Epigenetics and the environment: emerging patterns and implications

Robert Feil[1] *and Mario F. Fraga*[2,3]

Abstract | Epigenetic phenomena in animals and plants are mediated by DNA methylation and stable chromatin modifications. There has been considerable interest in whether environmental factors modulate the establishment and maintenance of epigenetic modifications, and could thereby influence gene expression and phenotype. Chemical pollutants, dietary components, temperature changes and other external stresses can indeed have long-lasting effects on development, metabolism and health, sometimes even in subsequent generations. Although the underlying mechanisms remain largely unknown, particularly in humans, mechanistic insights are emerging from experimental model systems. These have implications for structuring future research and understanding disease and development.

Epigenetic modifications
Chemical additions to the DNA and histones that are stably maintained and do not change the primary DNA sequence.

Epigenomes
The overall epigenetic modifications of cells. An organism has multiple, cell type-specific, epigenomes

[1]*Institute of Molecular Genetics (IGMM), CNRS UMR-5535 and the University of Montpellier, 1919 route de Mende, 34293 Montpellier, France.*
[2]*Department of Immunology and Oncology, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain.*
[3]*Cancer Epigenetics Laboratory, Institute of Oncology of Asturias (IUOPA-HUCA), University of Oviedo, Spain.*
Correspondence to R.F.
e-mail: robert.feil@igmm.cnrs.fr
doi:10.1038/nrg3142
Published online 4 January 2012

In eukaryotic cells, DNA is packaged into chromatin, and covalent modifications on the histone proteins of the chromatin and modifications on the DNA itself can influence the expression of genes. When heritable from one cell generation to the next, such modifications are referred to as epigenetic modifications and can bring about lasting changes in gene expression[1,2]. Epigenetics is the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence[3].

Genome-wide patterns of DNA and chromatin modifications ('epigenomes') do not persist throughout life, but undergo precise, coordinated changes at defined stages of development, particularly in mammals. These transitions contribute to the lineage- and tissue-specific expression of genes[2,4–6]. In mammals, and to a lesser extent in plants, epigenetic modifications are reset upon passage through the germ line. This epigenetic reprogramming prepares the germ cells for development in the next generation. Besides the developmental ontogeny of epigenetic modifications, there is also considerable stochastic variation, often without apparent biological purpose[7–10]. It is thought that stochastic changes are mediated both by extrinsic (environmental) factors and intrinsic factors, but their relative contributions remain largely unknown (FIG. 1).

The possible impact of the environment on epigenetic regulation has attracted considerable interest. In many studies, environmentally induced changes in gene expression are associated with altered DNA methylation patterns or with altered histone modifications. From work on model systems it is now clear that epigenetic alterations can indeed be involved in the environmentally triggered phenotypes. To date, much of the focus in 'environmental epigenetics' has been on DNA methylation, which is essential in development and is involved in processes such as genomic imprinting and silencing transposable elements[2,6,11]. In mammals and insects, cytosine methylation is found almost exclusively in the context of CpG dinucleotides, but in plants it also occurs in CHG and CHH contexts (where H = A, T or C). The DNA methyltransferases (DNMTs) that establish and maintain the patterns of DNA methylation have been studied in detail[2]. Less well understood are the processes that dictate where in the genome DNA methylation is established and subsequently maintained, and it is these processes on which environmental signals could have a considerable impact.

Histone methylation patterns also can be somatically stable, and can mediate the silencing or activation of genes during development. Dimethylation and trimethylation of lysine-4 of histone H3 (H3K4me2/3), for example, are associated with the stable expression of developmental genes[12]. There is evidence that histone-methylation-dependent gene regulation can be perturbed by external factors, but for most histone modifications it is unclear whether they are epigenetic[1] and contribute to environmentally induced phenotypes. Non-coding RNAs, including small RNAs, also contribute to the regulation of the epigenome[13], particularly at repeated DNA sequences. However, besides the involvement of small interfering RNAs (siRNAs) in heat-stress

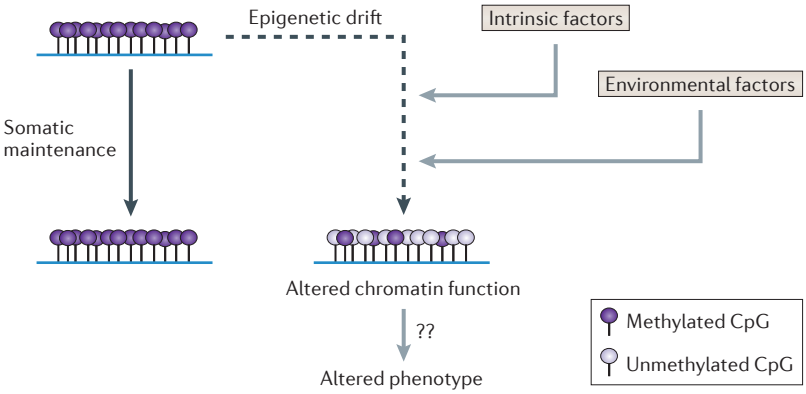


Figure 1 | **Factors that lead to epigenetic variation over time.** Many epigenetic modifications become biologically stabilized at a particular stage of development, and are maintained subsequently throughout the lifetime of the organism (shown by the solid black arrow). However, at certain genomic loci, epigenetic marks can readily change over time (shown by the discontinuous black arrow)[7,9,10,120,121]. This 'epigenetic drift' is thought to depend both on environmental and intrinsic factors. This diagram shows an example of how epigenetic drift can lead to the loss of DNA methylation, with consequences for gene expression and cellular and organismal phenotypes.

responses in plants[14,15], it remains largely unknown whether small RNAs could be linked to environmental effects as well.

The theme of 'environment and epigenetics' evokes excitement because environmentally triggered phenotypes are found to be associated with DNA methylation and chromatin alterations. So far, however, few studies have provided clear mechanistic insights. Particularly in mammals, it remains often unknown whether there are substantial epigenetic changes and whether these actually contribute to the environmentally induced phenotypes[16]. Here we consider our current knowledge, from different animal and plant species, of the effects that are brought about by diet, temperature changes, chemical pollutants and other external stresses. This Review does not cover epigenetic effects that are induced by socioeconomic aspects of the environment; this is a fascinating topic that is discussed elsewhere[17,18]. Our discussion is structured according to addressing key questions: which are the developmental and metabolic phenotypes that are brought about by specific environmental cues; what do we know about the mechanistic links between the environmental trigger and the observed epigenetic alterations; are some individuals more susceptible to specific environmental stresses than others; and finally, how should data from experimental models be extrapolated, and what could be the implications for human health?

Long-term effects on development

The environment can have long-lasting phenotypic effects without apparent underlying genetic change. Striking examples are provided by natural phenomena, including developmental and reproductive transitions that are brought about by environmental cues. Studying such processes may help to pinpoint the mechanisms that underlie aberrant, pathological effects that are brought about by the environment.

Arthropods. In aphids, the presence of predators, crowding or other external stresses induces a shift in the population from wingless animals to winged animals. This developmental switch occurs during the early stages of development, through unknown mechanisms. Similarly, in *Daphnia* (water flea) species, external stress (that is, water-borne chemical cues from predators) brings about dramatic morphological changes during development, and these persist over several generations in the population[19]. Aphids and *Daphnia* spp. have orthologues of vertebrate DNMTs, but it is unclear whether their pronounced morphological transitions involve alterations in DNA methylation[19]. Substantial changes in DNA methylation are associated with an environmentally induced developmental transition in honey bees (*Apis mellifora*). Female bees have two alternative forms, sterile workers and fertile queens, which develop from genetically identical larvae through receiving different foods. Only larvae that are fed 'royal jelly' develop into queens. Through yet-unknown mechanisms this brings about differential DNA methylation and the differential expression of many genes between queen and worker larvae, through the action of the *de novo* methyltransferase DNMT3 (REFS 20,21).

In mealybugs, which are *Pseudococcidae*, and a few other groups of insects, sex determination is triggered by environmental factors, probably through temperature changes during gametogenesis[22,23]. Though genetically identical, male and female mealybugs are morphologically highly divergent. Remarkably, the induction of male sex identity during early development involves the formation of stable heterochromatin and the silencing of the entire paternally inherited genome, which brings about dosage changes at crucial genes[22]. Whether temperature-dependent sex-determination in other groups of animals — including reptiles (such as turtles and crocodiles) and fish species — is also mediated through changes in chromatin and DNA methylation, remains to be explored[24].

Plants. Epigenetic transitions can arise in individual plants through environmental signals (BOX 1), particularly as a consequence of persistent temperature changes[25]. A striking example is vernalization (FIG. 2A), a natural process through which plants in temperate climates become instructed to flower early after having been exposed to the cold temperatures of winter[26]. Another classic example of phenotypic plasticity in plants is in *Linaria vulgaris* (yellow toadflax), for which flower symmetry can be heritably bilateral or radial. The change from one to the other flower type occurs occasionally, and involves alterations in DNA methylation at a gene encoding a transcription factor, without apparent genetic mutations[27]. Also, studies on plant populations in the wild have identified differential phenotypes that are associated with specific DNA methylation profiles; these presumably emerge through selection[28–33].

Mammals. Animal models have shown that nutrition and environmental exposures during development can lead to locus-specific changes in the epigenome[34]. Possibly the best-studied model is the agouti viable yellow (A^{vy})

Intrinsic factors
Factors that are inherent to the individual animal or plant. Genetically determined, intrinsic factors induce considerable stochastic variation, such as different behaviour between cells.

CpG dinucleotides
Indicates a cytosine followed (5′–3′) by a guanine. Cytosines at CpG dinucleotides constitute the principal target of DNA methylation in mammals. In plants, cytosine methylation occurs also in other sequence contexts.

Heterochromatin
A densely packaged, transcriptionally silenced type of chromatin. Constitutive heterochromatin is found close to centromeres in all tissues. Facultative heterochromatin, such as that commonly found at gene promoters, can be developmentally reprogrammed.

Phenotypic plasticity
The ability of a genotype to yield different phenotypes; for example, in response to environmental stimuli.

Box 1 | **Altered epigenetic states and phenotypes in plants**

In plants, there are many natural examples of stable alterations in DNA methylation that confer specific phenotypes. These include the *Colourless non-ripening* (*Cnr*) locus in tomato, at which heritable hypermethylation induces repression and hence, non-ripening of the fruit[72]. Another, classic example is flower symmetry in the toadflax *Linaria vulgaris*, which is heritably influenced by levels of DNA methylation at the *CYCLOIDEA* locus, which comprises a retrotransposon[27]. Also in the wild potato *Solanum ruiz-lealii*, flower morphology seems to be linked to differential DNA methylation[135]. In the melon, the DNA methylation status of a transposon at the *CmWIP1* transcription factor locus controls the transition from male to female flowers[31]. In these examples, it is unclear whether DNA methylation states are influenced by the environment. One way to address this important issue has been to compare natural populations in different environments (reviewed in REF. 71). In wild populations of the perennial violet, *Viola cazorlensis*, selection seems to be influenced by cytosine methylation levels at multiple gene loci[28]. Differential DNA methylation between populations growing in different microenvironments was also reported in mangrove trees[30] and in orchid species[29].

In wild populations, it has generally been challenging to ascertain that observed phenotypic variations are indeed caused by differential epigenetic states, rather than resulting from genetic mutations. Therefore, as an alternative approach plants have been directly subjected to specific stresses, and long-term epigenetic effects were explored[25]. In fascinating recent work on asexually reproduced dandelions (*Taraxicum officinale*), plants were subjected to four different stress treatments (high salt, low nutrients and two different stresses to evoke anti-pathogen and anti-herbivore defences). At many loci, altered DNA methylation was observed following these treatments, and this was often transmitted to the progeny of the stressed plants[32]. Because the dandelions reproduced from unfertilized seeds (through 'apomixis'), these striking epigenetic effects occurred in the absence of genetic variation.

A well-known response to heat stress is the activation of transposable elements[15]. In *Arabidopsis thaliana*, heat stress transiently activates specific retrotransposons[65], and this can lead to the accumulation of genetic mutations in the progeny through chromosomal insertions. The deleterious effects of heat stress on these heterochromatic repetitive elements are kept under control not only by regulators of DNA methylation, but also by components of the RNA interference pathway[14].

allele in the mouse. This locus comprises an upstream intracisternal A-particle (IAP) retrotransposon. When this retrotransposon is unmethylated, the *agouti* gene is aberrantly expressed, leading to a yellow coat colour, obesity and diabetes (FIG. 2B). A^{vy} has been called a metastable epiallele because its epigenetic state, though somatically maintained, has reduced stability and can readily switch from one generation to the next[34–36]. In humans, there is growing awareness that particularly nutrition, but also exposure to toxic components (TABLE 1), may have long-term phenotypic effects[37]. As is discussed below, human studies on the gestational effects of nutrition have thus far identified DNA methylation changes that are relatively small[38–40]. One challenge for the future will be to determine the biological relevance of these minor changes, and whether they are a cause, or a consequence, of the observed developmental and metabolic perturbations.

Developmental stage-dependent effects

Epigenetic alterations that arise around the time of conception, or during early embryogenesis, are amplified during development by cell division and somatic maintenance, and thus affect a high proportion of cells in the fully grown organism. By contrast, when epigenetic alterations occur in adult quiescent cells they remain restricted to those cells, and when they occur in adult stem cells they remain restricted to a specific tissue. Indeed, altered DNA methylation patterns that are acquired during early embryonic development are maintained throughout later development at imprinted genes and at some other loci[39,41–43]. However, other genes and certain repeat sequences readily change their methylation levels during adult life[7,10]. Further research may identify which sequences in the genome are epigenetically most stable over time, and to what extent epigenetic maintenance processes are comparable between tissues.

Gestational effects. The impact of extrinsic factors on the mammalian epigenome seems to depend on the developmental stage. During mouse gestation, dietary intake of folate together with other compounds that affect the methionine cycle (also known as the one-carbon cycle; see below) has clear effects on A^{vy} methylation and the health of developing offspring[44], but does not apparently affect A^{vy} methylation in the mother. Several lines of evidence suggest that the gestational period is particularly susceptible to epigenetic perturbation, and that the environment can have different effects on the placenta and embryo. An example is the increased incidence of type 2 diabetes in rats that were exposed to suboptimal nutrition during early development[45]. This was associated with the epigenetic repression, in pancreatic islets, of hepatocyte nuclear factor 4a (*Hnf4a*), which encodes a transcription factor that is involved in β-cell differentiation and glucose homeostasis. Further examples include global hyper-acetylation of histone H3 in the offspring of pregnant Japanese macaques that were fed high-fat diets[46], alterations in DNA methylation at imprinted genes[41] in mouse placentae as a result of a high-fat maternal diet[47], and severe health problems that were associated with reduced levels of DNA methylation at multiple CpG islands in the offspring of pregnant ewes that were fed restricted amounts of dietary folate, methionine and vitamin B12 (REF. 48). Several other studies reported effects on peroxisome proliferator-activated receptors (PPARs), proteins that are known to regulate lipid homeostasis and the activities of which are tightly controlled by nutrient availability. *Ppara* expression and promoter methylation were altered in the offspring of female rats that were fed a low-protein diet[49]. Remarkably, the offspring of male mice that were fed a low-protein diet also showed altered expression of *Ppara* and numerous other genes in the liver. These alterations were associated with minor DNA methylation changes, including reduced levels of methylation at a putative enhancer of *Ppara*[50]. This paternally induced, transgenerational effect on liver metabolism was not linked to changes in DNA methylation in the sperm of the males that were fed the low-protein diet.

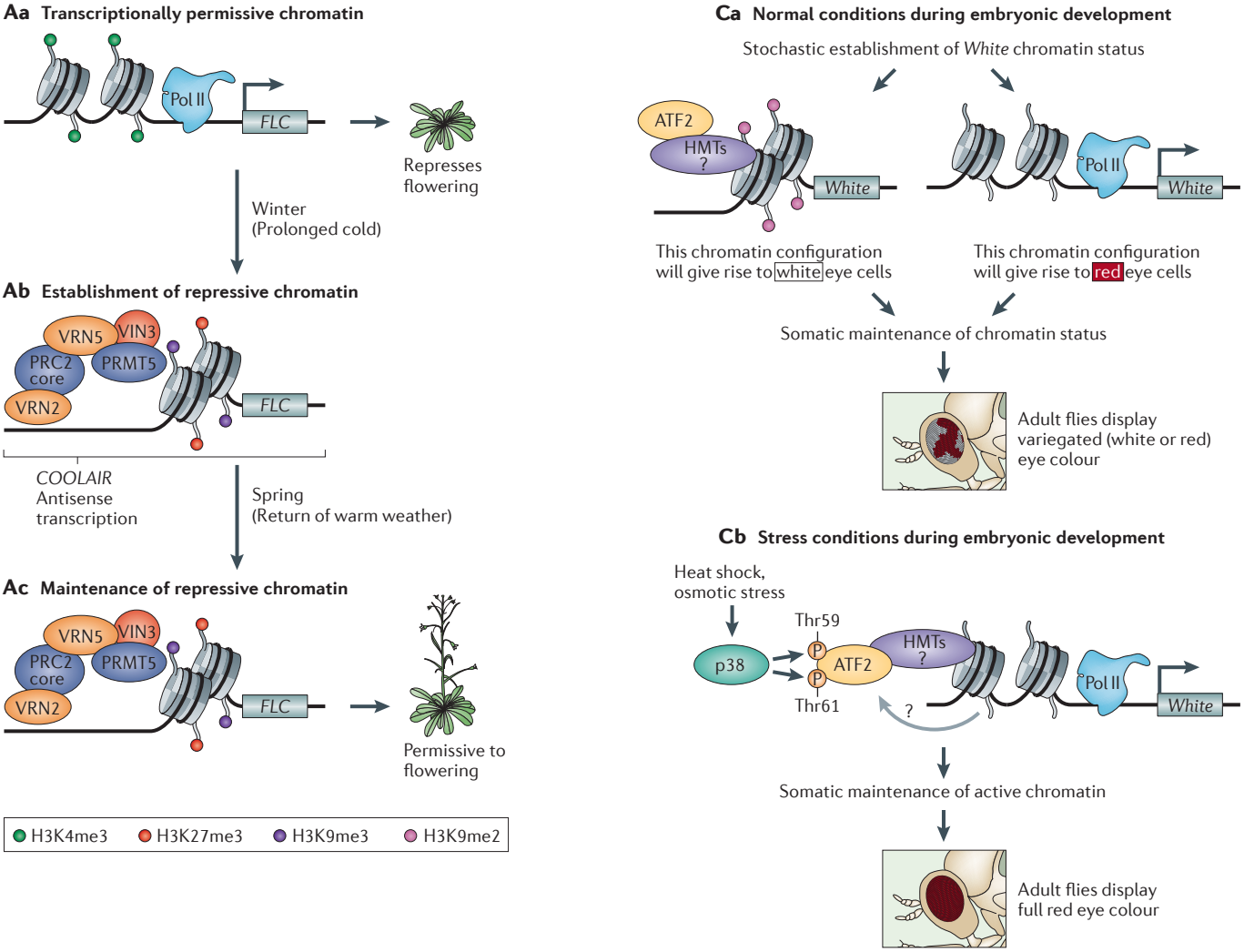
A hypothesis that emerged from epidemiological studies proposes that fetal metabolic adjustments to nutritionally unfavourable conditions can affect growth and development, and may substantially alter the risk of chronic disorders later in life[51]. During the last decade, this important framework of thought evolved into the general concept of 'developmental origins of health and disease' (DOHaD) (BOX 2). Retrospective studies have shown that nutritional conditions[38,40] and folic acid

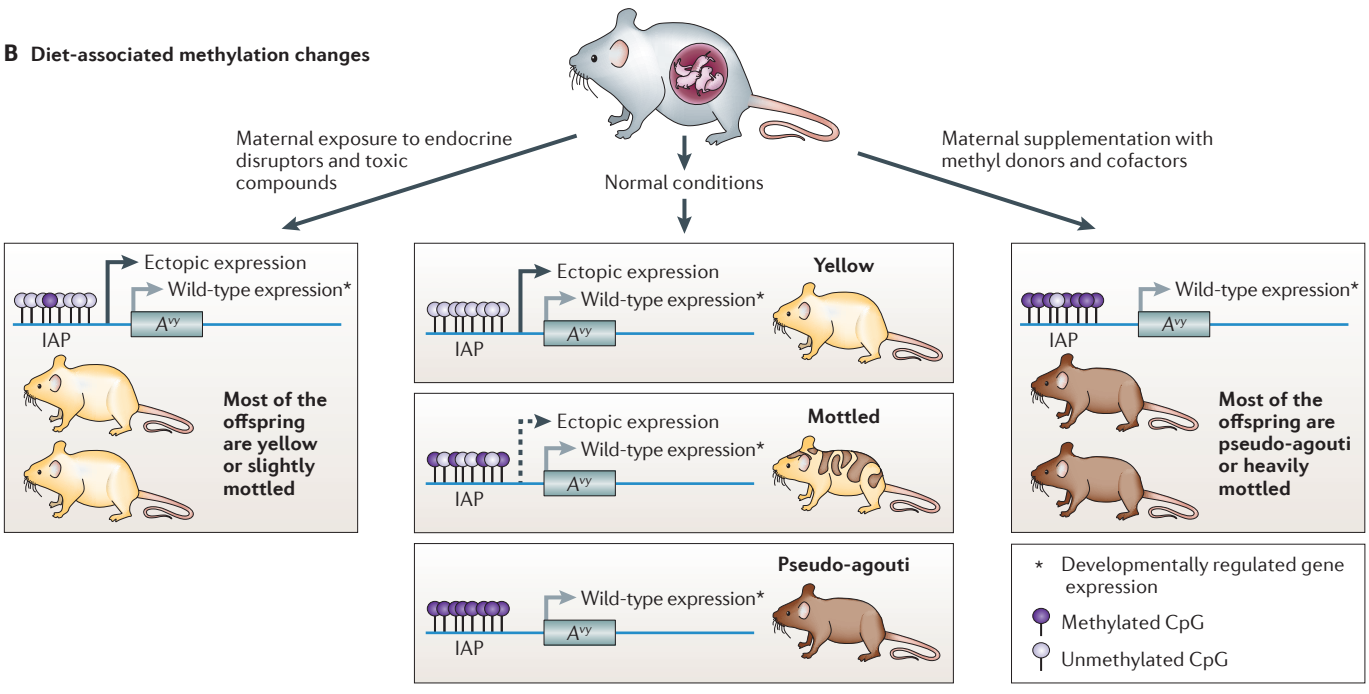
Metastable epiallele
An allele for which the expression depends on environmentally influenced, stochastic establishment of epigenetic states during early development.

Imprinted genes
Genes that express one of their two alleles only, in a parent-of-origin-specific manner.

Folate
(Vitamin B9). A water-soluble B vitamin that is abundant in green vegetables and fruits. Folate derivatives are important substrates in many one-carbon-transfer reactions.

Methionine
An essential amino acid that is abundant in fish, eggs and some seeds and vegetables.





◀ Figure 2 | **Environmentally induced epigenetic phenotypes in plants and animals.** **A** | Vernalization in plants. In *Arabidopsis thaliana*, the *FLOWERING LOCUS C (FLC)* gene encodes a transcription factor that functions as a repressor of flowering. Before vernalization, the active chromatin mark of histone H3 trimethylated on lysine-4 (H3K4me3; shown by green circles) is linked to *FLC* expression (**Aa**). Winter temperatures initially induce the transcription of *COOLAIR* antisense transcripts[90]. Subsequently, there is recruitment of different Polycomb repressive complex 2 (PRC2) components including VERNALIZATION 2 (VRN2) and the PHD finger proteins VRN5 and VERNALIZATION INSENSITIVE 3 (VIN3; expression of which is cold-induced[140]), and of protein arginine *N*-methyltransferase 5 (PRMT5; also known as SKB1) (**Ab**). This brings about repressive chromatin, which is characterized by H3K27me3, H3K9me3 and H4 arginine-3 symmetrical dimethylation (H4R3me2s). After the winter period the repressive chromatin is maintained, thus preventing *FLC* expression and allowing early flowering of the growing plant (**Ac**). **B** | Agouti viable yellow (A^{vy}) mice. The A^{vy} allele originated through retrotransposition of an intracisternal A-particle (IAP) upstream of the canonical wild-type transcription start site (shown by grey right-angled arrows) of the agouti gene[141]. These mice are heterozygous for the A^{vy} allele, and the other allele (*a*) produces no functional agouti protein. Alternative transcription (shown by black right-angled arrows) of the A^{vy} allele starts from a cryptic promoter at the IAP element, and is inhibited by methylation of surrounding CpG dinucleotides[142]. IAP methylation is established in the early embryo, and is somatically maintained throughout subsequent development (central panels). Aberrant (high) A^{vy} expression leads to the agouti (yellow) phenotype; unperturbed (low) expression results in the pseudo-agouti (brown) phenotype; and intermediate expression states produce a large variety of variegated phenotypes. Because A^{vy} expression depends on the methylation status of the IAP, the coat colour of A^{vy} mice ultimately depends on this epigenetic trait. Maternal diet supplementation with folate and other compounds that affect the methionine cycle leads to IAP methylation and A^{vy} repression in the offspring, which are consequently pseudo-agouti[95] (right panel). Maternal exposure to endocrine disruptors or toxic compounds can induce the overexpression of A^{vy} through hypomethylation of IAP (left panel)[96,97,99]. As a result, the offspring exhibit the agouti (yellow) phenotype. In the published studies, the mothers were generally pseudo-agouti or mottled. **C** | Eye colour changes in *Drosophila melanogaster*. Red eye colour in *D. melanogaster* depends on the expression of the *White* gene. Translocation of *White* next to pericentric heterochromatin (the speak white-mottled-4 mutation) leads to a mosaic pattern of expression of *White*, and thus a variegated (white or red) eye colour. In white cells, the stress-responsive transcription factor ATF2 mediates *White* repression. This process could be mediated by the recruitment of histone methyltransferases (HMTs) that establish repressive H3K9me2 marks[91] (**Ca**). Heat shock or osmotic stress during early larval development lead to p38-dependent ATF2 phosphorylation, release of ATF2 from the *White* promoter, and subsequent gene activation[91] (**Cb**). The resulting active chromatin state is somatically maintained and adult flies display completely red eyes. Pol II, RNA polymerase II.

supplementation[52,53] during gestation are associated with small alterations in DNA methylation at imprinted genes and at putative metastable epialleles in the peripheral blood of the offspring. The peri-conceptional period and early embryonic stages of development seem particularly sensitive. Studies on the Dutch hunger winter, at the end of the Second World War, indicate that famine exposure in the peri-conceptional period led to adverse metabolic and mental phenotypes in the next generation. Initial studies reported a minor, but significant, decrease in DNA methylation at a differentially methylated region (DMR) in the imprinted insulin-like growth factor 2 (*IGF2*) gene[38]. Further research[39] identified persistent, small alterations in DNA methylation at other imprinted genes such as insulin (*INS*), guanine nucleotide binding protein α-stimulating (*GNAS*) and maternally expressed gene 3 (*MEG3*). Importantly, such alterations were also found at some loci that are involved in growth and metabolic disease, such as interleukin 10 (*IL10*), leptin (*LEP*) and ATP-binding cassette A1 (*ABCA1*). Famine exposure later during gestation had no significant effects on DNA methylation[38]. Recently, Waterland and colleagues[40] identified various genes for which methylation levels varied considerably between individuals, even within pairs of monozygotic twins. To test whether peri-conceptional nutritional states could affect these putative metastable epialleles, the authors studied peripheral blood leukocytes in children from rural Gambia that were conceived during either the dry season or the rainy season. The rainy season is nutritionally challenging and children conceived during this season showed slightly increased CpG methylation at some of the loci. The genes that were found to be affected in the Dutch hunger winter cohorts were not altered in the Gambia study, indicating that different nutritional challenges have different epigenetic outcomes.

Postnatal effects. Environmental conditions can affect the epigenome postnatally as well. Studies in monozygotic twins revealed epigenetic changes that could, at least in part, be environmentally induced[9]. Data obtained in a recent longitudinal study in young monozygotic twins demonstrate that locus-specific inter-individual DNA methylation differences arise, in part, after birth[10].

One epidemiological study identified a possible link between particulate air pollution and global DNA methylation in blood cells[54]. Global methylation was also altered in the blood of individuals that had been exposed to the carcinogen benzene[55]. A possibly confounding factor is that environmental stress can alter the relative abundance of different cell types in the blood. Such shifts could lead to altered levels of measured DNA methylation for specific genes. In addition, DNA methylation itself is associated with cell fate determination in haematopoiesis, and its perturbation could affect the cell populations that constitute peripheral blood[11,56]. To avoid such confounding effects, ideally other cell lineages and tissues should be explored as well, if experimental design allows one to do so.

Epidemiological studies on skin and other tissues identified locus-specific DNA methylation changes associated with various environmental factors, including chronic exposure to sunlight, asbestos and tobacco smoke, consumption of alcohol and use of hair-dye[57–59]. The relationship between tobacco smoke and altered DNA methylation seems particularly relevant given that several tumour suppressor genes show increased promoter methylation in healthy lung cells of smokers and of former smokers[60]. Conversely, the coagulation factor II receptor-like 3 (*F2RL3)* gene locus is hypomethylated in the peripheral blood of smokers[61]. It remains to be determined whether these aberrant methylation patterns are involved in lung carcinogenesis. In these and similar studies (TABLE 1), it is also unknown whether the observed epigenetic changes are directly mediated by the toxic compound under study. Because not all tissues of an adult organism are equally exposed to chemical pollutants, and because sensitivity depends on the tissue type, epigenetic alterations in adults are probably tissue-specific in nature. This would be interesting to explore further, particularly because most human studies so far have only considered peripheral blood.

CpG islands
GC-rich DNA sequences (of 200–2,000 bp in length) that have a high density of CpG dinucleotides. Approximately half of the mammalian genes have a CpG island near the transcription start site, often with promoter activity.

Vitamin B12
(Cobalamin). A vitamin that is abundant in meat, seafood, eggs and dairy foods. It is a fundamental cofactor in the regeneration of methionine from homocysteine, and in other biochemical reactions.

Table 1 | **Chemicals and pollutants that affect health and induce epigenetic alterations**

Compound	Species	Ontogenic stage	Epigenetic alteration	Tissues or cell types affected	Phenotypic alterations	Refs
Tobacco smoke	Human	Adult life	Locus-specific DNA methylation and histone modifications; chromatin remodelling machinery	Lung, blood	Lung cancer?	60,61,143
Particulate air pollution	Human, Mouse	Adult life	DNA methylation	Blood, sperm	Unknown	54,69
Asbestos	Human	Adult life	DNA methylation	Pleural tissues	Susceptibility to different diseases	57
Bisphenol A (BPA)	Mouse	Embryonic development	Locus-specific DNA methylation	Systemic	Coat colour distribution of agouti viable yellow (A^{vy}) mice	99
Diethylstilbestrol (DES)	Mouse	Embryonic development	DNA methylation	Gonads	Male sexual function	144,145
Metal ions (such as chromium, cadmiun, nickel, arsenic and methylmercury)	Multiple species	Embryonic development, adult life	DNA methylation; histone modifications (for nickel)	Multiple tissues	Increased susceptibility to diseases such as cancer	Reviewed in REFS 146,147
Vinclozolin	Mouse, rat	Embryonic development	DNA methylation	Male germ cells	Altered gonad development and spermatogenesis in the male offspring	81,82
Methoxychlor	Mouse	Embryonic development, adult life	DNA methylation	Male germ cells	Altered male reproductive system	84
Silica	Human	Adult life	DNA methylation	Blood	Silicosis	148
Benzene	Human	Adult life	DNA methylation	Blood	Increased risk of AML	55
Di- and trichloroacetic acid, trichloroethylene	Mouse	Adult life	Locus-specific DNA methylation	Liver	Increased risk of hepatic cancer	Reviewed in REF. 147

AML, acute myeloid leukaemia

Explanations for differences between gestational and postnatal effects. In animals, susceptibility to epigenetic alterations could be linked to the degree of pluripotency. Whereas the epigenomes of fully differentiated cells seem to be relatively stable, the epigenome of mouse embryonic stem (ES) cells is particularly sensitive to *in vitro* culture conditions[43,62–64]. Also, culture of mammalian pre-implantation embryos can readily affect the establishment and maintenance of DNA methylation, particularly at imprinted genes[43,64]. Similarly, assisted reproduction in humans involves *in vitro* embryo culture, and is associated with an increased occurrence of imprinting-related epigenetic diseases[42]. Epigenetic instability at imprinted loci has not been reported in cultured differentiated cells.

In mammals, early embryonic cells show high expression levels of various regulators of DNA methylation and chromatin modifications, including the *de novo* DNA methyltransferases, DNMT3A and DNMT3B. The abundance of these regulatory machineries in early embryos, stem cells and germ cells is a possible reason for the susceptibility of these pluripotent cell types to environmental signals. In numerous adult cell types, many of the chromatin regulators are less active, and epigenetic marks have become stabilized (FIG. 3). In addition, stem cell differentiation itself entails stepwise chromatin transitions, so it is possible that the epigenome could be more readily perturbed by external cues during these transitions than in the fully differentiated state.

It is unclear whether embryonic and adult cells in plants also have differential susceptibility to environmental cues. Most organs of plants retain a certain degree of pluripotency, and epigenetic patterns are possibly susceptible to change in many different cell types. Indeed, temperature and osmotic stress affect the organization of heterochromatin and levels of DNA methylation at different loci in adult plants[65]. *In vitro* culture of potato cell suspensions (derived from tuber cortex) was shown to alter DNA methylation and acetylation on histones H3 and H4 (REF. 66). Given the considerable developmental plasticity in plants, it should be interesting to explore the extent to which the epigenetic (and genetic) effects of stress contribute to adaptation in subsequent generations[14].

Transgenerational inheritance

There is substantial interest in whether environmentally induced epigenetic alterations can be inherited from one generation to the next[36]. During gametogenesis, the epigenome is globally reprogrammed in mammals[4] and to a certain degree in plants as well[33,67,68]. The remodelling processes make gonadal cells particularly vulnerable to extrinsic factors, even in exposed adults[69], and may explain why apparent transgenerational effects are observed. However, true epigenetic transgenerational inheritance implies that the maternal or paternal animal or plant already had the epigenetic change and transmitted the change to its offspring, whose cells were not exposed. In mammals, only epigenetic marks

Box 2 | **Developmental origins of health and disease**

Early research revealed a relationship between obesity in adulthood and famine exposure both *in utero* and during early postnatal life[136], and demonstrated the importance of early environmental conditions for adult health and disease. Hales and Barker[51] conceptualized this idea and proposed the 'thrifty phenotype' hypothesis. Based on retrospective epidemiological studies, Hales and Barker argued that nutritional conditions during uterine development have effects much later in life, and influence the occurrence of adult metabolism and diseases[51]. They proposed that, under poor nutritional conditions, the fetal environment could modify the development of the embryo to prepare the offspring for a future environment with low resources during adult life (a thrifty phenotype). Although genetic factors also contribute[137], subsequent epidemiological studies and animal models sustained the hypothesis[37]. Exposure to other external factors — such as toxic compounds, alcohol and tobacco[96,138] — was found to affect fetal programming as well. Different nutritional cues during infancy and childhood have also been shown to have adverse effects during adult life[139]. Consequently, the thrifty phenotype theory has evolved into a more general theory known as the 'developmental origins of health and disease' (DOHaD)[37], which proposes that a wide range of environmental conditions during embryonic development and early life determine susceptibility to disease during adult life.

transmitted to the F3 generation are truly transgenerational, as the developing germ cells that give rise to the F2 generation are already present (and could thus have been exposed) during the embryonic development of the F1 generation. Although still largely unexplored, transgenerationally inherited epigenetic states could provide long-term adaptation to changing environmental conditions, particularly in plants[70,71].

Plants. In flowering plants, DNA methylation patterns at transgenes and endogenous loci are often inherited from one generation to the next[27,72–74]. This suggests that, in contrast to the germ lines in mammals[75], plants do not undergo extensive loss of DNA methylation during gametogenesis. Indeed, whereas CpG methylation is partially reprogrammed from one generation to the next[76], there could be substantial transmission of CHG and CHH methylation through plant gametogenesis[33,67,68]. By contrast, histone H3 modifications are globally reprogrammed during plant gametogenesis, and are therefore unlikely to contribute to transgenerational epigenetic inheritance[77].

Mammals. Only in exceptional cases are DNA methylation-associated phenotypes inherited from one generation to the next in mammals. The genome-wide DNA demethylation and chromatin remodelling that occur in the primordial germ cells of the developing embryo and in the pre-implantation embryo largely prevent this from happening[4,75]. However, some sequences in the genome are relatively resistant to the global DNA methylation reprogramming in mammals, including IAP elements[78]. Indeed, the A^{vy}-associated phenotypes in the mouse are determined by levels of DNA methylation at the IAP element in this locus (FIG. 2B), and can be transmitted to the next generation through the female germ line[79]. DNA methylation is probably not the primary element of heritability, however, given that this IAP element was found to be unmethylated at the blastocyst stage, and becomes remethylated only after implantation[80].

Endocrine disruptors
Chemical compounds that affect endocrine regulation and cause developmental alterations, cancer and other pathologies.

Some endocrine disruptors have phenotypic effects that can be perpetuated through the male germ line. Vinclozolin, a fungicide that is widely used in vegetable and fruit production, has anti-androgenic effects. In rats and mice, when administered by intravenous injection, vinclozolin affects gonad development and spermatogenesis in male offspring, leading to decreased sperm numbers and mobility. Intriguingly, on paternal inheritance, part of this phenotype is iterated in males of subsequent generations, up to the third generation[81,82]. In rats, this transgenerational effect correlates with small alterations in DNA methylation at about fifty different promoters in sperm[83]. It was not assessed whether altered methylation states were also present in early embryos (before germ-cell formation) and could thus be linked to the transgenerational inheritance. In mouse studies using vinclozolin, several imprinted genes showed decreases or increases in DNA methylation in sperm. Similar small effects on imprinted genes were observed in the sperm of male offspring following exposure of pregnant female mice to the endocrine disruptor methoxychlor[84]. However, somatic cells of different tissues showed completely normal levels of DNA methylation[82,84]. This leaves the question as to what could confer the transgenerational heritability, and whether this actually involves epigenetic modifications. One possible scenario is suggested by recent studies showing that at some mammalian genes, despite the genome-wide histone-to-protamine exchange during spermatogenesis[4], there is a persistence of histones and associated modifications in mature sperm[85,86]. In mammals, there is no evidence thus far that histone modifications could contribute to transgenerational epigenetic inheritance. In the nematode *Caenorhabditis elegans*, perturbation of the protein complex that regulates H3K4me3 was found to induce a transgenerational effect on longevity across three generations; the effect was linked to altered levels of this permissive modification at a subset of genes[87].

Mechanisms of alterations

How do environmental cues bring about epigenetic alterations? What are the mechanisms involved, and are their effects on the epigenome direct or indirect? It is here that knowledge is lacking, particularly in humans, thus hampering progress in the field. Nevertheless, experimental model systems have provided valuable insights into the molecular mechanisms that are involved in temperature- and diet-mediated effects.

Temperature effects. Plants and animals use various strategies to respond to changes in ambient temperature. Recent work in *Arabidopsis thaliana* shows that, in plants, short-term adaptation to temperature changes is partly mediated through a general mechanism that involves the variant histone H2AZ[88]. Nucleosomes containing H2AZ have DNA-unwrapping properties. Cooler ambient temperatures favour increased H2AZ incorporation, whereas temperature elevation leads to reduced incorporation of H2AZ. This differential histone-variant enrichment mediates levels of gene expression that are appropriate for the ambient temperature[88].

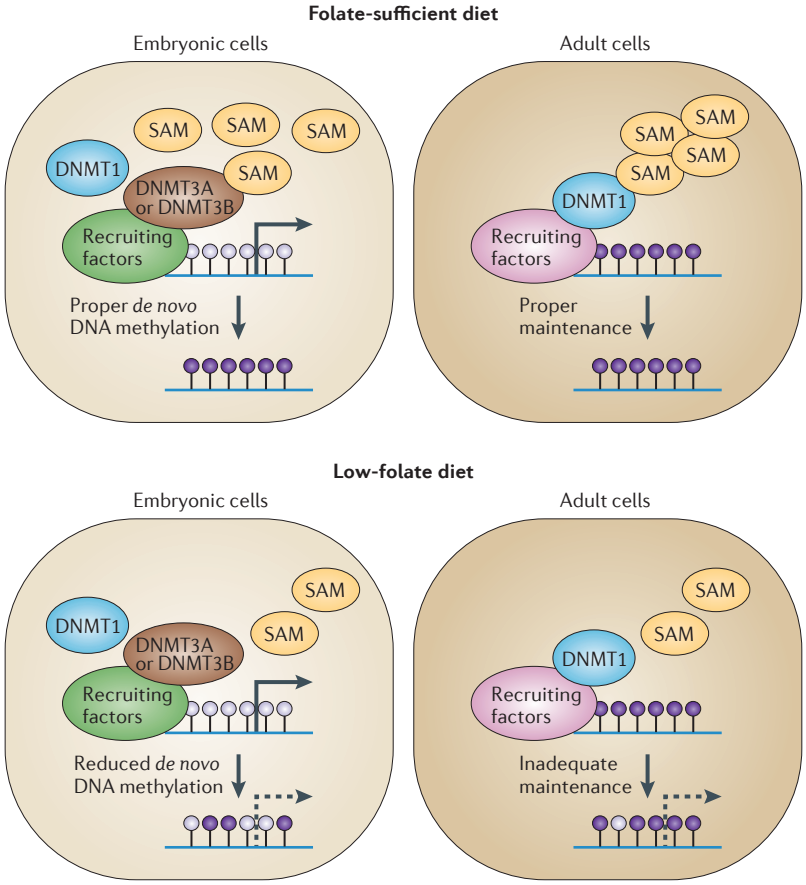


Figure 3 | **Time windows of environmental susceptibility in mammals.** Epigenetic transitions play crucial roles in development and in the differentiation of stem cells and primordial germ cells. Concordantly, the regulating enzymes are generally highly expressed in these pluripotent cells[6]. For example, the *de novo* methyltransferases, DNMT3A and DNMT3B, are highly expressed in the early embryonic cells in which *de novo* methylation is acquired. Low amounts of external methyl donor groups from dietary sources can reduce the concentrations of the universal methyl donor, *S*-adenosylmethionine (SAM), and can readily affect *de novo* DNA methylation. Also, aberrant gains of methylation may occur in early embryonic cells owing to other external triggers. In adult cells, the maintenance of DNA methylation is performed mainly by the maintenance methyltransferase, DNMT1, in a process that seems less sensitive to diet-induced changes in the abundance of methyl donors.

Polycomb group proteins
A family of chromatin-modifying proteins that are involved in chromatin silencing. They are organized into Polycomb repressive complexes (PRCs) that catalyse histone H3 lysine-27 trimethylation and histone H2A lysine-119 ubiquitination.

Vitamin B6
(Pyridoxal phosphate). A vitamin that is abundant in meat, fish and some tubers and fruits. It is a crucial cofactor in the trans-sulphuration of homocysteine and in other biological reactions.

As mentioned above, vernalization in plants is a long-term consequence of exposure to cold temperatures, and gives rise to the repression of specific genes that suppress flowering. In *A. thaliana*, the *FLOWERING LOCUS C (FLC)* gene encodes a MADS-box DNA-binding protein that functions as a repressor of flowering (FIG. 2A). Winter temperatures lead to the recruitment of Polycomb group proteins[89] and other histone-modifying enzymes, which together induce repressive chromatin. The earliest step that is known to trigger this silencing process is the expression of a long non-coding transcript that is antisense to the *FLC* gene[90]. After the winter period, the repressive chromatin is somatically maintained, thus preventing *FLC* expression and allowing early flowering of the plant (see REF. 26 for a review).

In *Drosophila melanogaster*, exposure to heat stress at a specific time during larval development triggers the loss of heterochromatin at several chromosomal domains[91]. Heat shock (and also osmotic stress) leads to phosphorylation of the transcription factor ATF2 by the stress-activated kinase p38. This results in the release of ATF2 from chromatin, which causes the loss of heterochromatin and gene derepression (FIG. 2C). Reminiscent of earlier studies in flies[92] that used the *White* eye-colour gene as a marker for heterochromatin formation in *D. melanogaster*, Ishii and colleagues[91] found that the effects of stress were sometimes transmitted to the next generation. This transgenerational inheritance occurred even when unstressed animals were mated with stressed animals, a phenomenon that could be similar to paramutation in plants (reviewed in REF. 93). Also, in the fission yeast *Schizosaccharomyces pombe*, the activity of the related protein Atf1 is modulated by stress-activated protein kinases, and stress can lead to defects in heterochromatin assembly[94].

Dietary components: DNA methylation. In mammals, many dietary components — including folate, vitamin B6, vitamin B12, betaine, methionine and choline — have been linked to changes in DNA methylation. These nutrients can all affect the pathways of one-carbon metabolism that determine the amount of available *S*-adenosylmethionine (SAM), which is the methyl donor for DNA methylation and histone methylation (FIG. 4A). Although the availability of methyl donors could be expected to have a global effect on the epigenome, locus-specific effects are reported in many studies. One such example is methylation at the A^{vy} allele in mice (see above and FIG. 2B), in which maternal nutrients that enhance the levels of available methyl donors result in increased levels of IAP element methylation[44,95]. The same phenotype was observed as a consequence of maternal ethanol consumption[96]. Also, the addition of genistein — a phyto-oestrogenic component of soy beans — to the maternal diet, led to increased DNA methylation at the locus in the offspring[97]. How genistein affects methylation is unknown, but its action seems independent of the methionine cycle because SAM and *S*-adenosylhomocysteine (SAH) concentrations remained normal[98].

Maternal food supplementation with bisphenol A (BPA) — a compound that is widely used in the manufacturing of polycarbonate plastics — led to decreased CpG methylation at the A^{vy} allele in the offspring, along with an increased frequency of yellow coat colour, obesity and diabetes[99]. BPA is an anti-androgen compound that affects endocrine regulation, and could therefore have locus-specific rather than global effects on the epigenome. Remarkably, the effects of this endocrine disruptor were counterbalanced by supplementation of the maternal diet with genistein[99]. This demonstrates how diet can protect against deleterious effects of chemical compounds, at least in this specific case. Undoubtedly, genome-wide studies will identify further loci that are particularly susceptible to changes in the methionine cycle and to endocrine disruptors such as BPA[100].

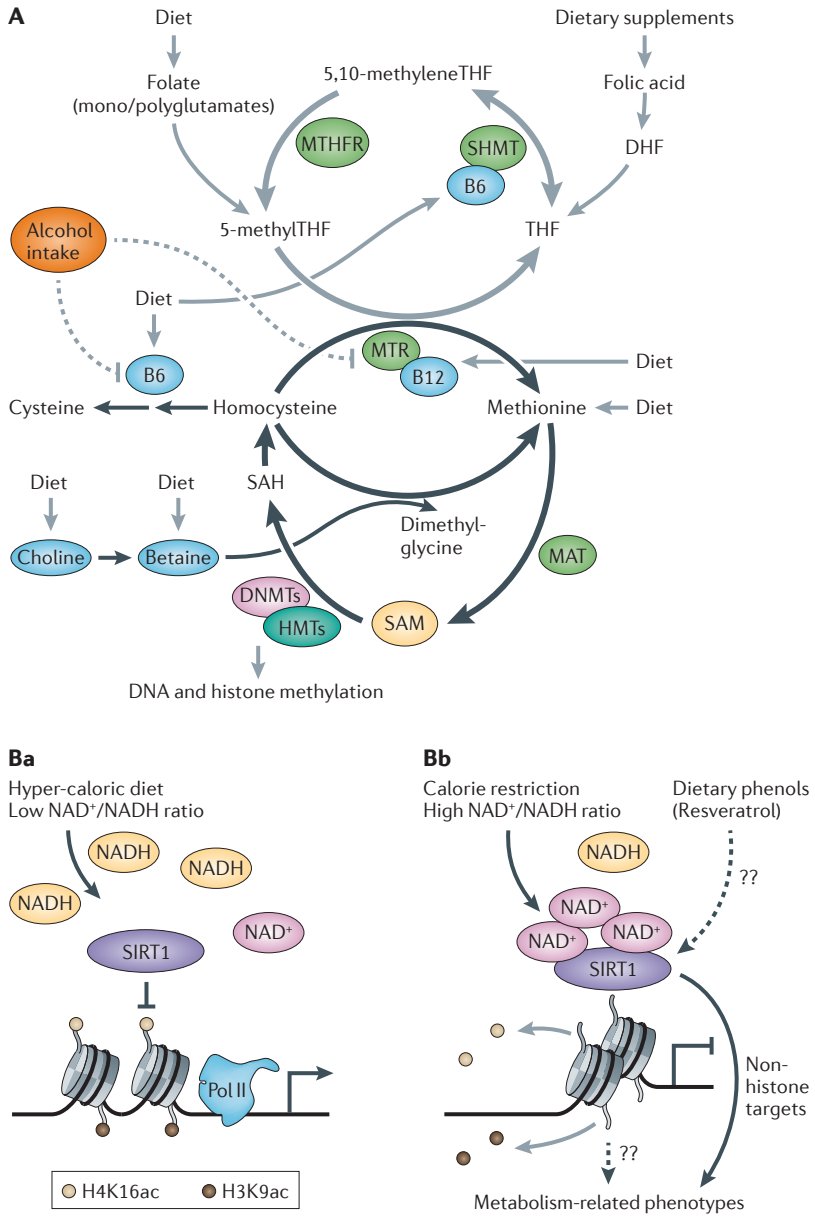


Figure 4 | **Molecular mechanisms that mediate environmental effects. A** | Levels of *S*-adenosylmethionine (SAM) affect global DNA and histone methylation. In cells, SAM is generated by the methionine cycle (also known as the one-carbon cycle; thick black arrows). The cycle incorporates methyl groups from dietary folate in another multistep cyclic pathway, called the folate cycle (thick grey arrows). The folate cycle includes the enzymes serine hydroxymethyltransferase (SHMT), methylenetetrahydrofolate reductase (MTHFR) and 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR). Before its incorporation into the folate cycle, folic acid (the synthetic form of natural folate) from dietary supplements must be converted to dihydrofolate (DHF) and then to tetrahydrofolate (THF). MTR uses methyl groups from the folate cycle to convert homocysteine to methionine. Methionine adenosyltransferase (MAT) catalyses the synthesis of SAM from methionine. SAM is then converted to *S*-adenosylhomocysteine (SAH) by DNA- and histone-methyltransferases (DNMTs and HMTs) that use its methyl group to methylate DNA and histones. SAH is hydrolysed to homocysteine to close the cycle. The methionine cycle can also incorporate methyl groups from betaine. Two important cofactors that are involved in SAM biosynthesis are vitamins B6 and B12. Vitamin B6 is involved in the conversion of homocysteine to cysteine, and of THF to 5,10-methyleneTHF. Vitamin B12 is a cofactor of MTR. Alcohol intake can have an effect on SAM production at least at two different levels: the conversion of homocysteine to methionine, and the conversion of homocysteine to cysteine (by altering the levels of vitamin B6). **B** | Sirtuins remove acetyl groups from histones and other proteins in a reaction that consumes NAD^+. Sirtuin 1 (SIRT1) specifically targets H4K16ac and H3K9ac. Hyper-caloric diets give rise to a low NAD^+/NADH ratio (**Ba**) and, consequently, low SIRT1 activity. Calorie restriction gives rise to a high NAD^+/NADH ratio (**Bb**), and can therefore increase the activity of SIRT1. Sirtuins have important roles in the establishment of the adaptive response to calorie restriction[108]. They can be activated in an indirect manner by dietary phenols such as resveratrol[109,110].

Betaine
A molecule that is abundant in whole-wheat foods and some green vegetables. Some organisms can synthesize betaine from choline.

Choline
A soluble molecule that is abundant in meat, fish, seafood, eggs, dairy foods and some vegetables, seeds and nuts.

Methyl donor
A chemical compound that can donate a methyl group. The universal methyl donor for DNA methylation and histone methylation is *S*-adenosylmethionine (SAM).

Dietary components: histone modifications. In mammals, dietary compounds have been shown to influence the activity of histone-modifying enzymes. Examples include inhibitors of histone deacetylases (HDACs), such as butyrate, which is produced from carbohydrates and organosulphur compounds from garlic and cruciferous vegetables[101]. Valproic acid, a drug that is used to treat neuronal disorders, also has a strong inhibitory effect on HDACs. In addition to effects that are mediated by histone-modifying enzymes, histone methylation may also depend on the availability of methyl donors. Although positive associations have been reported[102], further research is needed to unravel the molecular mechanisms.

Sirtuins are a class of HDACs that require nicotinamide adenine dinucleotide (NAD^+); their activity could therefore depend on nutritional and metabolic factors (FIG. 4B). In addition to roles in the deacetylation of various transcription factors that are involved in the regulation of glucose and fatty acid metabolic pathways, mammalian sirtuins target acetylated histones, such as H4K16ac[103,104]. In addition, a recent study has shown that sirtuin 1 (SIRT1) can deacetylate DNMT1 and thereby alter its activity[105]. These findings suggest that sirtuins could mediate environment-dependent epigenetic states (reviewed in REF. 106). Indeed, SIRT1 was found to regulate constitutive heterochromatin in response to calorie-restricted diets[107]. SIRT1 also targets non-histone proteins, such as PPARα[108]. Through an indirect mechanism[109], the action of sirtuins is enhanced by resveratrol[110], which is a natural polyphenolic compound that is abundant in plants and fruits (and in red wine), and that has gained considerable interest owing to its potential benefit to healthy ageing. Future research should elucidate precisely how sirtuins are involved in the long-term

metabolic and epigenetic effects of dietary conditions, and whether these metabolic sensor proteins indeed mediate stable chromatin alterations.

Genetic predisposition to environmental effects

Genetic differences between individuals can influence epigenetic regulation. For example, many autosomal mammalian genes show different levels of DNA methylation between males and females[39,111,112]. Similarly, nutritional effects on DNA methylation at specific autosomal genes can be strikingly different between males and females[39,48]. Studies on mice have also revealed marked differences in epigenetic responses to environmental factors between different inbred genotypes[34]. Furthermore, dizygotic twins show more epigenetic differences than monozygotic twins[113,114]. In plants, it is likely that genetic differences between ecotypes influence susceptibility and adaptation to environmental effects[71].

DNA sequence effects. The extent to which DNA sequence determines the levels of epigenetic modifications at specific loci is unclear. Studies on human blood cells show that SNPs can affect the level of methylation of nearby CpG dinucleotides[115–119]. In humans, at least 10% of common SNPs reside in regions that have a propensity for local DNA methylation differences between different alleles[116].

Studies on ageing indicate that epigenetic variation over time preferentially occurs at specific loci[120,121]. Certain loci are more susceptible than others, but the reasons for this remain poorly understood. However, based on various studies it seems that CpG-rich regions tend to gain DNA methylation over time, whereas CpG-poor sequences tend to lose methylation[7,57]. Furthermore, sequences that are prone to acquiring DNA hypermethylation during ageing are often enriched in 'bivalent chromatin'[120,121]. DNA methylation in some sequence contexts, including the CpG islands that control imprinted gene expression, is particularly stable during ageing[38,122]; this could be linked to particular chromatin configurations at these loci, which have similarities to pericentric heterochromatin[123]. The resilience of some sequences to changes over time could be linked to the mechanisms that maintain DNA methylation at these loci.

Many protein complexes are involved in the regulation of DNA methylation and histone modifications in plants and mammals[2]. Histone modifications, and possibly also DNA methylation[124], are controlled by the balance between activities that either establish or remove the marks. Levels of specific proteins may vary between individuals and also between individual cells, and this could shift the balance. Mouse studies have shown that artificially modified levels of *Dnmt1* expression give rise to altered levels of DNA methylation at specific gene loci[125,126]. Although this has not been studied systematically, smaller, naturally occurring variations in *Dnmt1* expression might also affect DNA methylation levels and may influence responses to the environment. Variations in other proteins are also likely to affect epigenetic responses. A classic example concerns 5,10-methylenetetrahydrofolate reductase (MTHFR), which is part of the folate metabolic cycle (FIG. 4A). SNPs in *MTHFR* give rise to less-active protein variants; these affect DNA methylation levels and susceptibility to nutritional and environmental cues[127]. Studies on pairs of monozygotic twins have shown that some twin couples present more epigenetic differences than others[9,10,128–130]. Besides the differences in environmental exposure between the two individuals in a pair, one explanation could be genetic variation between different pairs of twins at genes that code for chromatin-modifying enzymes and other key proteins[8]. In the design of human environmental studies, genetic susceptibility to epigenetic change needs to be taken into account.

Conclusions

Environmental factors can have long-lasting effects on gene expression and chromatin. Despite considerable progress during recent years, many questions remain. Apart from natural processes, including vernalization, it remains largely unknown how the environment triggers alterations in the epigenome. Unravelling the underlying molecular mechanisms will be a daunting task, but it is important to understand whether the observed methylation and chromatin alterations are directly or indirectly linked to the toxic, dietary or ambient factors that are being studied. Cell-based approaches could be instructive tools for such mechanistic studies, as was shown for HDAC inhibitors[101].

Another key issue concerns the biological relevance of the observed epigenetic alterations. In many animal and human studies, only minor changes at specific loci were detected, and it is unknown whether there could be broader epigenetic effects as well. Exploration of why specific loci are affected requires the identification and characterization of the factors that recruit chromatin-modifying enzymes to these loci. Because transcription and non-coding RNAs can be involved in the recruitment of epigenetic modifiers to specific loci[13], there is the intriguing possibility that the perturbation of transcription could give rise to altered epigenetic patterns. Whether such a process contributes, for example, to the locus-specific changes that arise following exposure to endocrine disruptors is unknown, but would be interesting to explore.

To conclude that the epigenome is altered because of the environmental factors themselves is not straightforward. Developmental and pathological phenotypes may readily change the relative contribution of cell populations in a tissue, and thus alter the measured levels of epigenetic modifications at specific loci. In the case of systemic epigenetic alterations, one way to overcome this hurdle is to analyse different tissues and cells; this approach has been taken in several recent studies[40,114,120]. As discussed above, genetic variation also needs to be considered. Studies in model organisms have the distinct advantage that inbred animals are often available. However, in some inbred mouse lines, environmental cues have stronger effects than in others[34]. Also, intrinsic epigenetic drift could depend on genetic factors and could give more variable outcomes in some genetic backgrounds than in others[131].

Butyrate
A short-chain carboxylic acid that is produced by bacteria in the gut as an end product of the fermentation of dietary carbohydrates.

Sirtuins
A family of proteins that couple lysine deacetylation to NAD^+.

Bivalent chromatin
Regions of chromatin that have co-occurrence of histone H3 trimethylated on lysine-27 (H3K27me3) and H3K4me2/3 during embryonic development.

Research will be boosted by the expanding use of next-generation DNA sequencing technologies[132]. Applications include chromatin immunoprecipitation followed by DNA sequencing (ChIP–seq) — to assess the genomic distribution of histone modifications, histone variants and nuclear proteins — and global DNA methylation analysis through the sequencing of bisulphite-converted genomic DNA. Combined with appropriate statistical and bioinformatic tools[133], these methods will permit the identification of all the loci that are epigenetically altered. It remains difficult to systematically assess the epigenetic statuses of repeat sequences in the genome, including retrotransposons, as repeat sequences are often excluded from bioinformatic analyses. In plants and mammals, DNA methylation at repeat sequences is regulated through specific mechanisms[2] and could be particularly susceptible to certain extrinsic factors. Genome sequences of many wild animal and plant species are rapidly becoming available. For example, the aphid and *Daphnia pulex* genomes were sequenced recently[19], opening the door for epigenomic exploration of the environmentally induced phenotypes in these arthropods.

It seems too early to apply epigenetic alterations that are induced by diet and chemical compounds as biomarkers in public health and medicine. Unlike in cancer[134], environmentally induced DNA methylation changes often are small, and variable among exposed individuals. In the coming years, genome-wide approaches will further define the loci that are susceptible to environmental insults and could yield important results for common diseases, such as obesity and diabetes, that are influenced by dietary conditions[37]. Combinations of loci that are susceptible to environmentally induced epigenetic changes could be explored in cohorts of exposed populations. Recent epidemiological studies in humans have identified lasting effects of nutritional intake on metabolism and development, often with associations to specific diseases. If epigenetic changes can be validated in large-scale human studies, they could be used to monitor the effects of diet and other extrinsic factors over time, possibly even across generations. There is still a long way to go, but the coming years will undoubtedly provide further insights into the involvement of epigenetics in environmentally triggered phenotypes and diseases.

1. Henikoff, S. & Shilatifard, A. Histone modification: cause or cog? *Trends Genet.* **27**, 389–396 (2011).
2. Law, J. A. & Jacobsen, S. E. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nature Rev. Genet.* **11**, 204–220 (2010).
3. Russo, V. E. A., Martienssen, R. A. & Riggs, A. D. *Epigenetic Mechanisms of Gene Regulation*, (Cold Spring Harbor Laboratory Press, New York, 1996).
4. Kota, S. K. & Feil, R. Epigenetic transitions in germ cell development and meiosis. *Dev. Cell* **19**, 675–686 (2010).
5. Okano, M., Bell, D. W., Haber, D. A. & Li, E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell* **99**, 247–257 (1999).
6. Reik, W. Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* **447**, 425–432 (2007).
7. Bjornsson, H. T. *et al.* Intra-individual change over time in DNA methylation with familial clustering. *JAMA* **299**, 2877–2883 (2008).
8. Fraga, M. F. Genetic and epigenetic regulation of aging. *Curr. Opin. Immunol.* **21**, 446–453 (2009).
9. Fraga, M. F. *et al.* Epigenetic differences arise during the lifetime of monozygotic twins. *Proc. Natl Acad. Sci. USA* **102**, 10604–10609 (2005).
10. Wong, C. C. *et al.* A longitudinal study of epigenetic variation in twins. *Epigenetics* **5**, 516–526 (2010).
11. Borgel, J. *et al.* Targets and dynamics of promoter DNA methylation during early mouse development. *Nature Genet.* **42**, 1093–1100 (2010).
12. Zhou, V. W., Goren, A. & Bernstein, B. E. Charting histone modifications and the functional organization of mammalian genomes. *Nature Rev. Genet.* **12**, 7–18 (2011).
13. Pauli, A., Rinn, J. L. & Schier, A. F. Non-coding RNAs as regulators of embryogenesis. *Nature Rev. Genet.* **12**, 136–149 (2011).
14. Ito, H. *et al.* An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. *Nature* **472**, 115–119 (2011).
This study explores the role of the siRNA pathway in preventing the transgenerational genetic effects of stress-induced alterations in plants.
15. Mirouze, M. & Paszkowski, J. Epigenetic contribution to stress adaptation in plants. *Curr. Opin. Plant Biol.* **14**, 267–274 (2011).
16. Jirtle, R. L. & Skinner, M. K. Environmental epigenomics and disease susceptibility. *Nature Rev. Genet.* **8**, 253–262 (2007).
17. Borrelli, E., Nestler, E. J., Allis, C. D. & Sassone-Corsi, P. Decoding the epigenetic language of neuronal plasticity. *Neuron* **60**, 961–974 (2008).
18. Hackman, D. A., Farah, M. J. & Meaney, M. J. Socioeconomic status and the brain: mechanistic insights from human and animal research. *Nature Rev. Neurosci.* **11**, 651–659 (2010).
19. Simon, J. C., Pfrender, M. E., Tollrian, R., Tagu, D. & Colbourne, J. K. Genomics of environmentally induced phenotypes in 2 extremely plastic arthropods. *J. Hered.* **102**, 512–525 (2011).
20. Kucharski, R., Maleszka, J., Foret, S. & Maleszka, R. Nutritional control of reproductive status in honeybees via DNA methylation. *Science* **319**, 1827–1830 (2008).
This study provides evidence that the nutrition-dependent phenotype determination in honey bees is highly dependent on DNA methylation.
21. Lyko, F. *et al.* The honey bee epigenomes: differential methylation of brain DNA in queens and workers. *PLoS Biol.* **8**, e1000506 (2010).
22. Khosla, S., Mendiratta, G. & Brahmachari, V. Genomic imprinting in the mealybugs. *Cytogenet. Genome Res.* **113**, 41–52 (2006).
23. Sanchez, L. *Sciara* as an experimental model for studies on the evolutionary relationships between the zygotic, maternal and environmental primary signals for sexual development. *J. Genet.* **89**, 325–331 (2010).
24. Marshall Graves, J. A. Weird animal genomes and the evolution of vertebrate sex and sex chromosomes. *Annu. Rev. Genet.* **42**, 565–586 (2008).
25. Chinnusamy, V. & Zhu, J. K. Epigenetic regulation of stress responses in plants. *Curr. Opin. Plant Biol.* **12**, 133–139 (2009).
26. Kim, D. H., Doyle, M. R., Sung, S. & Amasino, R. M. Vernalization: winter and the timing of flowering in plants. *Annu. Rev. Cell Dev. Biol.* **25**, 277–299 (2009).
27. Cubas, P., Vincent, C. & Coen, E. An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* **401**, 157–161 (1999).
28. Herrera, C. M. & Bazaga, P. Epigenetic differentiation and relationship to adaptive genetic divergence in discrete populations of the violet *Viola cazorlensis*. *New Phytol.* **187**, 867–876 (2010).
29. Paun, O. *et al.* Stable epigenetic effects impact adaptation in allopolyploid orchids (*Dactylorhiza*: Orchidaceae). *Mol. Biol. Evol.* **27**, 2465–2473 (2010).
30. Lira-Medeiros, C. F. *et al.* Epigenetic variation in mangrove plants occurring in contrasting natural environment. *PLoS ONE* **5**, e10326 (2010).
31. Martin, A. *et al.* A transposon-induced epigenetic change leads to sex determination in melon. *Nature* **461**, 1135–1138 (2009).
32. Verhoeven, K. J., Jansen, J. J., van Dijk, P. J. & Biere, A. Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytol.* **185**, 1108–1118 (2010).
Using genetically identical plants as experimental models, this study identified generalized stress-associated epigenetic changes and showed that they are frequently transmitted to the next generation.
33. Paszkowski, J. & Grossniklaus, U. Selected aspects of transgenerational epigenetic inheritance and resetting in plants. *Curr. Opin. Plant Biol.* **14**, 195–203 (2011).
34. Rosenfeld, C. S. Animal models to study environmental epigenetics. *Biol. Reprod.* **82**, 473–488 (2010).
35. Rakyan, V. K., Blewitt, M. E., Druker, R., Preis, J. I. & Whitelaw, E. Metastable epialleles in mammals. *Trends Genet.* **18**, 348–351 (2002).
36. Daxinger, L. & Whitelaw, E. Transgenerational epigenetic inheritance: more questions than answers. *Genome Res.* **20**, 1623–1628 (2010).
37. Gluckman, P. D., Hanson, M. A., Buklijas, T., Low, F. M. & Beedle, A. S. Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. *Nature Rev. Endocrinol.* **5**, 401–408 (2009).
38. Heijmans, B. T. *et al.* Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc. Natl Acad. Sci. USA* **105**, 17046–17049 (2008).
39. Tobi, E. W. *et al.* DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum. Mol. Genet.* **18**, 4046–4053 (2009).
40. Waterland, R. A. *et al.* Season of conception in rural gambia affects DNA methylation at putative human metastable epialleles. *PLoS Genet.* **6**, e1001252 (2010).
This study identifies for the first time putative metastable epialleles in humans, and shows how their methylation status is influenced by nutritional conditions during gestation.
41. Ferguson-Smith, A. C. Genomic imprinting: the emergence of an epigenetic paradigm. *Nature Rev. Genet.* **12**, 565–575 (2011).
42. Hirasawa, R. & Feil, R. Genomic imprinting and human disease. *Essays Biochem.* **48**, 187–200 (2010).
43. Khosla, S., Dean, W., Brown, D., Reik, W. & Feil, R. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biol. Reprod.* **64**, 918–926 (2001).
44. Waterland, R. A. & Jirtle, R. L. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol. Cell. Biol.* **23**, 5293–5300 (2003).

45. Sandovici, I. *et al.* Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the *Hnf4a* gene in rat pancreatic islets. *Proc. Natl Acad. Sci. USA* **108**, 5449–5454 (2011).
46. Aagaard-Tillery, K. M. *et al.* Developmental origins of disease and determinants of chromatin structure: maternal diet modifies the primate fetal epigenome. *J. Mol. Endocrinol.* **41**, 91–102 (2008).
47. Gallou-Kabani, C. *et al.* Sex- and diet-specific changes of imprinted gene expression and DNA methylation in mouse placenta under a high-fat diet. *PLoS ONE* **5**, e14398 (2010).
48. Sinclair, K. D. *et al.* DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proc. Natl Acad. Sci. USA* **104**, 19351–19356 (2007).
This broad study reports epigenetic and physiological alterations in the offspring of sheep that were fed diets poor in compounds that are involved in methyl-donor pathways.
49. Lillycrop, K. A., Phillips, E. S., Jackson, A. A., Hanson, M. A. & Burdge, G. C. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J. Nutr.* **135**, 1382–1386 (2005).
50. Carone, B. R. *et al.* Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. *Cell* **143**, 1084–1096 (2010).
51. Hales, C. N. & Barker, D. J. The thrifty phenotype hypothesis. *Br. Med. Bull.* **60**, 5–20 (2001).
52. Hoyo, C. *et al.* Methylation variation at *IGF2* differentially methylated regions and maternal folic acid use before and during pregnancy. *Epigenetics* **6**, 928–936 (2011).
53. Steegers-Theunissen, R. P. *et al.* Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the *IGF2* gene in the very young child. *PLoS ONE* **4**, e7845 (2009).
54. Baccarelli, A. *et al.* Rapid DNA methylation changes after exposure to traffic particles. *Am. J. Respir. Crit. Care Med.* **179**, 572–578 (2009).
55. Bollati, V. *et al.* Changes in DNA methylation patterns in subjects exposed to low-dose benzene. *Cancer Res.* **67**, 876–880 (2007).
56. Calvanese, V. *et al.* A promoter DNA demethylation landscape of human hematopoietic differentiation. *Nucleic Acids Res.* 12 Sep 2011 (doi:10.1093/nar/gkr685).
57. Christensen, B. C. *et al.* Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. *PLoS Genet.* **5**, e1000602 (2009).
58. Langevin, S. M. *et al.* The influence of aging, environmental exposures and local sequence features on the variation of DNA methylation in blood. *Epigenetics* **6**, 908–919 (2011).
59. Grönniger, E. *et al.* Aging and chronic sun exposure cause distinct epigenetic changes in human skin. *PLoS Genet.* **6**, e1000971 (2010).
This epidemiological study explored the epigenetic effects of sun exposure on the skin and compared these observed differences with those that arose on ageing.
60. Belinsky, S. A. *et al.* Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers. *Cancer Res.* **62**, 2370–2377 (2002).
This study established for the first time the association between smoking and aberrant hypermethylation of tumour suppressor genes in non-transformed lung cells.
61. Breitling, L. P., Yang, R., Korn, B., Burwinkel, B. & Brenner, H. Tobacco-smoking-related differential DNA methylation: 27K discovery and replication. *Am. J. Hum. Genet.* **88**, 450–457 (2011).
62. Dean, W. *et al.* Altered imprinted gene methylation and expression in completely ES cell-derived mouse fetuses: association with aberrant phenotypes. *Development* **125**, 2273–2282 (1998).
63. Doherty, A. S., Mann, M. R., Tremblay, K. D., Bartolomei, M. S. & Schultz, R. M. Differential effects of culture on imprinted *H19* expression in the preimplantation mouse embryo. *Biol. Reprod.* **62**, 1526–1535 (2000).
64. Young, L. E. *et al.* Epigenetic change in *IGF2R* is associated with fetal overgrowth after sheep embryo culture. *Nature Genet.* **27**, 153–154 (2001).
65. Pecinka, A. *et al.* Epigenetic regulation of repetitive elements is attenuated by prolonged heat stress in *Arabidopsis*. *Plant Cell* **22**, 3118–3129 (2010).
66. Law, R. D. & Suttle, J. C. Chromatin remodeling in plant cell culture: patterns of DNA methylation and histone H3 and H4 acetylation vary during growth of asynchronous potato cell suspensions. *Plant Physiol. Biochem.* **43**, 527–534 (2005).
67. Jullien, P. E. & Berger, F. DNA methylation reprogramming during plant sexual reproduction? *Trends Genet.* **26**, 394–399 (2010).
68. Teixeira, F. K. & Colot, V. Repeat elements and the *Arabidopsis* DNA methylation landscape. *Heredity* **105**, 14–23 (2010).
69. Yauk, C. *et al.* Germ-line mutations, DNA damage, and global hypermethylation in mice exposed to particulate air pollution in an urban/industrial location. *Proc. Natl Acad. Sci. USA* **105**, 605–610 (2008).
70. Johannes, F. *et al.* Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet.* **5**, e1000530 (2009).
71. Richards, E. J. Natural epigenetic variation in plant species: a view from the field. *Curr. Opin. Plant Biol.* **14**, 204–209 (2011).
72. Manning, K. *et al.* A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nature Genet.* **38**, 948–952 (2006).
73. Saze, H. & Kakutani, T. Heritable epigenetic mutation of a transposon-flanked *Arabidopsis* gene due to lack of the chromatin-remodeling factor DDM1. *EMBO J.* **26**, 3641–3652 (2007).
74. Teixeira, F. K. *et al.* A role for RNAi in the selective correction of DNA methylation defects. *Science* **323**, 1600–1604 (2009).
75. Sasaki, H. & Matsui, Y. Epigenetic events in mammalian germ-cell development: reprogramming and beyond. *Nature Rev. Genet.* **9**, 129–140 (2008).
76. Schmitz, R. J. *et al.* Transgenerational epigenetic instability is a source of novel methylation variants. *Science* **334**, 369–373 (2011).
77. Ingouff, M. *et al.* Zygotic resetting of the HISTONE 3 variant repertoire participates in epigenetic reprogramming in *Arabidopsis*. *Curr. Biol.* **20**, 2137–2143 (2010).
78. Lane, N. *et al.* Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* **35**, 88–93 (2003).
79. Morgan, H. D., Sutherland, H. G., Martin, D. I. & Whitelaw, E. Epigenetic inheritance at the agouti locus in the mouse. *Nature Genet.* **23**, 314–318 (1999).
80. Blewitt, M. E., Vickaryous, N. K., Paldi, A., Koseki, H. & Whitelaw, E. Dynamic reprogramming of DNA methylation at an epigenetically sensitive allele in mice. *PLoS Genet.* **2**, e49 (2006).
81. Anway, M. D., Cupp, A. S., Uzumcu, M. & Skinner, M. K. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* **308**, 1466–1469 (2005).
82. Stouder, C. & Paoloni-Giacobino, A. Transgenerational effects of the endocrine disruptor vinclozolin on the methylation pattern of imprinted genes in the mouse sperm. *Reproduction* **139**, 373–379 (2010).
83. Guerrero-Bosagna, C., Settles, M., Lucker, B. & Skinner, M. K. Epigenetic transgenerational actions of vinclozolin on promoter regions of the sperm epigenome. *PLoS ONE* **5**, e13100 (2010).
84. Stouder, C. & Paoloni-Giacobino, A. Specific transgenerational imprinting effects of the endocrine disruptor methoxychlor on male gametes. *Reproduction* **141**, 207–216 (2011).
85. Brykczynska, U. *et al.* Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. *Nature Struct. Mol. Biol.* **17**, 679–687 (2010).
86. Hammoud, S. S. *et al.* Distinctive chromatin in human sperm packages genes for embryo development. *Nature* **460**, 473–478 (2009).
87. Greer, E. L. *et al.* Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. *Nature* **479**, 365–371 (2011).
88. Kumar, S. V. & Wigge, P. A. H2A.Z-containing nucleosomes mediate the thermosensory response in *Arabidopsis*. *Cell* **140**, 136–147 (2010).
89. De Lucia, F., Crevillen, P., Jones, A. M., Greb, T. & Dean, C. A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of *FLC* during vernalization. *Proc. Natl Acad. Sci. USA* **105**, 16831–16836 (2008).
90. Swiezewski, S., Liu, F., Magusin, A. & Dean, C. Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. *Nature* **462**, 799–802 (2009).
91. Seong, K. H., Li, D., Shimizu, H., Nakamura, R. & Ishii, S. Inheritance of stress-induced, ATF-2-dependent epigenetic change. *Cell* **145**, 1049–1061 (2011).
This study describes an epigenetic, environmentally triggered phenotype in fruit flies that can be transgenerationally transmitted.
92. Cavalli, G. & Paro, R. The *Drosophila* Fab-7 chromosomal element conveys epigenetic inheritance during mitosis and meiosis. *Cell* **93**, 505–518 (1998).
93. Chandler, V. L. Paramutation's properties and puzzles. *Science* **330**, 628–629 (2010).
94. Jia, S., Noma, K. & Grewal, S. I. RNAi-independent heterochromatin nucleation by the stress-activated ATF/CREB family proteins. *Science* **304**, 1971–1976 (2004).
95. Wolff, G. L., Kodell, R. L., Moore, S. R. & Cooney, C. A. Maternal epigenetics and methyl supplements affect agouti gene expression in A^{vy}/*a* mice. *FASEB J.* **12**, 949–957 (1998).
96. Kaminen-Ahola, N. *et al.* Maternal ethanol consumption alters the epigenotype and the phenotype of offspring in a mouse model. *PLoS Genet.* **6**, e1000811 (2010).
97. Dolinoy, D. C., Weidman, J. R., Waterland, R. A. & Jirtle, R. L. Maternal genistein alters coat color and protects A^{vy} mouse offspring from obesity by modifying the fetal epigenome. *Environ. Health Perspect.* **114**, 567–572 (2006).
98. Ross, S. A. & Milner, J. A. Epigenetic modulation and cancer: effect of metabolic syndrome? *Am. J. Clin. Nutr.* **86**, s872–s877 (2007).
99. Dolinoy, D. C., Huang, D. & Jirtle, R. L. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc. Natl Acad. Sci. USA* **104**, 13056–13061 (2007).
This work shows that nutritional interventions during gestation can counteract some deleterious epigenetic effects that are induced by specific endocrine disruptors during embryonic development.
100. Weinhouse, C. *et al.* An expression microarray approach for the identification of metastable epialleles in the mouse genome. *Epigenetics* **6**, 1105–1113 (2011).
101. Dashwood, R. H. & Ho, E. Dietary histone deacetylase inhibitors: from cells to mice to man. *Semin. Cancer Biol.* **17**, 363–369 (2007).
102. Zhou, W. *et al.* Requirement of *RIZ1* for cancer prevention by methyl-balanced diet. *PLoS ONE* **3**, e3390 (2008).
103. Imai, S., Armstrong, C. M., Kaeberlein, M. & Guarente, L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **403**, 795–800 (2000).
104. Vaquero, A. *et al.* Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol. Cell* **16**, 93–105 (2004).
105. Peng, L. *et al.* SIRT1 Deacetylates the DNA methyltransferase 1 (DNMT1) protein and alters its activities. *Mol. Cell. Biol.* **31**, 4720–4734 (2011).
106. Vaquero, A. & Reinberg, D. Calorie restriction and the exercise of chromatin. *Genes Dev.* **23**, 1849–1869 (2009).
107. Bosch-Presegue, L. *et al.* Stabilization of Suv39H1 by SirT1 is part of oxidative stress response and ensures genome protection. *Mol. Cell* **42**, 210–223 (2011).
108. Rodgers, J. T. *et al.* Nutrient control of glucose homeostasis through a complex of PGC-1α and SIRT1. *Nature* **434**, 113–118 (2005).
109. Beher, D. *et al.* Resveratrol is not a direct activator of SIRT1 enzyme activity. *Chem. Biol. Drug Des.* **74**, 619–624 (2009).
110. Kaeberlein, M. *et al.* Substrate-specific activation of sirtuins by resveratrol. *J. Biol. Chem.* **280**, 17038–17045 (2005).
111. El-Maarri, O. *et al.* Gender specific differences in levels of DNA methylation at selected loci from human total blood: a tendency toward higher methylation levels in males. *Hum. Genet.* **122**, 505–514 (2007).
112. Waxman, D. J. & O'Connor, C. Growth hormone regulation of sex-dependent liver gene expression. *Mol. Endocrinol.* **20**, 2613–2629 (2006).
113. Kaminsky, Z. A. *et al.* DNA methylation profiles in monozygotic and dizygotic twins. *Nature Genet.* **41**, 240–245 (2009).
114. Ollikainen, M. *et al.* DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. *Hum. Mol. Genet.* **19**, 4176–4188 (2010).
115. Gertz, J. *et al.* Analysis of DNA methylation in a three-generation family reveals widespread genetic influence on epigenetic regulation. *PLoS Genet.* **7**, e1002228 (2011).

116. Hellman, A. & Chess, A. Extensive sequence-influenced DNA methylation polymorphism in the human genome. *Epigenetics Chromatin* **3**, 11 (2010).
117. Kerkel, K. *et al.* Genomic surveys by methylation-sensitive SNP analysis identify sequence-dependent allele-specific DNA methylation. *Nature Genet.* **40**, 904–908 (2008).
118. Murrell, A. *et al.* An association between variants in the *IGF2* gene and Beckwith-Wiedemann syndrome: interaction between genotype and epigenotype. *Hum. Mol. Genet.* **13**, 247–255 (2004).
119. Schilling, E., El Chartouni, C. & Rehli, M. Allele-specific DNA methylation in mouse strains is mainly determined by *cis*-acting sequences. *Genome Res.* **19**, 2028–2035 (2009).
120. Rakyan, V. K. *et al.* Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. *Genome Res.* **20**, 434–439 (2010).
121. Teschendorff, A. E. *et al.* Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. *Genome Res.* **20**, 440–446 (2010).
122. Heijmans, B. T., Kremer, D., Tobi, E. W., Boomsma, D. I. & Slagboom, P. E. Heritable rather than age-related environmental and stochastic factors dominate variation in DNA methylation of the human *IGF2/H19* locus. *Hum. Mol. Genet.* **16**, 547–554 (2007).
123. Henckel, A. *et al.* Histone methylation is mechanistically linked to DNA methylation at imprinting control regions in mammals. *Hum. Mol. Genet.* **18**, 3375–3383 (2009).
124. Feng, S., Jacobsen, S. E. & Reik, W. Epigenetic reprogramming in plant and animal development. *Science* **330**, 622–627 (2010).
125. Biniszkiewicz, D. *et al. Dnmt1* overexpression causes genomic hypermethylation, loss of imprinting, and embryonic lethality. *Mol. Cell. Biol.* **22**, 2124–2135 (2002).
126. Weaver, J. R. *et al.* Domain-specific response of imprinted genes to reduced DNMT1. *Mol. Cell. Biol.* **30**, 3916–3928 (2010).
127. Marini, N. J. *et al.* The prevalence of folate-remedial MTHFR enzyme variants in humans. *Proc. Natl Acad. Sci. USA* **105**, 8055–8060 (2008).
128. Baranzini, S. E. *et al.* Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. *Nature* **464**, 1351–1356 (2010).
129. Javierre, B. M. *et al.* Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. *Genome Res.* **20**, 170–179 (2010).
130. Schneider, E. *et al.* Spatial, temporal and interindividual epigenetic variation of functionally important DNA methylation patterns. *Nucleic Acids Res.* **38**, 3880–3890 (2010).
131. McLaren, A. Too late for the midwife toad: stress, variability and Hsp90. *Trends Genet.* **15**, 169–171 (1999).
132. Park, P. J. ChIP-seq: advantages and challenges of a maturing technology. *Nature Rev. Genet.* **10**, 669–680 (2009).
133. Pepke, S., Wold, B. & Mortazavi, A. Computation for ChIP-seq and RNA-seq studies. *Nature Methods* **6**, S22–S32 (2009).
134. Baylin, S. B. & Jones, P. A. A decade of exploring the cancer epigenome — biological and translational implications. *Nature Rev. Cancer* **11**, 726–734 (2011).
135. Marfil, C. F., Camadro, E. L. & Masuelli, R. W. Phenotypic instability and epigenetic variability in a diploid potato of hybrid origin, *Solanum ruiz-lealii*. *BMC Plant Biol.* **9**, 21 (2009).
136. Ravelli, G. P., Stein, Z. A. & Susser, M. W. Obesity in young men after famine exposure *in utero* and early infancy. *N. Engl. J. Med.* **295**, 349–353 (1976).
This is one of the first reports in humans showing the possible long-term effects of prenatal and early-life nutrition on adult health and disease.
137. Dunger, D. B. *et al.* Association of the *INS* VNTR with size at birth. *Nature Genet.* **19**, 98–100 (1998).
138. Baccarelli, A. *et al.* Neonatal thyroid function in Seveso 25 years after maternal exposure to dioxin. *PLoS Med.* **5**, e161 (2008).
139. Bhargava, S. K. *et al.* Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. *N. Engl. J. Med.* **350**, 865–875 (2004).
140. Sung, S. & Amasino, R. M. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature* **427**, 159–164 (2004).
141. Yen, T. T., Gill, A. M., Frigeri, L. G., Barsh, G. S. & Wolff, G. L. Obesity, diabetes, and neoplasia in yellow A^{vy}/- mice: ectopic expression of the *agouti* gene. *FASEB J.* **8**, 479–488 (1994).
142. Michaud, E. J. *et al.* Differential expression of a new dominant agouti allele A^{iapy} is correlated with methylation state and is influenced by parental lineage. *Genes Dev.* **8**, 1463–1472 (1994).
143. Hussain, M. *et al.* Tobacco smoke induces polycomb-mediated repression of Dickkopf-1 in lung cancer cells. *Cancer Res.* **69**, 3570–3578 (2009).
144. Sato, K. *et al.* Neonatal exposure to diethylstilbestrol alters expression of DNA methyltransferases and methylation of genomic DNA in the mouse uterus. *Endocr. J.* **56**, 131–139 (2009).
145. Volle, D. H. *et al.* The orphan nuclear receptor small heterodimer partner mediates male infertility induced by diethylstilbestrol in mice. *J. Clin. Invest.* **119**, 3752–3764 (2009).
146. Calvanese, V., Lara, E., Kahn, A. & Fraga, M. F. The role of epigenetics in aging and age-related diseases. *Ageing Res. Rev.* **8**, 268–276 (2009).
147. Baccarelli, A. & Bollati, V. Epigenetics and environmental chemicals. *Curr. Opin. Pediatr.* **21**, 243–251 (2009).
148. Umemura, S. *et al.* Aberrant promoter hypermethylation in serum DNA from patients with silicosis. *Carcinogenesis* **29**, 1845–1849 (2008).

Acknowledgements
We thank F. Berger, M. Constância, the reviewers and all members of our teams for helpful comments and discussions. We apologize to our colleagues whose research we were unable to review owing to the focus on selected model systems, and because of space limitations. M.F.F. is grant supported by the Spanish Ministry of Health (PS09/02454) and the 'Obra Social Cajastur'. R.F. acknowledges grant funding from the 'Institut National du Cancer', the 'Ligue Contre le Cancer', the 'Agence Nationale de la Recherche' and the UK Agency for International Cancer Research. He is affiliated to the European network EpiGeneSys.

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
Robert Feil's departmental homepage:
http://www.igmm.cnrs.fr
Mario F. Fraga's departmental homepages:
http://www.cnb.uam.es and http://www.unioviedo.es/IUOPA

ALL LINKS ARE ACTIVE IN THE ONLINE PDF