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Metallothioneins may not be enough – the role of phytochelatins in invertebrate metal detoxification

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How do animals respond to environmental pollution by potentially toxic elements (PTEs), and what detoxification pathways do they use? This is a key question: not only to understand the fundamental biological responses of organisms in contaminated environments, but also to assess if these responses can also have practical uses, for instance as biomarkers of pollution.

Metallothioneins have been especially widely studied in this context. Metallothioneins are small cysteine-rich proteins that strongly bind soft metal ions[1] – indeed, they were originally incorrectly believed to be cadmium-requiring enzymes, as they sequester cadmium so readily from the environment. They have repeatedly been shown to be strongly metal-inducible in many different animal species, and there is ample evidence (again, in multiple species) that knocking out metallothioneins reduces tolerance to PTEs such as cadmium.[2]

However, it is also clear that metallothioneins alone are not the sole players in detoxification, and that metallothioneins have many biological roles beyond detoxification[1]. As a result, the baseline variability of metallothioneins in the natural environment can be high, which can also complicate their use as biomarkers of pollution.[2] What other biological systems are involved in responses to PTEs? There is growing evidence that phytochelatins may be important in many different animal species. Phytochelatins, like metallothioneins, are cysteine-rich peptides; unlike metallothioneins, they are not genetically encoded, but are non-ribosomal peptides produced from glutathione by the enzyme phytochelatin synthase (PCS).[1] Originally thought to be found only in plants and yeast, PCS genes have since been found in species that span almost the whole animal tree of life (with some important exceptions, such as the phylum Arthropoda, and – mentioned here for reasons of parochial interest – the sub-phylum Craniata). Biochemical studies have also shown that these PCS genes are functional: the *Caenorhabditis elegans* PCS enzyme produces phytochelatins when it's expressed in an appropriate host, and knocking out the gene increases the sensitivity of *C. elegans* to cadmium.[1]

However, do phytochelatins have real-world relevance to PTE detoxification in animals? For *C. elegans*, at least, the answer is clear: phytochelatins are produced *in vivo* after exposure to cadmium, and – at least for cadmium – they are more important than metallothioneins, as knocking out the PCS gene has an even bigger effect on cadmium lethality than knocking out the metallothionein genes.[3] Could phytochelatins turn out to be of general importance for dealing with PTEs across many animal species? The PCS protein is generally constitutively expressed, and it has a very high turnover rate, so it can respond quickly to sudden increases in metal ion concentrations.[1] This suggests a possible functional interaction with metallothioneins for PTE detoxification: phytochelatins would be synthesized rapidly on exposure to PTEs, and could play a holding role, mopping up free metal ions until the (relatively slow) induction and synthesis of metallothionein proteins. Metallothioneins could then take over the main detoxification role.

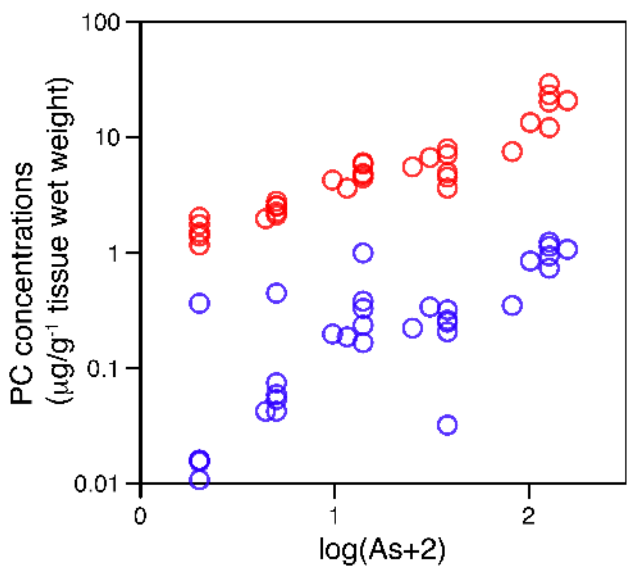
Sadly, real life appears to be less straightforward. Recently, the PCS from the human parasite *Schistosoma mansoni* was also shown to synthesize phytochelatins when cloned into yeast. This extended the number of animal phyla containing species with confirmed functional PCS enzymes to two, Platyhelminthes and Nematoda. However, and contrary to our simplistic metal-detoxification hypothesis, *S. mansoni* doesn't synthesize phytochelatins when exposed to the classic inducer cadmium[4] – maybe phytochelatins are here playing an alternative biological role, such as maintaining metal homeostasis or scavenging free radicals.[4] Other studies have also shown that phytochelatins and metallothioneins may have different specificities and hence different ecological functions – for example, in plants, metallothioneins may be more important for detoxifying copper and phytochelatins for detoxifying cadmium.[1]

Clearly we need more information on what might happen in other animals, ideally from different taxonomic groups, and exposed to a range of different metal ions. A recent study has now given us a third data point: the earthworm *Lumbricus rubellus* (phylum Annelida) produced phytochelatins in response to arsenic, both in laboratory exposures (Figure 1) and in worms sampled from contaminated field sites.[5] Admittedly, this means we still only have data from three phyla, but it does demonstrate that phytochelatin responsiveness to PTEs is not unique to nematodes.

What are the implications for environmental scientists? Metallothioneins have been very widely studied, and phytochelatin responses in plants have also been widely studied, but so far there is barely a double-handful of papers relevant to phytochelatins in animals. We argue that studying phytochelatin responses in animal species, and their interactions with metallothioneins, should be an important future goal. PCS genes are found in animal species from eight phyla so far that we are aware of. These include species that are widely used in environmental toxicity testing and environmental monitoring – e.g. the oyster *Crassostrea gigas*, as well as nematodes and earthworms. Given the taxonomic spread of the PCS gene, and the decreasing cost of sequencing a novel genome, we have no doubt that many more animals with PCS genes will be identified in the future. Phytochelatins can be analysed relatively easily by liquid chromatography coupled to an appropriate detector. This direct analysis of the peptides themselves is probably necessary, as so far there is little evidence that phytochelatin responses are mediated by gene expression.[5] Hence, at least for species that are known or suspected to contain PCS genes, studying metallothionein responses alone to PTEs may not be enough to understand metal handling: knowledge of phytochelatin responses may also be needed to complete the picture.

We propose four questions for future research: which animal species with PCS genes make phytochelatins in response to PTEs? What happens to metal ions once they have been bound by phytochelatins? Do phytochelatins interact with metallothioneins to help detoxify PTEs? And could phytochelatin levels potentially be used as biomarkers of environmental pollution? Answering these questions would be an important step forward in understanding how pollution by PTEs affects key invertebrate species in the environment.

Figure 1. The metabolites phytochelatin-2 (red symbols, $(GluCys)_2Gly$) and phytochelatin-3 (blue symbols, $(GluCys)_3Gly$) both increase in a dose-responsive manner to 28-day soil exposure to arsenic in the earthworm *Lumbricus rubellus*. Figure originally published in Liebeke et al.[5]



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