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Editorial

The Need for Integrative Models of Metal Transport and Transfer in Marine Invertebrates

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Anthropogenic pollution continues to be a major stressor of concern in the fields of environmental science and ecology. Although major point-source discharges have been curbed, ecosystems continue to be incrementally degraded by non-point sources. Metals in particular are continuing to bioaccumulate and impact the physiology and metabolism of a variety of terrestrial and aquatic organisms.

A great deal of this ecotoxicological research has been done on the uptake, bioaccumulation, organ distribution, and toxicity of various metals in marine invertebrates. Although metals are known to bioaccumulate from both food and water, the sub-cellular processes involved are poorly understood. Some individual mechanisms have been studied, including transmembrane transport of metal ions via channels and carriers [1], intracellular binding of metals to metallothioneins and Ca concretions [2,3], and ion regulation within extracellular fluids (Na^+, K^+, Ca^{2+}) [4]. Because very few studies have simultaneously examined two or more of these processes, we currently lack an integrative model of metal regulation and transport. This is particularly needed for the largest group of invertebrates – those that possess circulatory systems (annelids, molluscs, arthropods, echinoderms, chordates and some coelomate worms). For this group, we are faced with a major gap in our knowledge: we know little about the fundamental process(es) whereby metals are *transported* in invertebrate circulatory systems and selectively *transferred* to tissues where the metals are either needed or sequestered/eliminated. Differential distribution of metals to various organs implies selective and coordinated mechanisms involving metal recognition (likely linked to metal speciation), protein-to-protein communication, transmembrane metal transfer, and intracellular processing. Our understanding of these processes, and the coordination among them, is quite limited. The long-term goal of my research group is to better understand the processes of metal circulatory transport and transfer to tissues of invertebrates with blood systems. For practicality however, our work has been limited to two phylogenetically different species of marine bivalve molluscs (the blue mussel *Mytilus edulis* and the quahog *Mercenaria mercenaria*), and to a subset of metals (primarily Cd, but also Ca and Pb) that are each differentially accumulated by different bivalve organs. Bivalves were chosen because they are ecologically important (often dominant in marine benthic environments and able to filter seawater of contaminant-laden particles), and economically important (commercial and subsistence fisheries). Cd was chosen because it has a low natural background concentration in bivalve blood (< 0.01 µg/g whole blood [5,6], and it out-competes (i.e. binds more strongly) the more abundant essential metals such as Zn and Ca. Nevertheless, our findings likely have relevance to other metals and other bivalve orders, classes of molluscs and invertebrate phyla.

Over the past 15 years, our laboratory has identified and characterized what we have shown to be a major plasma metal binding protein in bivalve molluscs – histidine-rich glycoprotein (HRG) [7-9]. HRG is a ~63 kDa, polymorphic N-glycosylated dimer with 10-11 % carbohydrate (mostly hexose, with ~17 % fucose) [7]. It accounts for ~60 % of all the plasma proteins present in the blood plasma of seven marine bivalve species we have so far examined (ELISA analysis) [10]. HRG's 13.6 % histidine content accounts for its divalent metal-binding properties [7], although an additional Ca binding site has been predicted from primary structure [11]. HRG can bind a variety of Class A (Ca, Mg), Class B (Hg, Pd) and Borderline metals (Cd, Ni, Zn; IMAC experiments [13], biological metal classification of Nieboer and Richardson [12]). Cd binds to two types of sites (conditional log Ks = 7.65 and 5.41 M^{-1}; ISE titration experiments) [9]. The vast majority of the Cd (and Ca) in bivalve blood is *not* free, but is bound to HRG [7,14]. The wealth of information that we have amassed on HRG provides us with a strong foundation on which to now address the more involved questions on how various metals are recognized and then transferred to specific tissues of marine bivalve molluscs.

Our initial findings have shown that mussel HRG is involved in a metal transfer mechanism that rapidly moves Cd from blood to kidney [8]. Figure 1 depicts our current conception of this mechanism. Briefly, HRG may be synthesized in hemocytes [15] and either released into the blood as an apoprotein or else complexed with cations such as Ca or Zn prior to release ①. Since Cd has a higher affinity for histidine groups than either

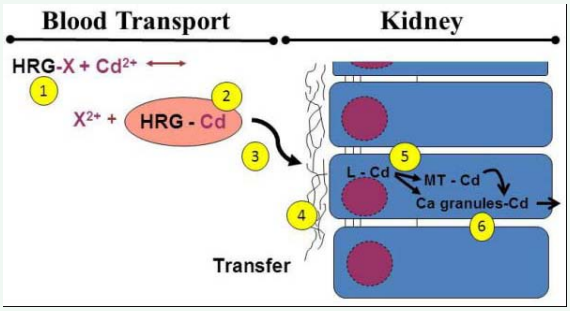

Figure 1 Model of Cd circulatory transport, recognition and transfer to kidney cells across the basal lamina in marine bivalve molluscs.

Ca or Zn, it replaces these metals forming an HRG-Cd complex ②. Our experimental results indicate that the HRG-Cd complex is differentially recognized by kidney cells ③, not by cells of other tissues), and is involved in the rapid transfer of Cd (and possibly Ca and other metals) into kidney cells. We hypothesize that this complex is recognized by glycosaminoglycans (GAGs) situated on the basal lamina of the kidney epithelium ④. Upon recognition, either the HRG-Cd complex is transferred intact into the kidney cells, or the Cd is transferred from HRG to a membrane transporter, leaving the HRG in the blood. Once inside kidney cells, Cd ⑤ becomes incorporated into both cytosolic metallothionein and Ca concretions ⑥ [16-20].

This conceptual model has been an invaluable tool, helping us to formulate hypotheses and design experiments to fill in the gaps between what we already know and what we need to address. Ultimately we hope to move from a purely conceptual model to a quantitative model. We urge others to think of metal uptake, transport, transfer, sequestration, metabolism and elimination holistically, and to develop the integrative models that will enhance our mechanistic understanding of metal processing in a much broader range of marine invertebrate species.

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