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SYMPOSIUM

Patterns of DNA Methylation in Animals: An Ecotoxicological Perspective

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Synopsis DNA methylation refers to the addition of a methyl group to nucleotides within DNA. As with other epigenetic endpoints, patterns of DNA methylation are susceptible to alterations due to exposure to environmental stressors, including contaminants. These alterations can persist in the absence of the initial stressor as cells divide, and can even be inherited between generations if they occur in the germ line. Although our knowledge concerning patterns of DNA methylation in animals is increasing, there remains a gap in the literature when it comes to species outside of those typically used for biomedical research. Here, I review the literature relating to DNA methylation in an array of taxa (mammals, fish, birds, amphibians, reptiles, and invertebrates) and discuss these data from an ecotoxicological perspective. The pattern and extent of DNA methylation is well conserved across species of vertebrates; methylation appears mainly on cytosine residues within a CpG context, and much of the genome is methylated, with the notable exception of cytosines within CpG islands in the promoters of genes. Highly methylated genes in vertebrates tend to be transcriptionally repressed. However, large differences occur between classes of vertebrates in terms of the timing and nature of reprogramming and genomic imprinting: epigenetic processes that establish patterns of DNA methylation in the early embryo and which are sensitive to environmental stress. In invertebrates, patterns of DNA methylation are extremely variable and differ significantly from the condition observed in vertebrates. Some invertebrate genomes exhibit no DNA methylation while others are methylated to a level that is comparable to vertebrates. Additionally, DNA methylation may have different functions in invertebrates, e.g., alternative splicing. This variability in basic patterns of DNA methylation among species during sensitive periods of development suggests that responses to epigenetically active environmental contaminants may be similarly variable. For example, the timing of exposure to a contaminant may be a critical factor when considered in the light of variable reprogramming schedules among species. With this in mind, I review data relating to the effects of contaminants on DNA methylation in animals, focusing on non-model organisms and on exposures in natural environments, when possible. An ecotoxicological perspective on patterns of DNA methylation in animals may improve our understanding of the range and diversity of epigenetic phenomena in the natural world.

Introduction

Epigenetics is the study of alterations of patterns of gene expression that are heritable but occur outside of changes in the DNA sequence itself. DNA methylation is one of several mechanisms by which epigenetic inheritance can occur (others are histone modification, chromatin remodeling, and non-coding RNA). These endpoints are sensitive to environmental stimuli such as exposure to contaminants, physiological stress, parental behavior, and nutritional deficits, but alterations to them can also be heritable as cells divide. This implies that experiences early in an individual's life can have consequences for health in later life, or even in future generations (if the epigenetic alteration happens in the germ line). These concepts have been demonstrated in animal models (Jirtle and Skinner 2007) and have important implications for the field of ecotoxicology.

In previous reviews, Head et al. (2012) and Vandegehuchte and Janssen (2011, 2014) have discussed the relevance of epigenetics to the field of

ecotoxicology. These papers review what is known about the epigenetic effects of environmental contaminants in non-model organisms and address their implications for risk assessment. Ecotoxicological models can also be useful for furthering our understanding of epigenetic phenomena in natural populations. Epigenetically active contaminants can be studied in a range of species with wide-ranging physiologies and ecological adaptations.

The aforementioned reviews and a paper by Lloyd et al. (2012) bring attention to the fact that there is a significant gap in the available information about basic epigenetic phenomena in most species. The purpose of this article is to summarize what is known about DNA methylation in diverse species and to discuss this information within the context of how organisms respond to environmental contaminants. I first review the scientific literature describing patterns of DNA methylation in animals, highlighting species other than those typically used for biomedical research (non-rodent mammals, fish, birds, amphibians, reptiles, and invertebrates). When possible, I focus on reprogramming and genomic imprinting, processes that occur early in development at a time when patterns of DNA methylation are first established. Then I consider the capacity of contaminants to disrupt patterns of DNA methylation in animals within the context of variation in basic patterns of epigenetic inheritance among species.

Patterns of DNA methylation across animals

A surge of interest in the field of epigenetics over the past decade has brought about an exponential increase in scientific publications concerning epigenetic phenomena. The majority of these papers report on research carried out on rodents or humans. This body of work has established the basis of our understanding of the function of DNA methylation and of processes by which patterns of DNA methylation are established, maintained throughout the life of the organism, and transmitted between generations. A more complete understanding of the role of DNA methylation in mediating effects of environmental contaminants depends upon knowledge about these basic cellular processes. Here, I briefly review DNA methylation in mammals (more detailed descriptions can be found elsewhere, e.g., Bird 2002; Law and Jacobsen 2010), and then provide additional information that is available for species other than those typically used for biomedical research. Where possible, I highlight data relating to reprogramming and genomic imprinting, processes that occur at a stage of embryonic development that is sensitive to the effects of environmental contaminants.

Overview from literature on rodents

In mammals, DNA methylation occurs primarily on cytosine residues that are followed by a guanine (CpG), although evidence is mounting that methylation also occurs in other contexts (Lister et al. 2013). The extent to which the mammalian genome is methylated is variable, but is estimated to fall within the range of 60–90% (Glastad et al. 2011). The majority of CG sites that are not methylated are found clustered together within ''CpG islands'' near the promoter regions of genes (Bird 1985). These unmethylated, CG rich areas, are important for regulating gene expression because DNA methylation inhibits transcription by blocking transcription factors from binding to promoters (Watt and Molloy 1988). More recently, researchers have identified non-methylated islands that are associated not only with promoters, but also with other regulatory elements, and are found across vertebrate taxa (Long et al. 2013).

Patterns of DNA methylation are established in the mammalian embryo early in development through a process called reprogramming (Fig. 1). This is also when genomic imprinting, the silencing of either the paternal or maternal allele of a gene, occurs. Reprogramming involves two genome-wide cycles of demethylation and *de novo* remethylation. The first cycle takes place in primordial germ cells. In mice, methylation marks in the sperm are re-established, starting at embryonic day 14, whereas marks in the egg are not re-established until ovulation. This is also when parental imprints are set. The second cycle occurs shortly after fertilization, but before implantation, when the entire genome is demethylated with the exception of imprinted genes and some classes of repetitive elements. In mice, the paternal DNA is demethylated first, a few hours after fertilization, followed by the maternal DNA after the first cellular replication occurs. Finally, at about the time of implantation, the genome is re-methylated according to a somatic pattern of methylation that will persist throughout the individual's life. In mammals, exposing a pregnant female (F0) to a chemical stressor can affect both waves of demethylation because the first wave occurs in the primordial germ cells that will eventually become the F2 individual and the second wave occurs in the F1 embryo itself (by convention the order is described from the perspective of the individual being reprogrammed, not

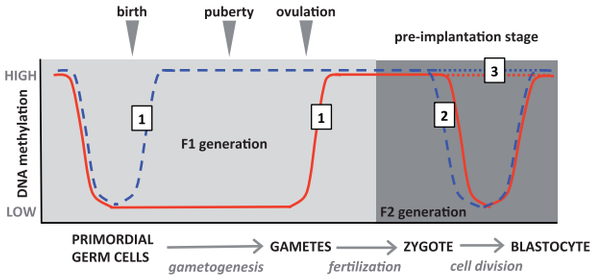

Fig. 1 Schematic of the reprogramming of DNA methylation as described for mice (adapted from Frésard et al. 2013) (Solid line represents females, dashed line represents males, and dotted line represents imprinted genes.). In mice, two cycles of reprogramming occur; the first in primordial germ cells of the F1 embryo (which will eventually become F2), and the second early in embryogenesis of the F2 individual (see "Overview from literature on rodents" section of the text). Less is known about the reprogramming of DNA methylation in other species, but several points of difference emerge from the literature. These are highlighted numerically in the figure. (1) In mice, imprints are established in male and female germ cells as they are remethylated in the first cycle of reprogramming. Genomic imprinting occurs in most mammals, but it is not yet clear whether or not it occurs in other vertebrates. (2) The second cycle of reprogramming occurs after fertilization. In mice, the paternal genome is demethylated before the first replication of DNA, whereas the maternal genome is only demethylated after replication has occurred. In other species (e.g., zebrafish, some mammals), high levels of methylation of paternal DNA are maintained throughout embryogenesis. (3) Imprinted genes are protected from the second cycle of demethylation. This may not occur outside of mammals (Reik et al. 2001; Santos et al. 2002; Faulk and Dolinoy 2011).

of the pregnant female) (Reik et al. 2001; Santos et al. 2002; Faulk and Dolinoy 2011).

Patterns of DNA methylation are established and maintained in mammals by a group of methylating enzymes called DNA methyltransferases (DNMTs). Detection of a full set of DNMTs in the genome has been used as evidence that a species has a functioning system of DNA methylation, although, DNMTs are frequently lost and gained throughout evolution (Ponger and Li 2005; Glastad et al. 2011). In mammals, DNMT1 is responsible for maintenance of DNA methylation, whereas DNMT3A, DNMT3B, and DNMT3L deal with *de novo* methylation. The mechanism for active demethylation during reprogramming was only described recently with the discovery of Ten-Eleven translocation (TET) enzymes with 5 mC oxidase activity (Wu and Zhang 2014).

In the next sections, I summarize what is known about DNA methylation in animal species other than the mammalian models typically used in biomedical research. I focus on epigenetic processes during embryogenesis that may be sensitive to the effects of environmental contaminants (reprogramming of DNA methylation; genomic imprinting) and highlight the extent of variability in patterns of DNA methylation among animals.

Other mammals

DNA methylation has been studied in a number of non-rodent mammals, including some agricultural species. Although the extent to which mammalian genomes are methylated is rather consistent, pathways by which the methylome is reprogrammed during embryogenesis may be more variable. For example, it is unclear whether the widespread demethylation of both parental genomes observed in the mouse embryo occurs to the same extent in cattle, sheep, pigs, and rabbits (Morgan et al. 2005; Dobbs et al. 2013). Genomic imprinting appears to be conserved across most mammals, including marsupials, but not in monotremes (Renfree et al. 2009).

A large part of our current knowledge about patterns of DNA methylation in mammals comes from research using inbred strains of laboratory mice. It has been proposed that deer mice (genus *Peromyscus*) would make excellent models for exploring epigenetics in a natural context (Shorter et al. 2012). This genus is indigenous to North America and widely distributed. There are already a number of studies exploring epigenetic phenomena in *Peromyscus* species and they have also been used for ecotoxicological studies.

Fish

DNA methylation is well described in fishes, particularly in the zebrafish (*Danio rerio*), a model organism for epigenetics and ecotoxicology. Zebrafish have been used as an epigenetic model for mammals, in part because patterns of DNA methylation in zebrafish's embryos closely resemble those in mice (Feng et al. 2010). Although initially controversial, it has now been established that, like mammals, zebrafish undergo demethylation of the genome early in embryogenesis, followed by *de novo* methylation (Mhanni and McGowan 2004; Mackay et al. 2007; Fang et al. 2013). In fact, general mechanisms governing demethylation were originally identified using a zebrafish model (Rai et al. 2008). However, an important difference recently was reported between mammalian and zebrafish reprogramming of DNA methylation. In zebrafish, the paternal pattern of methylation is maintained during early embryogenesis while the relatively hypomethylated maternal

DNA is reprogrammed to resemble the pattern seen in sperm (i.e., the methylome of the sperm is directly inherited, but not that of the oocyte) (Jiang et al. 2013; Potok et al. 2013). This contrasts with the pattern in mice, in which the paternal DNA also undergoes active demethylation (see "Overview from literature on rodents" section). Genomic imprinting is not well studied in fish although the absence of a DNMT3L ortholog (an enzyme that is implicated in imprinting in mammals) may suggest that genomic imprinting does not occur (Yokomine et al. 2006).

Data on patterns of DNA methylation in piscine species other than zebrafish are limited, but the extent of DNA methylation within the genome of various fishes has been relatively well described (Varriale and Bernardi 2006a). Fish tend to have higher percentages of global DNA methylation than do other vertebrates such as mammals or birds, a difference that has been attributed to a greater deamination rate of 5 mC to thymine (and therefore lower 5 mC levels) in endothermic animals (Jabbari et al. 1997; Head et al. 2014). This hypothesis is supported by the observation that 5 mC levels are inversely proportional to body temperature in polar and temperate/tropical species of fish (Varriale and Bernardi 2006a).

Birds

There is a relative lack of information in the literature concerning patterns of DNA methylation in birds; however, a few recent publications provide a basic picture of the avian methylome. A genome-wide map of DNA methylation from liver and muscle from week-old chicks showed hallmarks of the classic vertebrate patterns of DNA methylation, including unmethylated CpG islands in gene promoters, and enrichment in gene bodies and repetitive sequences (Li et al. 2011). DNA methylation is associated with decreases in transcription in birds. For example, the extent of methylation of CpG sites in the promoter of the CD4 gene correlates with its expression in the spleen of chickens (Luo et al. 2011).

The question of whether genomic imprinting exists in birds has not been addressed conclusively but several recent findings point to the absence of imprinted genes in chickens. For example, genes known to be imprinted in mammals have been found to be expressed biallelically in chickens (reviewed by Frésard et al. 2013), and a recent study found no evidence of genomic imprinting, using a whole-genome sequencing approach (Frésard et al. 2014). Moreover, as with fish, DNMT3L has not been identified in chickens, suggesting that genomic imprinting may not occur (Yokomine et al. 2006).

Little is known about the patterns of DNA methylation in avian species other than chickens. In general, the extent of DNA methylation in the genomes of birds is similar to that in mammals and lower than that of fish (Jabbari et al. 1997; Head et al. 2014), but variability is evident among species. For example, Head et al. (2014) reported that 56% and 71% of CpG sites are methylated in the occipital cortex of the embryos of chickens and Japanese quail, respectively.

Amphibians

DNA methylation has been studied in the model frog, *Xenopus*. In contrast to mice, in *Xenopus laevis* the embryos maintain high levels of DNA methylation throughout early embryogenesis (Veenstra and Wolffe 2001). Promoter DNA methylation is not associated with transcriptional repression until *Xenopus tropicalis* embryos reach gastrulation. It has been hypothesized that this indicates that a more relaxed interpretation of DNA methylation marks occurs during embryogenesis in *X. tropicalis* (Bogdanović et al. 2011). Little is known about genomic imprinting in amphibians, but a recent paper suggests that it may occur (Michalak 2014).

A discrepancy exists between two studies that described the extent of global DNA methylation in *Xenopus* compared with other vertebrates. Head et al. (2014) measured high levels of global DNA methylation in *X. laevis*, above values that were seen in fish. In contrast, Jabbari et al. (1997) observed more moderate levels, lower than fish but higher than that observed in mammals and birds. This difference may have been related to the fact that different methodologies were used to assess DNA methylation in the two studies (Head et al. 2014). A relationship between DNA methylation and ploidy was hypothesized, based on observations in *X. laevis* (tetraploid) and *X. tropicalis* (diploid). Global DNA methylation was ~13–15% higher in liver and brain tissue from the tetraploid when compared with the diploid frog (Head et al. 2014).

Reptiles

Data on the reprogramming of DNA methylation and genomic imprinting in reptilian species are very limited. Basic patterns of DNA methylation appear to be similar to those seen in other vertebrates, e.g., the presence of non-methylated islands in gene promoters (Long et al. 2013). The relationship between body temperature and DNA

methylation described for fish is complicated in reptilian species by variable strategies of thermoregulation. In fact, the percentage of the genome that is methylated ranges widely in reptiles, spanning levels found in fish, mammals, and birds (Varriale and Bernardi 2006b).

One interesting epigenetic phenomenon in reptiles relates to sex determination. Temperature-dependent sex determination was first identified in reptiles and is also observed in some species of fish (Bull 1980; Devlin and Nagahama 2002). The mechanism of this phenomenon is not known, but two studies implicate temperature-dependent DNA methylation of the gonadal aromatase promoter in red-eared slider turtles and in European sea bass (Navarro-Martín et al. 2011; Matsumoto et al. 2013). Another recent and intriguing study suggests that temperature-dependent sex determination can be inherited transgenerationally (Warner et al. 2013).

Invertebrates

Patterns of DNA methylation differ significantly between vertebrates and invertebrates. While DNA methylation covers most of the genome in vertebrates (referred to as global DNA methylation), invertebrate genomes can have long sections of methylated DNA interspersed with unmethylated regions (referred to as mosaic methylation) (Bird 2002). Methyl marks tend to appear on gene bodies and not on intergenic regions. Additionally, the degree of methylation of transposable elements and repetitive sequences is low to non-existent (Glastad et al. 2011). These alternate patterns of DNA methylation and the enormous amount of variability among species suggest a function for invertebrate DNA methylation outside of transcriptional repression (Suzuki and Bird 2008).

Insects

Patterns of DNA methylation are extremely variable among insects, both in terms of quantity and of location within the genome. Initial reports suggested that *Drosophila melanogaster* had no DNA methylation, but in 2000 Lyko et al. reported extremely low levels (<1% of cytosines methylated) early in development. The nature of DNA methylation in *Drosophila* is debated, but recent data suggest that it occurs in certain tissues and at certain developmental stages (Dunwell et al. 2013). The degree of variability among other insect species ranges from the flour beetle, *Tribolium castaneum*, which has no detectable DNA methylation (Zemach et al. 2010), to the moth *Mamestra brassicae* which has a high level of cytosine methylation, similar to that of vertebrates (Mandrioli and Volpi 2003). Other insects, including ants, aphids, bees, flies, beetles, crickets, and stick-insects also exhibit substantial levels of DNA methylation (reviewed by Glastad et al. 2011; Dunwell et al. 2013). Social insects such as bees, ants, and wasps are particularly interesting examples of invertebrates with well-developed DNA-methylation systems and potentially genomic imprinting (Kronforst et al. 2008). For example, DNA methylation plays an important role in establishing differences between castes in honey bee. Queen and worker bees have identical genetic backgrounds but develop different reproductive status through nutritional input in a process that is mediated by DNA methylation (Kucharski et al. 2008). Caste-specific patterns of DNA methylation have also been observed in ants (Bonasio et al. 2012). It has been hypothesized that these caste-specific effects are regulated through alternative splicing, with different versions of genes being expressed, rather than different levels (Lyko et al. 2010; Bonasio et al. 2012). Others also have suggested that DNA methylation may have a role in regulating alternative splicing in invertebrates (Park et al. 2011; Flores et al. 2012). Our understanding of patterns of DNA methylation in insects is increasing rapidly. Theories about how reprogramming of DNA methylation occurs are being developed (Patalano et al. 2012), and a study that observed monoallelic DNA methylation in ants supports the idea that parental or caste-specific genomic imprinting occurs in insects (Bonasio et al. 2012).

Other invertebrates

DNA methylation is equally variable in invertebrate models other than insects. The nematode worm *Caenorhabditis elegans* has no cytosine methylation and no conventional DNMT enzyme. In contrast, DNA methylation was recently observed in the parasitic nematode *Trichinella spiralis*, suggesting that patterns of DNA methylation are variable within the phylum Nematoda (Gao et al. 2012). Two species of water flea, *Daphnia pulex* and *Daphnia magna*, are commonly used in ecotoxicology and have been studied with respect to patterns of DNA methylation. DNMT enzymes have been identified in *D. pulex* (Glastad et al. 2011), and cytosine methylation was reported in *D. magna* (Vandegehuchte et al. 2009). Methylated cytosines also have been detected in several species of mollusk, a phylum that frequently is used for biomonitoring. The Pacific oyster (*Crassostrea gigas*), Japanese scallop (*Chlamys farreri*), and a salt-water clam (*Donax trunculus*) all exhibit DNA methylation (Gavery and Roberts 2013).

Effects of environmental contaminants on DNA methylation in animals

The epigenome is responsive to many environmental contaminants, particularly during embryogenesis when patterns of DNA methylation are being established. This topic has been reviewed previously (Baccarelli and Bollati 2009; Hala et al. 2012), and several papers have focused on effects in species with relevance to the field of ecotoxicology (Vandegehuchte and Janssen 2011, 2014; Head et al. 2012). Environmental epigenetics is a rapidly expanding area of research and new examples of the epigenetic effects of contaminants are continuously being published. Here I describe selected compelling and/or recent examples of the effects of environmental contaminants on DNA methylation in animals.

Most of our current knowledge relating to the epigenetic effects of environmental contaminants comes from rodent models. Many classes of contaminants have been shown to alter DNA methylation in rodents (either globally or at individual loci) including: metals, endocrine disrupting compounds, pesticides, air pollutants, and persistent organic pollutants (reviewed by Vandegehuchte and Janssen 2011, 2014; Baccarelli and Bollati 2009). The sheer variety of contaminants on this list suggests that hypermethylation and hypomethylation of DNA are generalized responses, but a recent study demonstrates that classes of contaminants can also have specific DNA-methylation signatures. Manikkam et al. (2012) showed that characteristic regions of the genome were differentially methylated by test mixtures of chemicals containing hydrocarbons, dioxins, plastics, or pesticides. These differentially methylated regions were proposed as biomarkers for ancestral exposures to particular classes of chemicals.

Several studies have linked altered methyl status at individual loci and resulting phenotypes in the whole organism. For example, Onishchenko et al. (2008) showed that gestational exposure of male mice to methylmercury resulted in reduced brain-derived neurotrophic factor (BDNF) gene expression and in depressive symptoms. This was linked to a repressive chromatin state at the BDNF promoter, which included DNA hypermethylation. In two plant models, white clover (*Trifolium repens*) and industrial hemp (*Cannabis sativa*), heavy metals induced hypomethylation at specific DNA sequences and not at random (Aina et al. 2004).

Molecular mechanisms by which contaminants effect changes in DNA methylation are unknown, but it has been hypothesized that the methionine cycle is involved (Lee et al. 2009). DNA methylation occurs when DNMT catalyzes the transfer of a methyl group from the universal methyl donor, *S*-adenosylmethionine (SAM), to DNA. SAM is regenerated from homocysteine via the methionine cycle. Homocysteine also feeds into the synthesis of glutathione, a conjugate that is critical for the biotransformation of many xenobiotics. Lee et al. (2009) proposed that contaminant exposure can result in a shortage of SAM due to homocysteine being shuttled into glutathione synthesis. Indeed, several studies have shown that contaminants decrease SAM levels in cells and tissues (Baccarelli and Bollati 2009). The question of how contaminants affect cytosine methylation at particular loci rather than randomly throughout the genome is not addressed by this proposed mechanism, and further research is needed in this area.

Transgenerational effects are a special category of the effects of contaminants on the epigenome, and they warrant further discussion. The term transgenerational (to be distinguished from ''multi-generational'') refers to effects that persist into un-exposed generations (Skinner 2008). Chemically-induced alterations to DNA methylation can persist in somatic tissues as cells divide in the absence of the initial chemical stressor, but when these alterations occur in the germ line there is the added potential for transgenerational effects to occur. The most prominent example of this comes from a series of studies describing effects of endocrine-disrupting chemicals in rats in which the effects of the fungicide vinclozolin on male reproductive parameters were observed into the F4 generation (Anway et al. 2005; Manikkam et al. 2012). It remains to be seen whether transgenerational epigenetic effects of chemicals are observed in natural environments under environmentally-relevant scenarios of exposure, and if so, how common they are. This is an important question to address since the discovery of transgenerational effects of contaminants in natural populations would have an enormous impact in the field of ecotoxicology.

Although there is a growing body of evidence to suggest that chemicals impact the rodent epigenome, examples with direct ecotoxicological relevance are less common. Effects of environmental contaminants on DNA methylation have been assessed in a large number of fish species including bluegill sunfish, false kelpfish, goldfish, stickleback, and zebrafish (reviewed by Vandegehuchte and Janssen 2011), and more recently in yellow perch (Basu et al. 2013b), medaka (Contractor et al. 2004), Nile tilapia (Flohr et al. 2012), flatfish (Mirbahai et al. 2011), and eels (Pierron et al. 2014). There is however, a significant

gap in the data for other animal classes. A series of papers described the effects of a variety of contaminants on DNA methylation in the water flea *D. magna* (reviewed by Vandegehucte and Janssen 2011). DNA methylation has been assessed in relation to arsenic in another invertebrate, the earthworm (*Lumbricus rubellus*) (Kille et al. 2013). In two non-rodent mammalian species, polar bears (*Ursus maritimus*) and American minks (*Neovison vison*), methylmercury was associated with hypomethylation of DNA (Pilsner et al. 2010; Basu et al. 2013b). However, when methylmercury was injected into chicken eggs, there was no effect on global DNA methylation in the developing embryo (Basu et al. 2013b). To our knowledge, no studies assessing effects of contaminants on DNA methylation have been published for amphibian or reptilian species.

Most of the studies mentioned above used laboratory animals subjected to controlled exposures, but a few reported on environmental exposures: (1) Pilsner et al. (2010) observed a significant dose-dependent decrease in global DNA methylation in male polar bears' brain tissue exposed to methylmercury, but not in the brains of females. Other contaminants likely to be present in brain tissue were not analyzed. (2) Caged mice environmentally exposed to air pollution had hypermethylated DNA in their sperm. Hypermethylation persisted even after the mice were removed from the contaminated area (Yauk et al. 2008). (3) In a strain of arsenic-tolerant earthworms, patterns of DNA methylation were associated with arsenic concentrations in the soil (Kille et al. 2013). (4) Liver tumors in flatfish caught from waters in the UK had distinctive patterns of DNA methylation that may be associated with exposure to marine pollutants (Mirbahai et al. 2011, 2013). Taken together, such findings provide evidence that contaminants impact patterns of DNA methylation in natural populations and diverse taxa.

Relevance to ecotoxicology

Alterations in patterns of DNA methylation are emerging as important mechanisms for the toxicological effects of environmental contaminants. As discussed, numerous contaminants have been shown to impact DNA methylation in mammals, fish, and some invertebrate species. However, data relating to DNA methylation as an ecotoxicological endpoint in birds, reptiles, and amphibians are sorely lacking. Similarly, the basic mechanisms by which patterns of DNA methylation are established and maintained in the genome have not been adequately described for these species. Describing the epigenome across animal classes will be critical to furthering our understanding of how epigenetic processes mediate organismal responses to exposure to contaminants. Similarly, studying the capacity of contaminants to disrupt patterns of DNA methylation may increase our knowledge of epigenetic pathways in diverse organisms.

The observation that there is an enormous amount of variability in the extent, pattern, and function of DNA methylation among species suggests that responses to epigenetically active environmental contaminants may be similarly variable. For example, methylmercury has been shown to affect DNA methylation in diverse species, but the direction and magnitude of changes in methylation are extremely variable (Basu et al. 2013a). This variability may reflect differences in epigenetic pathways among species, sexes, or stages of development. The large interspecific variability in how DNA methylation marks are established early in life seems particularly important. Fundamental deviations from the rodent model include the absence of genomic imprinting (monotremes and most non-mammalian vertebrates), temperature-dependent sex determination (fish and reptiles), and variable schedules of reprogramming. The majority of research relating DNA methylation to exposure to contaminants has been done in mammals and we have little perspective on how contaminants might act in, for example, oviparous species. Additionally, DNA methylation appears to have fundamentally different patterns and functions in vertebrates and invertebrates and these will certainly relate to how each group responds to contaminants.

With a few notable exceptions, nearly all of our current knowledge relating to effects of environmental contaminants on DNA methylation has been generated in the laboratory under controlled exposures and often with genetically uniform animals. With a surge of interest in epigenetic mechanisms in the fields of ecotoxicology, ecology, and evolutionary biology (reviewed by Vandegehuchte and Janssen 2011; Bossdorf et al. 2008; Crews and McLachlan 2006), we are sure to have more examples of how DNA methylation behaves in natural populations within the context of multiple stressors and variable genetic backgrounds in the near future. This will be enormously important for understanding the complex and dynamic interplay between the genetics, epigenetics, and environment.

Epigenetics is not a newly discovered phenomenon; we have known about the role of DNA methylation in regulating gene expression for over 35 years (Robertson and Jones 2000). What is new is

our developing epigenetic perspective on how early life experiences can have lasting impacts on health that may even be inherited by future generations. From an ecotoxicological perspective, we are currently lacking compelling examples of epigenetic effects of contaminants in natural populations. If these mechanisms are indeed common in nature, there is a potential for disruption of the linking of exposure and response that has characterized much of ecotoxicological research in recent decades. With epigenetic modes of action, level of exposure to contaminants, intermediary sub-clinical responses, and the overt toxic response may be temporally separated throughout an individual's lifetime, or even between generations, a possibility that most risk assessment does not take into account. Our ability to understand and observe these types of effects in nature will depend upon a solid foundation of knowledge in a variety of animal models outside of rodents.

Epigenetics holds promise as a means of explaining mechanisms that underlie persistent and multigenerational effects of environmental contaminants in natural populations. By the same token, contaminants may be useful as an experimental tool for uncovering basic differences in patterns of DNA methylation among species. In both respects, an ecotoxicological perspective may improve our understanding of the range and diversity of epigenetic phenomena in the natural world.

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