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Epigenetics

Environmental stressors and the epigenome

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Epigenetic modification and transgenerational transfer of phenotype at the individual or population level, particularly in response to environmental change, is at the forefront of biological investigation. The plasticity of this process allows an organism to respond to changes in environmental conditions, potentially conferring a survival advantage. In this review, we discuss epigenetic transgenerational phenomena in the specific context of environmental stressors including hypoxia and environmental toxicants.

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Introduction

Epigenetic mechanisms allow the stable transfer of potentially heritable traits to a subsequent generation or generations without requiring mutational events in genomic DNA [1,2]. The transgenerational effects that actually comprise epigenetic phenomenon are still contested, as is the very definition of 'epigenetics' [2–4]. Transgenerational transfer of traits can lower fitness, but they can also be adaptive by enabling an organism's survival and increasing its fitness when resources and/or stressor levels change [5].

Epigenetic mechanisms

Numerous mechanisms for non-genetic transgenerational transfer of traits have been identified, and new mechanisms are likely to emerge. Key mechanisms of epigenetic change include: covalent modification of dinucleotide sequences (CpG islands) in promoter regions of genes by DNA methyltransferase enzymes (DNMTs), direct modifications of histones and nucleosomes *via* deacetylation and/or methylation reactions by histone deacetylase (HDACs) and methyltransferase enzymes (HMTs) and post-translational modifications along with transcriptional repression of mRNAs and (retro)transposons by various interfering RNAs (or non-coding RNAs) [2,6–8]. Collectively, these processes and the components upon which they act constitute the epigenome (Fig. 1). Individually or in concert, they are important in 'imprinting' transcriptional regulatory states for various cell lineages and/or tissues over an organism's lifetime [9]. Additionally, epigenomic processes demonstrate 'plasticity' of induction, with differences occurring between various species and life-histories depending upon environment [10–12]. Consequently, the demonstration of stable transfer of altered gene-regulatory states across multiple generations may implicate a contribution of epigenetic processes in morphological evolution [13]. More specifically, to constitute stable epigenetic inheritance, the transmission of epigenetic markers is expected to comprise at least two to three generations [14]. Current research is pursuing mechanistic underpinnings of how the epigenome is activated and/or repressed by environmental stressors and how this translates to altered phenotypes and fitness.

Animal models for assessing the interactions of epigenetics and physiology

Not surprisingly, research using rodents dominates in epigenetic studies, as in most medical researches. A few studies

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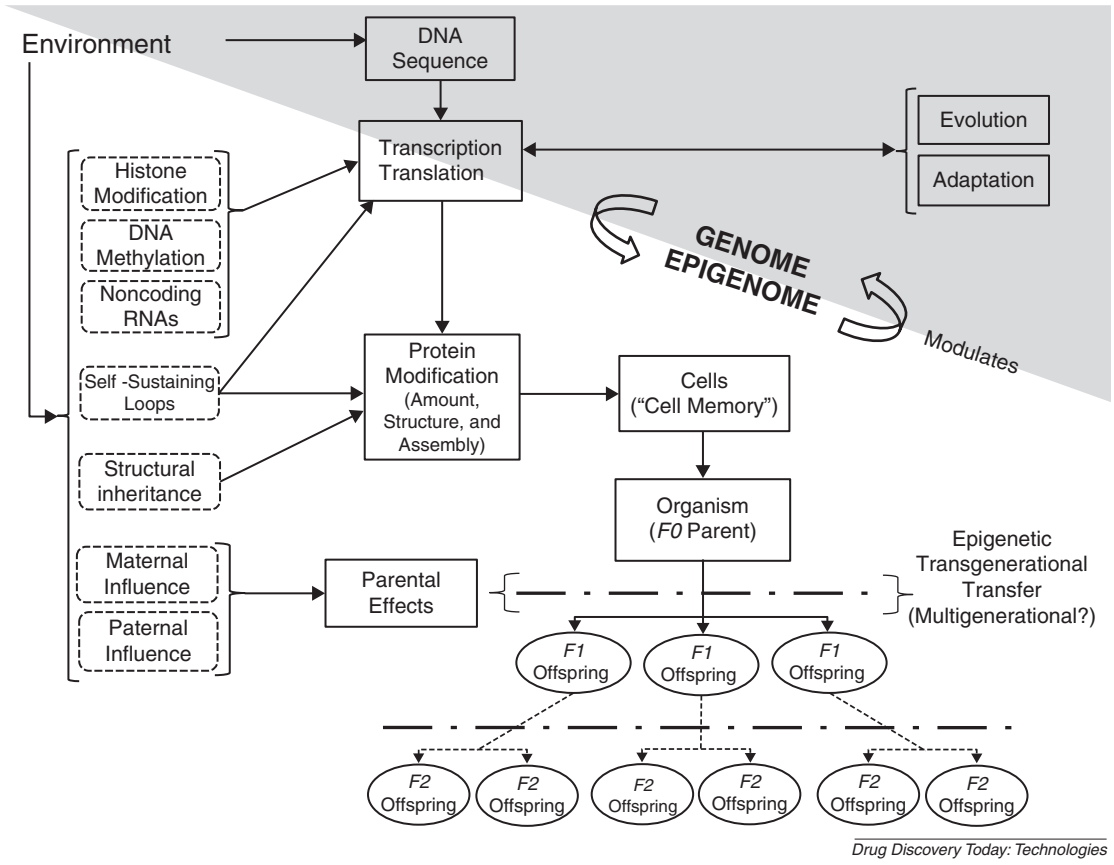

Figure 1. Relationship between the genome, the epigenome and the environment. Transgenerational effects can result from either parental effects or a variety of mechanisms that depend on modifications of protein expression.
Adapted from [2].

have blended epigenetics, toxicology and ecophysiology in aquatic organisms. Fishes, especially the zebrafish (*Danio rerio*), are now important in studies combining epigenetic transgenerational transfer and environmental stressors [15–17]. The water flea *Daphnia* is another highly promising aquatic model for studying the role of epigenetics in environmental phenotypic modification [18–20]. Zebrafish and *Daphnia* embody particularly useful characteristics such as short generation times and easy husbandry, making them useful models in ecology, evolution and toxicology. Daphnids are also parthenogenic, providing the ability to follow clonal populations across generations and eliminating genetic variation [20,21]. Other models, such as the avian embryo model, show promise for studying transgenerational transfer of altered phenotypes [22].

Effects of environmental contaminants on the epigenome

Various environmentally ubiquitous contaminants have been shown to alter methylation patterns in promoter regions of genes in mice (*Mus musculus*), rats (*Rattus norvegicus*) and various fish species [23–31]. Exposures have resulted in both hypo and hypermethylation changes in *in utero*, juvenile and adult life stages. Contaminants may act through induction/disruption of synthesis of the substrate S-adenosylmethionine (SAM) and its efficacy as a methyl donor to various methyltransferase enzymes (such as DNMTs). SAM synthesis is catalyzed by methionine adenosyltransferase enzyme, which transfers an adenosyl group from ATP to methionine, an essential amino acid that is also a limiting substrate for glutathione (GSH) synthesis [32]. In turn, GSH helps manage antioxidant responses (redox buffering) and toxicant biotransformation [33]. The potentially enhanced recruitment of GSH during toxicant detoxification may be the mechanism for competitive methionine depletion with consequent reduced SAM synthesis and lowered genome methylation (Fig. 2) [34]. There is evidence for contaminant induced disruption of SAM synthesis and genome-wide hypomethylation in liver tissue of false kelpfish (*Sebastiscus marmoratus*) exposed to organotins [29]. Exposure to environmentally relevant concentrations of organotins ($\leq$100 ng/L) induced DNA hypomethylation with concomitant reduction in SAM levels [29]. The disruption of SAM synthesis/activity may also be due to increased induction of catechol O-methyltransferases (COMTs) on exposure to elevated estrogens [35]. COMT enzymes convert catechol (hydroxylated)

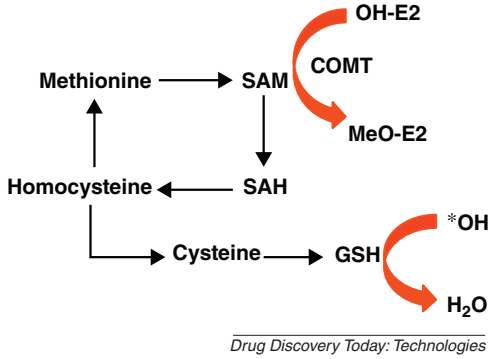

Figure 2. Relationship of SAM and GSH usage as produced from the methionine cycle, which may be modulated by estrogenic/contaminant (such as free radicals) stressors. These stressors are capable of disrupting methyltransferase activity of SAMs and/or its production, by either increasing its: (1) recruitment in the detoxification of estrogens (i.e. COMT mediated conversion of hydroxy-estrogens (OH-E2) to methoxy-estrogens (MeO-E2)); (2) availability for glutathione (GSH) production under increased oxidative stress.
Illustration adapted from [33,34,67].

estrogens to corresponding methyl ethers as a detoxification mechanism that minimizes formation of potentially reactive (oxidative) estrogen metabolites [36]. COMT facilitates the transfer of methyl groups from SAM to estrogens, hence xeno-estrogen exposures may lower SAM mediated DNMT formation (and in turn DNA methylation) due to elevated COMT recruitment (Fig. 2) [35]. Collectively, these effects lower effectiveness of SAM as a methylating agent, potentially influencing phenotype as methylation patterns are closely associated with transcriptional activity of genes, with heavily methylated regions generally corresponding to transcriptional silencing and *vice versa* for un-methylated regions [12].

Thus far, studies with rats and mice provide among the clearest cases relating xenobiotic exposures, with methylation (hypo/hypermethylation) changes leading to altered phenotype. Altered methylation patterns for the lysophospholipase (LPLase) gene occur in the germ cell lineage of male rats exposed developmentally (*in utero*) to the fungicide, vinclozolin (an anti-androgen) [26]. Altered methylation occurred in up to three generations of male offspring with observations of lowered sperm cell concentrations, motility and increased apoptosis. Furthermore, methylation changes in the LPLase gene (a key mediator of lipogenesis) were associated with lowered gamete viability as LPLase is involved with maintaining sperm lipid membrane viability in mammals [37]. Developmental exposures of rats to 17β-estradiol and the 'weakly' estrogenic (and ubiquitously present) plasticizer, bisphenol-A (BPA), have demonstrated transcriptional repression (*via* hypermethylation) of phosphodiesterase type 4 variant 4 gene (PDE4D4) with concomitant induction of precancerous prostatic lesions typical of un-inhibited cell proliferation [38]. The repression was specifically associated with the observed etiology as phosphodiesterase enzymes are involved with cyclic nucleotide monophosphate breakdown (such as cAMP). Hence, PDE4D4 silencing helped elevate intracellular cAMP levels, in turn enhancing the phosphorylation of kinases and transcription factors associated with cell proliferation (such as PKA and CREB) [38]. BPA exposure of gravid mice constituting the agouti viable yellow (A^{vy}) mouse model, have shown a change in coat color for offspring from pseudo-agouti brown to pure yellow [27]. The color change was due to hypomethylation of a retrotransposon region upstream of the agouti gene. Interestingly, maternal dietary supplementation with constituents capable of acting as methyl donors (e.g. folic acid and the phytoestrogen genistein), alleviate the hypomethylating effects of BPA, also highlighting a central role of nutritional status in the maintenance of a 'healthy' epigenome [27].

Effects of nutrition on altered epigenetic imprinting

Nutritional status effectively regulates the epigenome, contributing towards stress acclimation through metabolic homeostasis [5]. For example, reduced methylation of the PPARα receptor promoter region occurs in offspring of maternal rats fed a protein-restricted diet [39,40]. Lowered promoter methylation corresponded with increased PPARα mRNA expression, illuminating the epigenetic control of receptor expression. This regulation is important as PPARα constitutes a key nuclear receptor maintaining metabolic homeostasis of lipid biosynthesis (lipogenesis) and storage (adipogenesis) in vertebrates [41]. Epigenetic regulation of gluconeogenesis is also demonstrated under nutrient-impoverished conditions. Promoter hypomethylation of the glycolytic enzyme, phospoenolpyruvate carboxykinase (PEPCK) increases gluconeogenesis (conversion of pyruvate to glucose) and glycogen storage in baboons (*Papio* species) exposed *in utero* to nutrient limitation [42]. Epigenetic regulation is also assisted by intermediary metabolites produced during metabolic reactions. For example, acetyl-CoA, produced from citrate during glycolysis (by the enzyme ATP-citrate lyase), mediates gene activations by providing acetate units as substrate for histone acetyltransferases during histone modifications. Key metabolic genes regulated in this way include the glucose transporter (GLUT4), hexokinase 2, phosphofructokinase 1 and lactate dehydrogenase A [43]. Such epigenetic responses to nutritional state show the regulation of subsets of genes *via* promoter methylation changes, which in turn control 'far' ranging metabolic functions, potentially contributing to a more dynamic and widespread adaptive response [40].

Epidemiological evidence also associates prenatal malnutrition of humans with hypomethylation of a key growth factor gene, insulin-like growth factor II (or IGF-2) [44]. Hypomethylation of the IGF-2 gene is associated with growth-restricted fetal development and low birth weights

[45]. Low fetal birth weight is symptomatic towards development of metabolic syndrome and associated heightened risks of coronary heart disease, hypertension and non-insulin dependent diabetes later in life [46]. Unfortunately, we know little of the interplay of nutrient restriction and epigenomic changes on altered phenotype in wildlife species. The most comprehensive evidence linking nutrition and differential gene methylations to morphological/behavioral differences occurs in honeybees (*Apis mellifera*). Distinctive castes of queen and worker bees originate from the continual availability of the nutritional 'royal jelly' (containing the histone deacetylase inhibitors, phenyl butyrate and (*E*)-hydroxy-2-decenoic acid) to queen larvae, compared with the continual diet of pollen and nectar fed to worker larvae [47,48]. Furthermore, the existence of up to 560 differentially methylated genes in the genomes of queen and worker bees is proposed to contribute to differential gene patterning, accounting for the significant morphological and behavioral differences between queen and worker bees [49].

Hypoxia and epigenetic imprinting

Morphological plasticity is evoked by variations in environmental oxygen, with changes in total gill filament length and surface area improving oxygen uptake in fish raised in hypoxia [50,51]. Changes in gill morphology also correlate with increased head width and lengths [52]. Gene expression analysis of fishes (*Gillichthys mirabilis* and *Danio rerio*) and crustaceans (*Palaemonetes pugio*) exposed to hypoxia has also shown transcriptional changes indicating a shift in survival strategy from growth to survival under hypoxia (i.e. up-regulations of genes involved in erythropoiesis, angiogenesis and glycolysis) [53–55]. Most of these responses are inducible by the transcription factor, hypoxia inducible factor (or HIF-1α) [56]. HIF-1α induces histone demethylase enzyme gene expressions *in vivo* and *in vitro* in mammals. These genes include the jumonji domain containing family of enzymes constituting JMJD1A, 2A and 2B, which in turn activate metabolic regulators and proliferative factors (such as PPARα and the androgen receptor) [57,58].

Hypoxic exposure in *Daphnia magna* elevates hemoglobin concentrations and reduces body sizes in both parents and progeny (F1 and F2) [59]. Early-life (or developmental) hypoxia exposure in zebrafish (*Danio rerio*) results in arrested development, with skewed sex ratios towards a mainly male dominated population [53,54]. Altered sex ratios are due to altered steroidogenic enzyme gene expressions, such as the down-regulation of aromatase (cyp19a1a) enzyme activity and consequent increase of androgenic steroid concentrations in the developing fish [54]. A transgenerational zebrafish study showed that adult fishes exposed to moderate hypoxia (15% O_2) for 2–4 weeks and then returned to normoixa subsequently produced offspring that, at 8–12 days post fertilization, exhibited enhanced hypoxia resistance [17,60]. While population level impacts of hypoxia are well documented in fishes and dinoflagellates [61], further mechanistic investigations into its transgenerational impacts are warranted.

Predator stressors and epigenetic imprinting

Variations in rat maternal behavior of grooming pups cause increased methylation of the glucocorticoid receptor (GR) promoter and responsiveness to stress. Higher maternal grooming was correlated with 'less'-fearful responsiveness to stress with modest inductions of the hypothalamic-pituitary-adrenal (HPA) axis. By contrast, lower maternal grooming correlated with elevated H-P-A axis responsiveness and 'more' fearful response to stress [62]. Lowered GR promoter methylation was associated with higher maternal grooming and was hypothesized to lower corticotrophin releasing factor (CRF) production by feedback inhibition of the H-P-A axis, minimizing stress responses [62]. Humans also show increased maternal stress (due to domestic violence) to positively correlate with altered methylation of the GR promoter and compromised stress response in offspring. Such methylation changes may contribute to a defensive response through altered modulation of the H-P-A axis and release of corticotrophin releasing factors [63].

Daphnia showed the most prominent example of morphological plasticity in response to specific chemical cues released by predators, called kairomones. Early juvenile life stages exhibit greatest sensitivity, with morphological changes including the development of 'helmet' formation (exaggerated head shape) and 'neckteeth' (spines on the dorsal 'neck' region) [64]. Genomic analyses of *Daphnia pulex* exposed to kairomones show up-regulation of genes transcribing morphometric factors (Hox3 and extradenticle), juvenile hormone pathway enzymes (JHAMT and Met) and insulin signaling pathways (InR and IRS-1). In concert, these inductions may regulate adaptive (and survival) mechanisms [65]. From an epigenetic viewpoint, the induction of JHAMT (juvenile hormone acid methyltransferase) is of interest as it constitutes a key enzyme involved in juvenile hormone synthesis during metamorphosis. The predicted amino acid sequence for JHAMT contains a conserved S-adenosylmethionine (SAM) binding motif that allows its binding to SAMs, enabling methylation reactions during hormone synthesis [66]. This association of JHAMT and SAM implicates potential epigenomic regulation by a morphometric gene. The potential association of drastically altered phenotypes of *Daphnia* and epigenetic changes offer an exciting avenue of continued research.

Conclusion

The epigenome is a potent mediator of altered metabolic and physiological phenotypes over both single and multiple generation time scales. Susceptibilities to epigenetic mechanisms

can represent either adaptation or mal-adaptation of an organism experiencing changing environments. At present, the most comprehensive evidence for the association of altered epigenetic changes and disrupted phenotypes is provided from laboratory animal models and human epidemiological studies. These studies provide an extensive list of putative biomarkers that can be diagnostic of varying stressor scenarios. More specifically, direct epigenetic (hypo/hypermethylation) changes have been demonstrated for key metabolic enzyme genes (LPLase and PEPCK), nuclear receptors (PPARα and GR), an enzyme responsible for regulating intracellular cAMP levels (PDE4D4), and a proliferation (or growth) factor (IGF-2). In addition, inductions and/or disruptions of various 'effector' enzymes responsible for maintaining (or influencing) genome methylation levels are also key diagnostic candidates. For example, the concomitant measure of intracellular levels of SAM, DNMT, COMT, GSH, JMJD along with genomic methylation changes can inform of the status of altered epigenomes under contaminant (redox/biotransformation) and/or environmental (restricted nutrition and low oxygen) stresses. Investigations into the sensitivities of early life-stages to lasting epigenetic modifications and the implications of such changes to the fitness of a wider variety of aquatic vertebrate and invertebrate species are as yet unexplored and warrant further attention. Furthermore, investigation of stable transfer of epigenetic modifications over several generations (i.e. >2–3 generations) is also worthy of further investigation. From an ecological perspective, concerns over global climate and resultant habitat change, along with the emerging cornucopia of man-made contaminants, provides cause for concern of the role of the altered epigenome as a threat to species fitness or even survival.

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