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Review

Transcriptomic underpinning of toxicant-mediated physiological function alterations in three terrestrial invertebrate taxa: A review

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Environmental toxicology and transcriptomics in soil macroinvertebrates.

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ABSTRACT

Diverse anthropogenic activities often lead to the accumulation of inorganic and organic residues in topsoils. Biota living in close contact with contaminated soils may experience stress at different levels of biological organisation throughout the continuum from the molecular-genetic to ecological and community levels. To date, the relationship between changes at the molecular (mRNA expression) and biochemical/physiological levels evoked by exposures to chemical compounds has been partially established in a limited number of terrestrial invertebrate species. Recently, the advent of a family of transcriptomic tools (e.g. Real-time PCR, Subtractive Suppressive Hybridization, Expressed Sequence Tag sequencing, pyro-sequencing technologies, Microarray chips), together with supporting informatic and statistical procedures, have permitted the robust analyses of global gene expression changes within an ecotoxicological context. This review focuses on how transcriptomics is enlightening our understanding of the molecular-genetic responses of three contrasting terrestrial macroinvertebrate taxa (nematodes, earthworms, and springtails) to inorganics, organics, and agrochemicals.

1. Introduction

Responses to environmental stressors (van Straalen, 2003; Korsloot et al., 2004), including potentially toxic chemical compounds, are inevitably accompanied by changes in gene-expression profiles in the receptor organisms. Some responses are the direct consequence of toxicosis, other responses are indirect and compensatory (Ankley et al., 2006). The latter are homeostatic or restorative, reflecting either the repair of molecular damage or the upregulation of compromised target pathways, so that house-keeping functions are maintained, possibly with a fitness trade-off.

The term "toxicogenomics" was originally formulated to describe the application of genomics to toxicology (Nuwaysir et al., 1999), and rapidly diversified to encompass "ecogenomics" (Chapman, 2001) and "ecotoxicogenomics" (Snape et al., 2004) where the novel genomics tools were mobilized to address ecological (Chapman et al., 2006; Snoeren et al., 2006; Roelofs et al., 2007a) and environmental stress (Steinberg et al., 2008; Wang et al., 2008) questions. "Omics" technologies comprise multi-platform transcriptomics, proteomics, and metabolomics (van Straalen and Roelofs, 2006; Poynton et al., 2008), methods that are increasingly deployed cooperatively to good effect in 'systems biology' approaches (Miracle and Ankley, 2005; Bundy et al., 2008; Lemos et al., 2010). During the last decade, suites of molecular-genetic tools have been developed that allow the analysis of the transcriptomes of stressed organisms under laboratory and field exposure conditions (van Straalen and Roelofs, 2006; Hogstrand and Kille, 2008). Transcriptomics has progressed in step with advances in DNA sequencing capacity such that it is now routinely feasible to design and fabricate microarrays representing a high proportion of the genes in the whole genomes of even non-model, ecologically significant, organisms. Parallel informatic and statistics advances (Yu et al., 2004) allow the extraction of information from the multivariate datasets of unprecedented richness to describe how functionally interdependent networks of genes respond in concert to environmental toxicant perturbations (Menzel et al., 2009). In other words, it is now possible to profile discriminately the pattern of differential gene expression (i.e. which genes are upregulated, which are down-regulated, and which are unaltered), including interactive effects (Chapman et al., 2006), in organisms at known life-cycle phases under defined exposure conditions.

The high degrees of congruence observed between target gene expression levels measured by quantitative polymerase chain reaction results and microarray profiling (Nota et al., 2008; Owen

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et al., 2008; Wang et al., 2008) is most encouraging. It implies that high-density expression profiles can characterize disturbances in physiological pathways caused by exposures to individual chemical compounds with different intrinsic properties and biological affinities (e.g. metals and metalloids, and diverse organic residues, such as polycyclic aromatic hydrocarbons (PAHs), pesticides, and herbicides). Microarray-based transcriptomics offers the unparalleled advantage of tracking the activities of many annotated (and, depending on the subject species, many unannotated) genes simultaneously. Thus, this technological approach has the potential to be a tool of choice in ecotoxicology (Roh et al., 2009). Whilst the shortcomings of the biomarker approach to environmental risk assessment should not be underestimated (Forbes et al., 2006, 2008), transcriptomics can be anticipated in the near future to make important contributions within a tiered framework of environmental diagnostics (Ankley et al., 2006). These will probably include: (i) pinpointing exposures to specific classes of chemical stressors even without an analytical inventory of the local chemical contaminants; (ii) identifying the main stress-mediating factors in 'real world' contaminated samples; (iii) determining whether a measured chemical contaminant is bioavailable to the extent that its presence exerts a detectable change in the transcriptome profile; (iv) characterizing and deconvoluting the modes of action of the 'active ingredients' of chemical mixtures in abiotic field samples; (v) comparing the genomic responses of different species and ecotypes in and across a series of contaminated habitats.

Natural habitats, particularly soils, are spatially and compositionally heterogeneous at both local and landscape scales. The challenges that transcriptomics faces in delivering ecological risk assessment tools for terrestrial ecotoxicology are formidable, and will doubtless necessitate using an accumulated body of robust data derived from controlled laboratory exposures to assist in the interpretation of data derived from field populations. The advent of high-throughput sequencing technologies (Sundquist et al., 2007) will facilitate these initiatives by expanding present genomic knowledge on hitherto 'neglected' species. The present review brings together in an accessible fashion the state of knowledge on three different taxa of ecophysiologically-contrasting macroinvertebrates (nematodes, earthworms, and springtails) that each live in more-or-less intimate contact with soil. The overarching aim was to provide a heuristic perspective for the future development of transcriptomics-driven ecotoxicological monitoring. The review focuses mainly on the effects induced by three classes of contaminants: metals, PAHs, and agrochemicals (i.e. pesticides and herbicides), with some commentary where published data are available on metallo-nanoparticles and high explosive compounds.

2. Expression profile analyses in terrestrial invertebrates

2.1. Exposure to metals

Metals are the most studied chemical compounds in terrestrial invertebrates. Whilst metals modulate the transcriptional activity of a number of various genes, the expression of genes encoding members of the cysteine-rich metallothionein (MTs) family are strongly induced by S-seeking metals, such as Hg, Ag, Cu, Cd, and Zn, as well as the metalloid As^{3+}. MTs have been identified in all invertebrate species that have been examined. Analysis of their expression levels following exposure to metals has also been widely performed, with isoforms of MT genes displaying time- and dose-dependent upregulation of expression in various phyla. Thus, in the nematode species *Caenorhabditis elegans* (Freedman et al., 1993), the springtail *Orchesella cincta* (Insecta; Collembola) (Timmermans et al., 2005), the snail *Helix pomatia* (Mollusca; Gastropoda) (Chabicovsky et al., 2003) and the oligochaete annelid species *Lumbricus rubellus* (Stürzenbaum et al., 1998b; Galay-Burgos et al., 2003), *Lumbricus terrestris* (Asensio et al., 2007) and *Eisenia fetida* (Gruber et al., 2000; Demuynck et al., 2006, 2007), genes encoding MTs are considered to be good biomarkers of metal, especially Cd, exposure.

2.1.1. Nematodes

Caenorhabditis elegans is frequently used not only as model in biomedicine but also in environmental toxicology (Leung et al., 2008) because: (i) nematodes in general are widely distributed, species-rich, and often the most abundant animals in soils; (ii) the species has a short generation time, and is easily bred in laboratory; (iii) it is sensitive to various chemical compounds; and, (iv) its entire genome has been sequenced (*C. elegans* Sequencing Consortium, 1998; see also NEMABASE3 at http://www.nematodes.org/nemabase3/). With a fully sequenced genome and a well characterized biology, it is feasible with *C. elegans* to seek linkages between molecular-genetic responses and whole organism perturbations i.e. to pursue the principle of 'phenotypic anchoring' (Ankley et al., 2006; Tyler et al., 2008). A recent pilot study (Menzel et al., 2009) of the responses of *C. elegans* to three freshwater sediments contaminated to different degrees by mixtures of metals and organic residues illustrates not only the site-to-site discrimination power of global transcriptomics, but also the possibilities of linking toxic responses at different levels of biological organisation.

Liao and Freedman (1998) using Differential Display Reverse Transcription Polymerase Chain Reaction (DDRT-PCR) identified 32 *C. elegans* genes that were differentially expressed during exposure to 100 μM of $CdCl_2$ for 8 or 24 h. Amongst the key Cd-responsive genes identified in this targeted study were *type 1 metallothionein* (*mtl-1*), cadmium-responsive gene (*cdr*), heat shock protein (*hsp 70*), *dna gyrase*, *pyruvate carboxylase*, *β-adrenergic receptor kinase*, and genes encoding *collagen* (Table 1). A number of similar studies (Clemens et al., 1999; Ha et al., 1999; Dong et al., 2005) also assayed the expression levels of a small number of genes implicated directly in Cd sequestration or Cd-mediated cellular damage. In contrast, Cui et al. (2007) performed a microarray analysis of the global transcription profile change occurring in nematodes during short term (4 and 24 h) 100 μM Cd exposures. These authors observed a cluster of 290 differentially expressed genes (237 upregulated and 53 down-regulated) at both time intervals, and bioinformatic data interrogation using Gene Ontology (GO) (Ashburner et al., 2000) revealed that most of the genes upregulated after 4 h Cd exposure were involved in metal homeostasis (location and transport) pathways. Predominant amongst the functionally active genes participating in these pathways were *mtl-1*, *mtl-2*, *cdr-1*, and *pcs-1*. A study performed in 2005 by Novillo et al. confirmed the responsiveness of *cdr-1* and *mtl-2* (and *collagen*) over a protracted exposure period of 7 days to a range of Cd lower concentrations (0.1, 1 and 10 μM). On the basis of their own observations, Cui et al. (2007) postulated the following temporal pattern of Cd response: initial (i.e. well within 24 h) transcriptional adjustment to maintain ion homeostasis and energy metabolism, followed later (i.e. after approximately 24 h) by the activation of genes involved in proteolysis in response to accumulated damaged proteins contemporaneous with the down-regulation genes regulating cellular trafficking and fatty acid metabolism.

The involvement of the *cdr-1* gene in the Cd response is of particular interest because it has been shown to encode a lysosomal membrane protein (CDR-1) in the intestinal cells of *C. elegans* (Liao et al., 2002). RNA interference (RNAi) silencing of *cdr-1* demonstrated that the gene is essential for the development of *C. elegans* under Cd exposure (Liao et al., 2002). Moreover, the observation of fluid accumulation in the body of *C. elegans* deprived of *cdr-1* expression indicates an involvement in osmoregulation, possibly