contrast, a review by Hacisalihoglu & Kochian (pp. 341–350) examines aspects of Zn efficiency in crop plants. This review

highlights the depth of physiological and biochemical analysis and the sparsity of genetic and molecular tools in this field, and the authors argue for a molecular genetic model, perhaps in *Arabidopsis*, to reinforce these studies. Many studies in *Arabidopsis* use its strengths in molecular and genetic analysis but may lack the depth and sophistication of physiological analysis. As we move beyond the era of gene identification, clearly determining the roles of various functions involved in metal metabolism in plants will require a concerted and sophisticated physiological analysis of *Arabidopsis* mutants. No doubt some species are more amenable to physiological analysis and others are more amenable to molecular genetics. However, I suspect the technical difficulties in using *Arabidopsis* for physiological studies are less of an impediment than the absence of a sufficiently strong alliance between molecular geneticists and plant physiologists in pursuing these outcomes.

Metal hyperaccumulators ...

Much attention is being paid to metal accumulating plants that may be used for the phytoremediation of contaminated soils (Baker & Whiting, 2002). Some plants can accumulate remarkable levels of metals – 100–1000-fold the levels normally accumulated in most species. Metal hyperaccumulating species have been identified in at least 45 plant families and individual species (or even populations) can accumulate different metals such as Zn, Cd, Cu, Co, Ni, Se and As or particular combinations of these (Reeves & Baker, 1999). New metal hyperaccumulating species or populations continue to be identified (Kraemer, 2003). This issue includes studies of Zn, Cu and Ni accumulation and tolerance in different *Thlaspi caerulescens* accessions (Assunção *et al.*, pp. 411–419) and Se uptake and tolerance in a range of species (Goodson *et al.*, pp. 391–401). A focus of other recent publications in *New Phytologist* has been the identification of As hyperaccumulator species (Meharg, 2002; Zhao *et al.*, 2002; Bleeker *et al.*, 2003; Meharg & Hartley-Whitaker, 2002).

Physiological, biochemical and in some cases molecular approaches continue to be applied to identifying the underlying mechanisms of metal hyperaccumulation and/or tolerance in these species. For example, we know from work in model species that PCs are essential for Cd and As detoxification *in vivo*. This has led to studies investigating whether or not PCs are responsible for Cd and/or As accumulation or tolerance in some hyperaccumulators (Zhao *et al.*, pp. 403–410; Hartley-Whitaker *et al.*, 2001; Ebbs *et al.*, 2002). Hyperaccumulation implies mechanisms of metal tolerance and these two characters may be genetically distinguishable in a hyperaccumulator species (Assunção *et al.*, pp. 383–390, in this issue; Bert *et al.*, 2003). A thorough genetic analysis of different hyperaccumulator species or populations

may allow the genetic basis for both hyperaccumulation and tolerance to be teased apart. The genetics of these traits may be complex (Pollard *et al.*, 2002). One challenge will be in establishing (near) uniform approaches for the measurement of the various parameters used to define metal tolerance and accumulation in order to make sensible comparisons across different studies with the same or related organisms.

... and new model species

Some hyperaccumulator species, including *Arabidopsis halleri* and *Thlaspi caerulescens*, are members of the Brassicaceae family. The availability of genomics and functional genomics tools in *Arabidopsis thaliana* has led a number of groups to develop these related species as new models for the molecular genetic analysis of metal hyperaccumulation and tolerance. In this issue, Assunção *et al.* (pp. 351–360) review the advantages of *Thlaspi caerulescens* as a model organism. In a parallel vein Peer *et al.* (421–430) have assessed a panel of *Brassica* species and accessions – mainly *Thlaspi* – according to a number of criteria in order to determine their likely suitability as new model species. These criteria include growth habit, flowering time, self-fertility, seed set, genome size and transformation efficiency. This work also points to *T. caerulescens* as best fulfilling those criteria. Personally, I am excited by the prospect of developing hyperaccumulator species as new models to study the genetic basis for hyperaccumulation and tolerance. What might we expect to learn from such new models?

The fundamental functions underlying metal accumulation and tolerance are likely to be no different in, say, *Thlaspi* than *Arabidopsis thaliana*. Essentially the same suite of genes will function in both and the physiological differences will likely depend on differences in patterns of gene expression and protein activity. This assumption clearly underlies the application of *Arabidopsis* cDNA arrays to the analysis of gene expression in related *Brassica* species. Nonetheless, particular gene duplications allowing the evolution of variant (although not fundamentally different) functions may be important, as has been observed with the amplification of an MT gene in a Cu-tolerant population of *Silene vulgaris* (van Hoof *et al.*, 2001). In addition, new models may identify mechanisms that cannot be identified through the isolation or characterisation of mutants in *Arabidopsis* because they play a relatively minor role in the latter and mutants have no discernible phenotype or alternatively require mutation of two or more genes to generate a phenotype. It is also important to consider that, in terms of understanding the fundamental basis for metal hyper-accumulation and/or tolerance, perhaps no one hyper-accumulating *Brassica* species is a 'model' for any other. It is likely that different species, or even populations, have evolved different mechanisms for metal hyperaccumulation and tolerance.

The isolation of mutants in a genetically defined background will be a fruitful approach in these new models as it is in *Arabidopsis*. Indeed, a high frequency of T-DNA transformation using applications of *Agrobacterium* to intact plants is an important criterion in the adoption of these models. A challenging task will be to distinguish between functions that may be essential for hyper-accumulation and the primary genetic difference/s that may underlie the hyperaccumulation trait. For example, increased expression of a root-specific Zn transporter, as observed in the hyper-accumulator *T. caerulescens* (Pence *et al.*, 2000), may be essential for hyper-accumulation. However, this may be caused by a genetic difference that results, for example, in altered expression of a transcriptional regulator influencing the expression of a suite of Zn-responsive genes. Distinguishing between these will require a thorough analysis of the genetic basis of hyper-accumulation and tolerance independent of, but integrated with, mutant identification.

Manipulating metal accumulation and tolerance for phytoremediation

Knowledge of the basic processes of metal transport, accumulation and tolerance in both plants and other organisms is leading to ingenious strategies for the manipulation of these traits in transgenic plants with the aim of maximising the capacity of plants for phytoremediation. Various strategies have been reviewed recently (Pilon-Smits & Pilon, 2002). For example, Dhankher *et al.* (2002) have used the combined expression of bacterial enzymes involved in glutathione biosynthesis and arsenate reduction significantly to enhance both arsenic accumulation and tolerance. A parallel paper from the same group in this issue (Dhankher *et al.*, pp. 431–441) demonstrates that the expression of arsenate reductase unexpectedly results in accumulation and tolerance of Cd.

Future prospects

Our knowledge of various aspects of metal metabolism in plants is increasing steadily, enhanced by the use of current model systems. However, I argue that greater use can be made of the 'forward' genetic approach through the isolation and analysis of mutants based solely on phenotype. The increasing identification and study of the remarkable natural variation in the capacity of plants to accumulate and tolerate metals is and will continue to provide a wealth of information to further enhance our understanding of these processes. The application of molecular genetic tools to new model organisms among these hyperaccumulating species and the more sophisticated application of physiological experimentation to current models such as *Arabidopsis* will further enhance these endeavours. Collectively, these approaches can

be expected to lead to new applications in the areas of mineral nutrition and phytoremediation.

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References

- Assunção AGL, ten Bookum WM, Nelissen HJM, Vooijs R, Schat H, Ernst WHO. 2003a. Differential metal-specific tolerance and accumulation patterns among *Thlaspi caerulescens* populations originating from different soil types. *New Phytologist* 159: 411–419.
- Assunção AGL, ten Bookum WM, Nelissen HJM, Vooijs R, Schat H, Ernst WHO. 2003b. A co-segregation analysis of Zn accumulation and Zn tolerance in the Zn hyperaccumulator, *Thlaspi caerulescens*. *New Phytologist* 159: 383–390.
- Assunção AGL, Schat H, Aarts MGM. 2003c. *Thlaspi caerulescens*, the ideal model species to study heavy metal hyperaccumulation in plants. *New Phytologist* 159: 351–360.
- Baker AJM, Whiting SN. 2002. In search of the Holy Grail – a further step in understanding metal hyperaccumulation. *New Phytologist* 155: 1–4.
- Bert V, Meerts P, Saumitou-Laprade P, Salis P, Gruber W, Verbruggen N. 2003. Genetic basis of Cd tolerance and hyperaccumulation in *Arabidopsis halleri*. *Plant and Soil* 249: 9–18.
- Bleeker PM, Schat H, Vooijs R, Verkleij JAC, Ernst WHO. 2003. Mechanisms of arsenate tolerance in *Cytisus striatus*. *New Phytologist* 157: 33–38.
- Clemens S, Palmgren MG, Kraemer U. 2002. A long way ahead: understanding and engineering plant metal accumulation. *Trends in Plant Science* 7: 309–315.
- Clemens S, Simm C. 2003. *Schizosaccharomyces pombe* as a model for metal homeostasis in plant cells: the phytochelatin-dependent pathway is the main Cd detoxification mechanism. *New Phytologist* 159: 323–330.
- Cobbett CS, Hussain D, Haydon MJ. 2003. Structural and functional relationships between type 1B heavy metal-transporting P-type ATPases in *Arabidopsis*. *New Phytologist* 159: 315–321.
- Delhaize E. 1996. A metal-accumulator mutant of *Arabidopsis thaliana*. *Plant Physiology* 111: 849–855.
- Dhankher OP, Li YJ, Rosen BP, Shi J, Salt D, Senecoff JF, Sashti NA, Meagher RB. 2002. Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and gamma-glutamylcysteine synthetase expression. *Nature Biotechnology* 20: 1140–1145.
- Dhankher OP, Shasti NA, Rosen BP, Fuhrman M, Meagher RB. 2003. Increased cadmium tolerance and accumulation by plants expressing bacterial arsenate reductase. *New Phytologist* 159: 431–441.
- Di Baccio D, Tognetti R, Sebastiani L, Vitagliano C. 2003. Responses of *Populus deltoides* × *P. nigra* (*P.* × *euramericana*) I-214 to high zinc concentrations. Implications for phytoremediation. *New Phytologist* 159: 443–452.
- Ebbs S, Lau I, Ahner B, Kochian L. 2002. Phytochelatin synthesis is not responsible for Cd tolerance in the Zn/Cd hyperaccumulator *Thlaspi caerulescens* (J. & C. Presl). *Planta* 214: 635–640.
- Goodson CC, Parker DR, Amrhein C, Zhang Y. 2003. Soil selenium uptake and root system development in plant taxa differing in Se-accumulating capability. *New Phytologist* 159: 391–401.
- Guerinot ML, Salt DE. 2001. Fortified foods and phytoremediation. Two sides of the same coin. *Plant Physiology* 125: 164–167.
- Guo W-J, Bundithya W, Goldsbrough PB. 2003. Characterization of the *Arabidopsis* metallothionein gene family: Tissue-specific expression and induction during senescence and in response to copper. *New Phytologist* 159: 369–381.
- Ha S-B, Smith AP, Howden R, Dietrich WM, Bugg S, O'Connell MJ, Goldsbrough PB, Cobbett CS. 1999. Phytochelatin synthase genes from *Arabidopsis* and the yeast, *Schizosaccharomyces pombe*. *The Plant Cell* 11: 1153–1164.
- Hacisalihoglu G, Kochian LV. 2003. How do some plants tolerate low levels of soil zinc? Mechanisms of zinc efficiency in crop plants. *New Phytologist* 159: 341–350.
- Hanikenne M. 2003. *Chlamydomonas reinhardtii* as a model for studies of heavy metal homeostasis and tolerance in plants. *New Phytologist* 159: 331–340.
- Hanson B, Garifullina GF, Lindblom SD, Wangeline AL, Ackley A, Kramer K, Norton AP, Lawrence CB, Pilon-Smits EA. 2003. Selenium accumulation protects *Brassica juncea* from invertebrate herbivory and fungal infection. *New Phytologist* 159: 461–469.
- Hartley-Whitaker J, Ainsworth G, Vooijs R, ten Bookum W, Schat H, Meharg AA. 2001. Phytochelatin is involved in differential arsenate tolerance in *Holcus lanatus*. *Plant Physiology* 126: 299–306.
- van Hoof NALM, Hassinen VH, Hakvoort HWJ, Ballintijn KF, Schat H, Verkleij JAC, Ernst WHO, Karenlampi SO, Tervahauta AI. 2001. Enhanced copper tolerance in *Silene vulgaris* (Moench) Garcke populations from copper mines is associated with increased transcript levels of a 2b-type metallothionein gene. *Plant Physiology* 126: 1519–1527.
- Huitson SB, Macnair MR. 2003. Does zinc protect the zinc hyperaccumulator *Arabidopsis halleri* from predation by snails? *New Phytologist* 159: 453–459.
- Kraemer U. 2003. Phytoremediation to phytochelatin – plant trace metal homeostasis. *New Phytologist* 158: 4–6.
- Larsen PB, Tai CY, Kochian LV, Howell SH. 1996. *Arabidopsis* mutants with increased sensitivity to aluminum. *Plant Physiology* 110: 743–751.
- Maser P, Thomine S, Schroeder JI, Ward JM, Hirschi K, Ze H, Talke IN, Amtmann A, Maathuis FJM, Sanders D, Harper JF, Tchieu J, Gribskov M, Persans MW, Salt DE, Kim SA, Guerinot ML. 2001. Phylogenetic relationships within cation transporter families of *Arabidopsis*. *Plant Physiology* 126: 1646–1667.
- Meharg AA. 2002. Arsenic and old plants. *New Phytologist* 156: 1–4.
- Meharg AA, Hartley-Whitaker J. 2002. Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. *New Phytologist* 154: 29–43.
- Murphy A, Taiz L. 1995. A new vertical mesh transfer technique for metal-tolerance studies in *Arabidopsis* – ecotypic variation and copper-sensitive mutants. *Plant Physiology* 108: 29–38.

- Peer WA, Mamoudian M, Lahner B, Reeves RD, Murphy AS, Salt DE. 2003. Development of a model plant to study the molecular genetics of metal hyperaccumulation. Part I: Germplasm analysis of 20 Brassicaceae accessions from Austria, France, Turkey, and USA. *New Phytologist* 159: 421–430.
- Pence NS, Larsen PB, Ebbs SD, Letham DLD, Lasat MM, Garvin DF, Eide D, Kochian LV. 2000. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proceedings of the National Academy of Sciences USA* 97: 4956–4960.
- Pilon-Smits E, Pilon M. 2002. Phytoremediation of metals using transgenic plants. *Critical Reviews in Plant Sciences* 21: 439–456.
- Pollard AJ, Powell KD, Harper FA, Smith JAC. 2002. The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences* 21: 539–566.
- Reeves RD, Baker AJM. 1999. Metal accumulating plants. In: Ensley BD, Raskin I, eds. *Phytoremediation of toxic metals: using plants to clean-up the environment*. New York, USA: John Wiley & Sons, 193–229.
- Rengel Z, Zhang W-H. 2003. Role of dynamics of intracellular calcium in aluminium toxicity syndrome. *New Phytologist* 159: 295–314.
- Rogers EE, Guerinot ML. 2002. FRD3, a member of the multidrug and toxin efflux family, controls iron deficiency responses in *Arabidopsis*. *The Plant Cell* 14: 1787–1799.
- Tan-Kristanto A, Hoffmann A, Woods R, Batterham P, Cobbett C, Sinclair C. 2003. Translational asymmetry as a sensitive indicator of cadmium stress in plants: a laboratory test with wild-type and mutant *Arabidopsis thaliana*. *New Phytologist* 159: 471–477.
- Zhao FJ, Dunham SJ, McGrath SP. 2002. Arsenic hyperaccumulation by different fern species. *New Phytologist* 156: 27–31.
- Zhao F-Z, Wang JR, Barker JHA, Schat H, Bleeker PM, McGrath SP. 2003. The role of phytochelatin in arsenic tolerance in the hyperaccumulator *Pteris vittata*. *New Phytologist* 159: 403–410.

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