PHILOSOPHICAL TRANSACTIONS B

royalsocietypublishing.org/journal/rstb

Review

Cite this article: Vukic M, Wu H, Daxinger L. 2019 Making headway towards understanding how epigenetic mechanisms contribute to early-life effects. *Phil. Trans. R. Soc. B* **374**: 20180126.
http://dx.doi.org/10.1098/rstb.2018.0126

Accepted: 10 December 2018

One contribution of 18 to a theme issue 'Developing differences: early-life effects and evolutionary medicine'.

Subject Areas:
genetics

Keywords:
epigenetics, early-life effects, DNA methylation, histone modifications

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THE ROYAL SOCIETY PUBLISHING

Making headway towards understanding how epigenetic mechanisms contribute to early-life effects

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It has become clear that in addition to the DNA sequence there is another layer of information, termed epigenetic modifications, that can influence phenotypes and traits. In particular, environmental epigenomics, which addresses the effect of the environment on the epigenome and human health, is becoming an area of great interest for many researchers working in different scientific fields. In this review, we will consider the current evidence that early-life environmental signals can have long-term effects on the epigenome. We will further evaluate how recent technological advances may enable us to unravel the molecular mechanisms underlying these phenomena, which will be crucial for understanding heritability in health and disease.

This article is part of the theme issue 'Developing differences: early-life effects and evolutionary medicine'.

1. Introduction

The term epigenetics was originally introduced by Conrad Waddington in the early 1940s. The prefix epi- (in Greek—over, on) implies that something is 'on top of' genetics. He defined the task of epigenetics as being to 'discover the processes involved in the mechanism by which the genes of the genotype bring about phenotypic effects' [1]. What Waddington wanted to understand is how the human body, which consists of nearly 400 different cell types, could develop from a single fertilized egg. During development, the cells of our body acquire different phenotypes, which we now know is the result of differences in their gene expression patterns. Epigenetic state can influence the accessibility of DNA to transcription factors and members of the transcriptional machinery, thereby influencing gene expression patterns. The state of the epigenome is determined by epigenetic marks, which can be placed directly on the DNA or on its associated histone proteins. The existence of DNA methylation was discovered almost simultaneously with the identification of DNA [2], while the first histone modifications were discovered some 20 years later [3]. At that time, however, the importance of these chemical modifications on and near the DNA was not understood. The first evidence that epigenetic marks play a role in regulating gene expression was discovered in the late 1970s and early 1980s. Experiments performed at that time showed that cells that differently expressed certain genes had different DNA methylation patterns [4]. In addition, it was found that 5-azacytidine, a DNA methyltransferase inhibitor, could induce gene expression changes [5], and *in vitro* studies reported the first observations that nucleosome positioning at the promoter can block transcription [6]. In the 1990s, it became evident that histone tails are important for gene activation [7].

2. Epigenetic modifications

Today, DNA methylation and histone modifications are known as the two main mechanisms of epigenetic regulation. In addition, RNA molecules are now being recognized as an additional layer in the regulation of gene expression.

DNA methylation involves the addition of a methyl group to the fifth carbon in the cytosine pyrimidine ring. In mammals, DNA methylation largely occurs on CpG nucleotides and it is thought that up to 80% of the mammalian CpGs are methylated, with the main exception being CpG islands [8]. Historically, DNA methylation has been associated with transcriptional repression and high levels can be found in the heterochromatic regions of our genome and at repetitive elements [9]. More recently, DNA methylation over gene bodies has been linked to active gene transcription in the mouse and human genomes [10]. The DNA methyltransferases Dnmt3a and Dnmt3b are responsible for the establishment of DNA methylation patterns, whereas Dnmt1 is referred to as the maintenance DNA methyltransferase [11]. Together with partner proteins such as Pcna and Uhrf1, Dnmt1 can act on hemi-methylated DNA, thereby enabling the faithful inheritance of DNA methylation patterns after each cell cycle [9,12]. DNA methylation can also be removed, which is particularly important during epigenetic reprograming (discussed briefly below). Removal of DNA methylation can be either passive or active. Passive DNA demethylation occurs during the process of DNA replication, when Dnmt1 is not present, and leads to a cell-cycle-dependent dilution of DNA methylation [13,14]. The ten–eleven translocation (TET) family of proteins is responsible for mediating active DNA demethylation. The TET family has three members, TET1, TET2 and TET3, which can catalyse conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) through their oxidizing activity [15,16]. The oxidized groups (5fc and 5caC) are excised and replaced by unmodified cytosine via either thymine-DNA-glycosylase (TDG) catalysed base excision or DNA base excision repair (BER) [15,17–21]. Therefore, this process is defined as active DNA demethylation that is independent of DNA replication.

Post-translational modifications that can primarily be found at the amino-terminal ends, the tails, of histone proteins are called histone modifications. They have been associated with both transcriptional activation and repression and are established, recognized and erased by a diverse group of proteins often referred to as 'writers', 'readers' and 'erasers'. Histone modifications can be found throughout the mammalian genome, but particular marks have been associated with particular genomic elements and genomic contexts [22]. For instance, the lysine methylation marks H3K4me2/3 are usually found around the transcription start sites of actively transcribed genes [23], while H3K4me1 together with H3K27ac marks active enhancers [24–26]. H3K9me2/3 and H3K27me3 are referred to as repressive histone marks and can be found at repetitive elements and around transcriptionally silent genes, respectively [27].

While originally not considered to be part of the epigenetic machinery, RNA molecules, which can be located inside and outside the nucleus, are increasingly being recognized as major players in epigenetic processes. In particular, the discovery of a set of small RNA populations in mammalian sperm, including microRNAs (miRNAs) and tRNA-derived small RNAs (tsRNAs), has sparked great interest in their potential function as mediators of environmentally induced phenotypes across generations [28].

3. Epigenetic reprogramming

Epigenetic regulation can influence gene expression patterns throughout the life of an organism, but is particularly important during the earliest stages of embryonic development when mammals undergo two rounds of epigenetic reprogramming. Epigenetic reprogramming, which refers to the genome-wide erasure of epigenetic marks, occurs in the developing primordial germ cells (PGCs) during early to mid-gestation *in utero*, and in the embryo early in development. During PGC reprogramming, global erasure of DNA methylation and histone modifications takes place [29,30]. In females, this process continues even after birth in the growing oocytes of juvenile mice [31,32]. The second wave of reprogramming takes place during pre-implantation development and involves the clearing of the epigenetic marks that defined the gene expression patterns of the mature parental gametes, i.e. the sperm or the oocyte [33,34]. This enables the zygote to return to a state of totipotency, and allows the cells of the early embryo to differentiate into any of the different cell types in our body. Until recently, it was thought that because of the two phases of reprogramming, there is little chance for the inheritance of epigenetic marks [35]. However, there are now reports that, in both rodents and humans, reprogramming is not always complete [36–38]. For example, retrotransposons that belong to the LINE/LTR family, and also imprinted regions, were found to be protected from the global demethylation during PGC reprogramming in the mouse [38]. Similar results have been reported for humans, where it has become evident that evolutionarily young repetitive elements but also some single-copy genes can escape from reprogramming during early human [37] and PGC development [36].

4. Environmental epigenetics

Many lines of evidence indicate that early-life events are of critical importance to adult health. In addition, it is now widely accepted that a balanced lifestyle can help to prevent or treat certain diseases. Although genetic variation is the main contributor to an individual's phenotypic response to environmental factors, particularly for common diseases, it has remained difficult to identify the underlying DNA-sequence differences. Given its vital role in regulating gene expression patterns, epigenetics could be an important contributor. Indeed, it has become clear that environmental challenges such as nutrients, stress, maternal behaviour, immune challenges or chemicals can alter epigenetic marks, and researchers from many different disciplines including epidemiology, behavioural sciences and molecular biology have been investigating the relationships between early-life events and the epigenome.

The effects of environmental influences on an organism's phenotype can be more profound if they occur during certain periods of life. These stages of increased susceptibility appear to overlap with the critical time windows of epigenetic reprogramming. For example, environmental insults experienced by the mother during pregnancy could potentially influence offspring phenotype, either directly through exposure while in the mother's womb or indirectly through inheritance of an altered (epi)genetic state [39]. A large number of studies have focused on investigating maternal/gestational effects, summarized in a recent review [40]. Similarly, the father's phenotype or lifestyle can influence offspring phenotype. This can be mediated through sperm or seminal fluid during mating [41]. A number of studies have been exploring paternal effects and they have recently been reviewed in [28].

5. Nutrition and the epigenome

In determining the effects of the environment on the epigenome during early development, scientists took advantage of the existence of alleles that display the unusual characteristic of variable expressivity in the absence of genetic heterogeneity and are termed metastable epialleles (MEs) [42]. In the mouse, one of the most carefully studied MEs is *agouti viable yellow* (A^{vy}). The expression of the *agouti* locus depends on the methylation of the 3′ end of the intracisternal A-particle (IAP) retrotransposon, which inserted upstream of the *agouti* gene [43,44]. Using A^{vy} as an epigenetically sensitive reporter, different studies showed that nutritional composition [45–50] and exposure to certain chemicals [51] during gestation can affect the epigenome of the developing embryo, and result in abnormal phenotypes. For example, one study showed that consumption of ethanol by the dam during pregnancy increases the chances of hypermethylation of the A^{vy} locus in her offspring, suggesting that alcohol consumption can influence epigenetic state [51]. Furthermore, the offspring of alcohol-treated dams showed developmental abnormalities such as microcephaly and midfacial dimorphisms, a phenotype that is also observed in children diagnosed with fetal alcohol spectrum disorder.

The concept of MEs has also been investigated by researchers studying whether distinct seasonal changes in food availability around the time of conception and during early pregnancy can be linked to epigenetic and phenotypic differences in a rural Gambian population. In the initial study, genomic regions showing variable DNA methylation between individuals that were independent of genetic differences and showed little variation between tissues were identified. The authors termed them 'human metastable epialleles' [52]. Studies on the Gambian population have since focused on identifying links between DNA methylation differences and season of conception. Indeed, the wet season could be associated with higher DNA methylation levels at six tested MEs when compared with the dry season [53]. In addition, the imprinted non-coding VTRNA2-1 was identified as a human ME, and found to be hypermethylated in offspring conceived during the wet season [54].

Another well-known example of how nutritional influences during embryonic development may impact adult life is the Dutch Hunger Winter of 1944–1945, a six-month famine at the end of World War II that particularly affected the western part of The Netherlands. The offspring of females who were pregnant during the Dutch famine have been shown to have altered birthweight and increased neonatal adiposity [55,56]. One of the explanations for these observations could be epigenetics. Indeed, it was found that the imprinted IGF2 gene was hypomethylated in the blood of individuals born from mothers undernourished during pregnancy when compared with controls [57,58].

6. Stress and the epigenome

In mammals, traumatic stress experienced in early or adult life can influence social, emotional and cognitive behaviours and may influence disease risk [59]. For example, rats that are nurtured by stressed mothers are likely to be stressed [60]. This has been shown to involve the hypothalamic–pituitary–adrenal HPA axis, and DNA methylation changes at the glucocorticoid receptor gene, a critical component of the stress response, in the hippocampi of these rats have been reported [61]. Furthermore, brain-derived neurotrophic factor (BDNF), a gene involved in neural plasticity, has been linked to traumatic stress. A study performed in outbred mice showed that when pregnant mothers were exposed to stress during pregnancy, their offspring had decreased BDNF expression. This correlated with increased DNA methylation levels at the promoter of this gene. In addition, depression-like and anxiety-like phenotypes were observed [62]. On the other hand, exposure to a stimulating social environment during the early postnatal phase had an influence on the expression of BDNF in the brain of adult mice and this transcriptional change was accompanied by increased histone acetylation levels [63].

Collectively, the aforementioned studies provide solid support that environmental influences can shape an organism's phenotype and that epigenetics plays a crucial role in these processes. The challenge now is to delineate the molecular pathways affected by early-life experiences and their causal contribution to phenotypic outcome. Considering the wide range of epigenetic modifications and the fact that cross-talk occurs between DNA methylation, histone modifications and RNA molecules to regulate gene expression patterns, this is not a trivial task.

7. Towards a better molecular understanding

Over the past decade, major advances in next-generation sequencing technologies, including single-cell approaches, have greatly contributed to our understanding of the molecular processes important for epigenetic reprogramming in the mouse and human. In 2009, the use of single-cell RNA-sequencing was reported for the first time [64]. Soon thereafter, many laboratories developed additional methods to measure the distribution of DNA methylation [65–67], histone modifications [68], chromatin accessibility [69–71] and chromosome conformation [72,73] in single cells. Furthermore, technologies that can co-capture different layers of gene regulatory information in one cell through the combination of genome sequencing, transcriptome analysis, nucleosome occupancy and epigenome studies have been developed [74–82]. This has enabled the identification of previously unknown cell types, a detailed description of gene expression patterns and epigenetic states, and the prediction of developmental trajectories [83,84]. For example, DNA methylation, transcriptome and chromatin accessibility data have become available for human pre-implantation embryos until the blastocyst stage (zygote, 2-cell stage, 4-cell stage, 8-cell stage, morula) and also for sperm and oocytes [85–88]. One study reported that DNA demethylation is a much more dynamic process than was previously thought, with three waves of demethylation occurring in human pre-implantation embryos [85]. Another study investigated chromatin accessibility and revealed that during the earliest cell divisions, until the 4-cell stage, the paternal genome is in a more open state when compared with the maternal genome [88], and this correlates with global DNA methylation levels [85,88]. Single-cell studies focusing on epigenetic regulation in human PGCs revealed similarities but also differences between mouse and human germline development [36,89,90]. Overall, it was found that the mechanism of global erasure of DNA methylation is remarkably conserved between the two species. Some differences were observed in the deposition of the repressive

histone modification H3K27me3. In the mouse, this mark is found persistently enriched during PGC reprogramming [91], whereas in human PGCs, a global loss was reported. In all these studies, regions rich in repetitive elements were found to be more resistant to the reprogramming events, and repetitive elements are emerging as good mechanistic candidates underlying environmentally induced phenotypes. Of note, the repetitive compartment of the genome is often excluded from standard bioinformatics analyses, and therefore, further investigations or even re-analysis of published datasets are needed to determine whether repeats indeed play a central role in these processes.

8. Conclusion

Numerous studies have demonstrated that environmental challenges experienced early in life can influence an individual's phenotype, and much has been learned about the underlying molecular processes. In particular, monozygotic twins, which are essentially genetically identical, are an ideal human model system and have provided valuable insight into the genetic versus environmental contribution to human (disease) phenotype [92]. However, many studies in this area are complicated by the genetically heterogeneous nature of the human population and establishing causative links between environmental conditions, epigenetic state and phenotypic outcome has remained difficult in many cases [35,93–95]. For human cohort studies, the careful analysis of samples taken immediately before and after exposure, or the involvement of (grand)parental generations may not always be an option. Furthermore, it is known that epigenetic marks differ from tissue to tissue and indeed, we still know very little about the locus-specific regulation of epigenetic marks. Drawing conclusions based on blood, fibroblasts or saliva about other tissues can therefore be challenging. In addition, cell type heterogeneity in tissues can present an obstacle when interpreting results from bulk sequencing data [96,97]. Studies in rodents have mainly faced criticism because of varying experimental design, differences in data interpretation or the lack of causal evidence for the transmission of 'a mark' across generations. Importantly, these challenges are being recognized by the scientific community, and guidelines on how to address these issues in future studies have been published [96–99]. Well-controlled experiments using mammalian models and large, carefully characterized human cohorts in epidemiological studies will certainly shed some light on the contribution of epigenetics to early-life effects.

Data accessibility. This article has no additional data.

Competing interests. The authors declare that they have no competing interests.

Funding. L.D. is supported by a LUMC Fellowship, and The Netherlands Organisation for Scientific Research (ZonMw-VIDI 91718350).

Acknowledgements. The authors thank members of the Daxinger laboratory for helpful discussions.

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