

Environmental Epigenetics in Soil Ecosystems

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One of the major emerging concerns within ecotoxicology is the effect of environmental pollutants on epigenetic changes, including DNA methylation, histone modifications, and non-coding RNAs. Epigenetic mechanisms regulate gene expression, meaning that the alterations of epigenetic marks can induce long-term physiological effects that can even be inherited across generations. Many invertebrate species have been used as models in environmental epigenetics, with a special focus on DNA methylation changes caused by environmental perturbations (e.g., pollution). Among soil organisms, earthworms are considered the most relevant sentinel organisms for anthropogenic stress assessment and are widely used as standard models in ecotoxicological testing of soil toxicity.

Keywords: earthworms ; epigenetics ; DNA methylation

1. Epigenetics

The research field of epigenetics, directly translated as “above genetics” explores the non-genetic inheritance of variation in gene functions (phenotypes) occurring via mitosis or meiosis and describes the mechanisms behind this inheritance. Epigenetic phenomena are mediated through epigenetic marks which include DNA methylation, histone modifications, and non-coding RNAs ^[1]. These epigenetic marks shape the chromatin structure and are interconnected in a network that dictates the gene expression ^[2]. The best studied epigenetic mechanism is DNA methylation, which is a naturally occurring alteration that involves a methyl group addition to the cytosine (at the fifth carbon atom). This process involves the DNA methyltransferase (DNMT) enzyme which adds the methyl group (CH₃) from S-adenosyl-L-methionine (SAM). The presence or absence of methyl groups can affect the coiling of DNA around histones and therefore the accessibility and binding of transcription factors ^[3]. DNA methylation most often occurs within C-G nucleotide pairs (CpG dinucleotides). This process is well-studied in vertebrates, which have highly methylated genomes with up to 70–80% of CpG methylation. On the other hand, invertebrate genomes are mostly sparsely methylated exhibiting a mosaic pattern of methylation with most of the methylation restricted to gene bodies (gene body methylation-gbM) and silenced repetitive elements ^[4]. The level of DNA methylation in invertebrates ranges from the complete absence or very low levels of DNA methylation in *Caenorhabditis elegans* and dipteran insects to high levels of methylation in some species of sponges ^{[4][5][6]}.

The main role of DNA methylation in vertebrates is the silencing of inactive regions in the genome (transposable and viral elements). Unmethylated CpGs in vertebrates are mostly located in gene promoter regions within so-called CpG islands and are responsible for the regulation of gene transcription. In invertebrates, the prevalent type of CpG methylation is gbM ^[7]. GbM in invertebrates regulates transcriptional activity, alternative exon splicing, repression of intragenic promoter activity and reduces the efficiency of transcriptional elongation ^{[8][9][10][11]}. In invertebrates, gene bodies are either heavily or sparsely methylated which has been linked to high or low expression of genes, respectively ^[12]. It has been hypothesized that gbM could promote the predictable expression of essential genes for basic biological processes and modulate gene expression plasticity ^{[8][13]}. Furthermore, heavily methylated genes have been described as highly and broadly expressed, whereas lowly methylated genes are tissue-specific ^[12]. Across different invertebrate taxa, it was evident that genes with housekeeping functions, and constitutive and ubiquitous functions tend to be more methylated than those with inducible functions ^[13]. For example, in corals, weak methylation patterns increased the gene expression plasticity in response to environmental stress. It was speculated that the potential mechanism behind this phenomenon could be that weak methylation allows greater access to alternative transcription start sites, enabling in this way flexibility in gene expression plasticity ^[14]. This flexibility in gene expression was also confirmed in marbled crayfish, where gbM was found to be linked to stable gene expression and lowly methylated genes showed increased variation in expression levels ^[15]. Moreover, housekeeping genes of marbled crayfish that are unexpressed have low methylation of gene bodies, whereas those with moderate gene expression have higher levels of gbM ^[16]. Functional analyses in oysters revealed that the high expression level of genes correlates with high methylation ^{[17][18]}. Furthermore, hypermethylated genes in oysters

are enriched for biological processes related to metabolism and housekeeping functions, whereas hypomethylated genes are associated with developmental processes, cellular communication, and adhesion [19].

2. Environmental Epigenetics

Environmental epigenetics studies the cause-effect relationships between various environmental factors such as nutrition, temperature, exposure to pollutants, etc., and the epigenetic modifications and changes of the organism phenotypes. These modifications can enable the adaptation of organisms to novel environmental conditions. However, in some cases, changes in epigenetic marks can lead to detrimental phenotypic endpoints, can persist for a long time, and even be heritable [20]. Since epigenetic marks respond to environmental pollution resulting in potentially heritable effects, those marks have been suggested as suitable candidates for the development of biomarkers of environmental exposure [20][21].

Changes in epigenetic marks, mainly DNA methylation, under various environmental stressors have been studied in different invertebrate taxa, including polychaetas, insects, corals, mollusks, and crustaceans, as summarized by several review articles [22][23][24]. Among these, the most data of high resolution obtained through WGBS are available for *D. magna*, corals and mollusks. In *D. magna* WGBS approach detected alterations in DNA methylation patterns in individuals exposed to gamma irradiation and the toxic cyanobacterium *Microcystis aeruginosa* [25][26]. In coral *Stylophora pistillata* exposed to low-pH environments, WGBS revealed changes in pathways regulating cell cycle and body size and suggested that DNA methylation enables fine-tuning of gene expression as a response to changing environmental conditions [27]. In pacific oysters *Crassostrea gigas* exposed to the herbicide diuron, an intergenerational epigenetic effect was revealed using a whole genome approach. Changes in DNA methylation patterns within coding sequences of unexposed mussels, coming from exposed genitors were revealed, indicating DNA methylation as an important pathway in phenotypic changes induced by environmental pollution [28].

Although invertebrates have much lower levels of DNA methylation in comparison to vertebrates, they present promising models for environmental epigenetic studies. Additionally, their wide distribution, accessibility, easy laboratory maintenance, and limited ethical issues in comparison to vertebrate models, make invertebrate models even more attractive for environmental epigenetic studies. On the other hand, limited genomic data apart from commonly used model organisms, makes high-resolution methylation studies unavailable for many species. However, this is rapidly changing, as the sequencing costs are dropping and bioinformatics tools as well as genomic resources of many non-commonly used model organisms are becoming available.

3. Earthworms as models in epigenetic research

Most commonly used earthworm models in environmental epigenetics are the species from the genus *Lumbricus*. In *L. rubellus* earthworms collected from sites across former arsenic (As) and copper (Cu) mines, an association of methylation patterns with soil arsenic concentrations in one earthworm lineage has been revealed [29]. The authors suggest that these earthworms could utilize epigenetic mechanisms to adapt and cope with the contamination. In a laboratory exposure experiment of *L. rubellus*, there were no methylation changes evident upon exposure to As and cadmium (Cd), however, fluoranthene was able to alter DNA methylation patterns evident using methylation-sensitive amplification polymorphism (MSAP) technique [30]. The same author examined also earthworm DNA methylation patterns in specimens collected from sites close to zinc (Zn), lead (Pb), and Cd smelter, however, no marked changes in DNA methylation were observed in comparison to earthworms from the control location [31]. In a series of experiments on *L. terrestris* exposed to environmentally relevant concentrations of Cd (10–25 mg/kg), DNA hypermethylation was recorded at several time points during 12-week exposure experiments [32][33]. However, these changes in DNA methylation could not be explained by common mechanisms involved in DNA methylation and demethylation, including the expression and activity of DNMT and TET genes [32]. Additionally, at the level of methalothionein gene (MT2), gene body methylation did not show any changes caused by exposure to low environmentally relevant Cd concentrations [32]. Moreover, the promoter region of the MT2 gene in *L. terrestris* does not possess any methylated cytosines [34]. To further explore the mechanisms between DNA methylation and the Cd detoxification process, *L. terrestris* earthworms were acutely exposed to high Cd concentration (200 mg) and demethylating agent (5-aza-20-deoxycytidine (Aza)) over the period of 48 h, however, no relationship was discovered [35].

Although commonly used for toxicological testing, earthworm species *E. fetida* was not often used for the assessment of epigenetic endpoints. Exposure of this earthworm species to serial concentrations of bisphenol A (BPA) for 28 days caused a decrease in DNMT1 and DNMT3b gene expression, indicating the BPA effect on the DNA methylation process [36]. Another earthworm species used for evaluation of DNA methylation included *Octolasion lacteum* exposed to ionizing radiation within the Chernobyl exclusion zone. DNA methylation profiles assessed using methylation-sensitive

amplification polymorphism (MSAP) did not differ in comparison to the earthworms from clean locations [37]. In earthworms collected from gold and silver mines, the percentage of global DNA methylation was inversely correlated to total tissue concentrations of several metals [38].

4. Conclusions

Earthworms have been successfully used as models to study epigenetic alterations triggered by environmental pollution. For an invertebrate species, their genome is relatively highly methylated and the availability of molecular resources data, such as genome and transcriptome, for some earthworm species, makes it possible to use NGS approaches in the future. Furthermore, earthworm generational studies can be easily conducted in laboratory settings making them adequate models to study epigenetic inheritance. However, to adequately use earthworms as models in environmental epigenetics, there are still several knowledge gaps that need to be explored, such as the effects of pollutants on multigenerational and transgenerational epigenetic inheritance, the responsiveness of other epigenetic mechanisms apart from DNA methylation, the connection between epigenetic effects and phenotype as well as the use of NGS approaches for a detailed exploration of the earthworm methylome and its modifications in stressful conditions.

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