REVIEW

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# Advances in epigenetics link genetics to the environment and disease

Giacomo Cavalli[1]* & Edith Heard[2,3]*

**Epigenetic research has accelerated rapidly in the twenty-first century, generating justified excitement and hope, but also a degree of hype. Here we review how the field has evolved over the last few decades and reflect on some of the recent advances that are changing our understanding of biology. We discuss the interplay between epigenetics and DNA sequence variation as well as the implications of epigenetics for cellular memory and plasticity. We consider the effects of the environment and both intergenerational and transgenerational epigenetic inheritance on biology, disease and evolution. Finally, we present some new frontiers in epigenetics with implications for human health.**



Biologists have long sought to understand how a fertilized egg can form an organism composed of hundreds of specialized cell types, each expressing a defined set of genes. Cellular identity is now accepted to be the result of the expression of specific combinations of genes (Fig. 1). This expression pattern must be established and maintained—two distinct, but connected, processes. The pluripotency of the initial cell and the establishment of cell types depend to a large extent on the coordinated deployment of hundreds of transcription factors that bind to specific DNA sequences to activate or repress the transcription of cell lineage genes[1]. This establishment phase corresponds most closely to what is generally cited as the first definition of epigenetics by Conrad Waddington, namely the study of the mechanisms by which the genotype produces the phenotype in the context of development[2]. The maintenance phase often involves a plethora of non-DNA sequence specific chromatin cofactors that set up and maintain chromatin states through cell division and for extended periods of time—sometimes in the absence of the initial transcription factors[3]. This phase is more akin to a definition of epigenetics put forward by Nanney[4], then elaborated on by Riggs and Holliday[5–7] and further modified by Bird[8] and others[9] to mean the inheritance of alternative chromatin states in the absence of changes in the DNA sequence. DNA methylation was proposed early on as a carrier of epigenetic information with subsequent work revealing that chromatin proteins and noncoding RNAs are also important for this process[10–14]. For example, histone variants and histone modifications can influence local chromatin structure, either directly or indirectly. Such modifications can be heritable but reversible and are governed by a series of writers (that deposit them), readers (to interpret them) and erasers (to remove them). Finally, higher-order 3D chromosome folding is also thought to modulate gene expression and might contribute to inheritance[15].

Since 1942, when the word was first coined, epigenetics has been redefined multiple times[16] (Table 1). In this Review, we use epigenetics to mean "the study of molecules and mechanisms that can perpetuate alternative gene activity states in the context of the same DNA sequence". This operational definition has several implications. First, it encompasses transgenerational inheritance as well as mitotic inheritance and the persistence of gene activity or chromatin states through extended periods of time, even without cell division—for instance, in long-lived post-mitotic cells such as adult neurons. Second, the DNA sequence to be considered depends on the biological system. In mitotic inheritance, one should consider the genomic sequence of individual cells, whereas in transgenerational inheritance one should consider the DNA of the whole organism (including its microbiota, if this can contribute to inheritance). Finally, this definition explicitly extends the usage of 'epigenetic' to regulatory processes that involve molecules known to participate in epigenetic inheritance, even when not addressing the epigenetic memory function per se. We argue that this common practice should be accepted, as it conveys to non-specialists the broader field of epigenetic research. We also note that cases of inheritance that do not involve chromosomal components have been documented[14,17,18] and it will be important to study how widespread they are and whether similar phenomena occur in humans.

Here, we review the interplay between regulatory plasticity and stable epigenetic heritability, including cell fate and reprogramming events that occur during development, in response to physiological stimuli, and in disease. We discuss how noncoding RNAs, DNA methylation, heterochromatin, Polycomb and Trithorax proteins and 3D genome architecture (Box 1) can regulate both inheritance and gene expression plasticity, and how new technologies allow these phenomena to be analysed in a spatiotemporal fashion, in small numbers of cells or even single cells, and at multiple scales from the nucleotide to the chromosome (Box 2). We discuss evidence for a hotly debated topic—epigenetic inheritance across generations—particularly focusing on mammalian examples because of the potential biomedical implications. We also consider two other important new research areas: the potential influence of the environment and the effects of epigenetic changes on genome integrity. In closing, we highlight how epigenetic research may benefit human health.

## Epigenetic inheritance versus plasticity

An appreciation of the role of chromatin as a carrier of epigenetic information that can propagate active and silent activity states during cell division came from the study of different biological processes and model organisms. These include, to name but a few, heterochromatin inheritance in yeast, X-chromosome inactivation (the process by which one of the copies of the female X chromosome is silenced), or genomic imprinting (the parent-of-origin-specific repression of certain genes) in mammals; vernalization (the induction of flowering by exposure to prolonged cold during winter) in plants; position effect variegation

[1]Institute of Human Genetics, CNRS and University of Montpellier, Montpellier, France. [2]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany. [3]Collège de France, Paris, France.
*e-mail: giacomo.cavalli@igh.cnrs.fr; edith.heard@embl.org

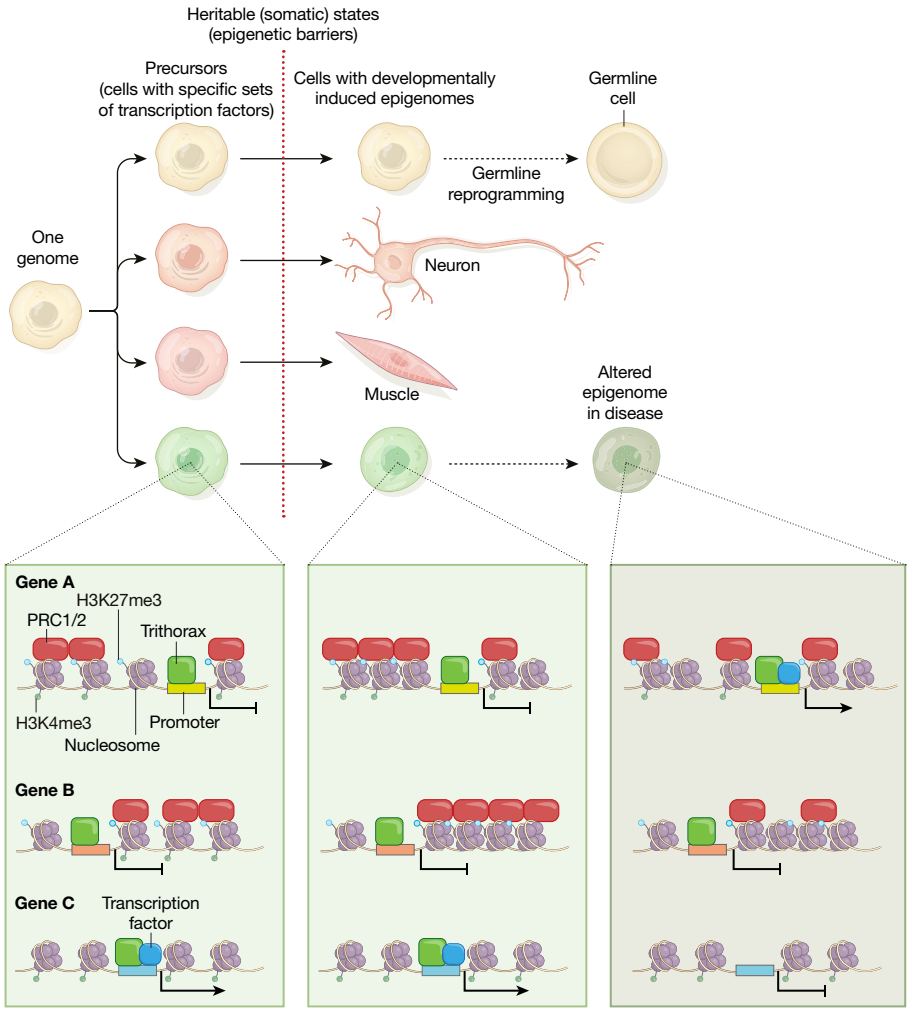


**Fig. 1 | Epigenetic mechanisms that maintain cell identities during development and throughout life.** Starting from the zygotic genome, stage- and cell-type-specific transcription factors initiate regulatory cascades that induce cell differentiation. Epigenetic components (for example, Polycomb PRC1/2 and Trithorax group proteins) maintain the 'off' states of certain genes and the 'on' states of others, in a cell-type- and time-specific manner (the bottom panels show three genes, depicted schematically as chromatinized templates, in which transcription is triggered by specific transcription factors and silent or active states are maintained by PRC1/2 or Trithorax proteins, respectively). In doing so, they constitute barriers against accidental reprogramming that maintain developmental and physiological homeostasis. Altered epigenomes can lead to changes in programmed cell differentiation or, when accidental, to disease (bottom right). Germline reprogramming resets the majority (but not all) of the epigenome to achieve reproduction (top right).

(the silencing of a gene in some cells through its abnormal juxtaposition to heterochromatin) in *Drosophila*. These studies demonstrated that differentially expressed states can be transmitted across cell divisions, once they are established and in the absence of the original signal. Studies of cellular reprogramming in the germline and early embryogenesis[19–22], during induced pluripotency (iPS)[23,24], or upon somatic nuclear transfer[25,26] have shown that chromatin and DNA methylation act as important 'epigenetic barriers' (Fig. 1) that prevent changes in gene expression and cell identity.

Epigenetic systems (Box 1) include heterochromatin (HP1 and H3K9me3 (trimethylation of histone 3 lysine 9)), Polycomb (PRC1 and PRC2) and Trithorax (COMPASS (complex proteins associated with SET1)) complexes. These complexes are thought to perpetuate functional responses by modifying histone proteins in chromatin and by binding their own histone marks in order to convey stable inheritance. Indeed, nucleosomes are subject to constant remodelling, histones are exchanged and all DNA and histone marks discovered so far are reversible, although the rates of exchange and the stability of the marks vary in different genomic domains[27]. Therefore, most regulatory signals would be rapidly lost in the absence of tight self-reinforcing loops that maintain the memory of the chromatin state[28]. Furthermore, the inheritance of epigenetic marks through cell division requires that they survive DNA replication and mitosis (Fig. 2). This is particularly relevant for histone modifications, because nucleosomes do not have a DNA template-based duplication system. Deposition of parental H3 and H4 histones occurs within few hundred base pairs of their pre-replication position and, upon replication, they are roughly equally distributed to the leading and the lagging strand daughter DNA molecules, through the action of dedicated molecular complexes[29,30]. Chromatin maturation factors, including DNMT1–UHRF1, EZH2 and HP1, use the proliferating cell nuclear antigen (PCNA; a DNA clamp that is essential for replication) or origin recognition complex (ORC) proteins as tethering components[31–34] (Fig. 2a). In addition, Polycomb components utilize their DNA-anchoring factors to propagate mitotic memory. Loss of the target DNA sequence elements results in loss of PcG proteins and of gene silencing within a few cell divisions in *Drosophila*[35,36], although sequence-independent propagation of silencing can be maintained in mammalian cell culture[37]. Mitotic retention of regulatory components (Fig. 2c), including transcription factors and some of the epigenetic machineries described above[38,39], has been well-documented in recent years[40,41]. Inheritance through meiosis is also possible at least to some extent, as shown

**Table 1 | Summary of the history and definitions of epigenetics**

| Authors | Epigenetics is the study of: | References |
|---|---|---|
| Waddington | the processes by which the genotype brings the phenotype into being | 2 |
| Nanney | the systems that regulate the expression of the 'library of specificities' (that is, the genetic material, which is meant to be the DNA or RNA sequence) | 4 |
| Riggs, Holliday, Martienssen, Russo | mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence | 5,151 |
| Bird | structural adaptations of chromosomal regions so as to register, signal or perpetuate altered activity states | 8 |
| Greally, Lappalainen | properties of a cell, mediated by genomic regulators, that confer on the cell the ability to remember a past event. | 59 |
| Nicoglou | various intracellular factors that have an effect on the stability of developmental processes through their action on genome potentialities | 16 |

by the ability of maternally deposited H3K27me3 to control DNA methylation-independent imprinting[42,43]. An additional possibility is that only a fraction of the marks can be meiotically transmitted, but this might be sufficient to reconstruct chromatin organization in the subsequent generation[44].

Owing to the lack of a precise 'replication' process for parental nucleosomes and to the loss of many DNA-binding factors and chromatin-associated components during mitosis and meiosis, the inheritance of single nucleosome marks poses specific challenges[28]. Mathematical modelling and biological evidence suggest that chromatin heritability requires the establishment of domains of several or even hundreds of kilobases in size[45–47]. Indeed, the genome is now known to be hierarchically organized in a series of 3D structures, starting from nucleosome clutches, to chromatin loops, to chromosomal domains called topologically associating domains (TADs), and finally to active or repressive compartments and chromosome territories[15,46,48–50]. TADs and compartments might stabilize functional states and drive their own inheritance. Furthermore, multiple epigenetic machineries often act together to stabilize heritable states. For example, PRC2 collaborates with PRC1 complexes and DNA methylation is sustained by heterochromatin proteins and/or small RNA pathways[51]. In summary, epigenetic inheritance can involve multiple layers; and usually entails the cooperation of partially overlapping signals, initially dependent on DNA sequence (elicited by transcription factor binding or RNA-mediated mechanisms). Each of these layers adds a degree of stability, but each of them is also reversible, allowing plasticity in the presence of regulatory cues[47,52]. The inheritance of chromatin states in the absence of chromatin domains, or without self-reinforcing mechanisms, is more challenging[28]. This might require retention of transcription factors, histone variants and histone modifiers during DNA replication and mitotic bookmarking[53].

## Epigenetics and DNA sequence variation

DNA sequence variation and epigenetics are inextricably linked. Chromatin states can influence transcription factor binding[54], and DNA sequence polymorphism influences chromatin states. Chromatin and DNA methylation display extensive variation in humans[55]. Furthermore they regulate genome stability and mutability. Transposable elements are frequent targets of epigenetic silencing that can sometimes be environmentally influenced and can influence gene expression as well as genome integrity.

## Genetic effects on epigenetics

The genome of each individual experiences both natural and environmentally induced mutations. While most mutations are neutral,

**Box 1**

## Major carriers of epigenetic information

**Heterochromatin components**

Pericentric heterochromatin contains a large number of proteins, but its most distinctive feature is the presence of megabase-sized repetitive DNA domains coated in a specific histone H3K9 trimethylation mark, which is deposited by the enzymes SUV39 and SETDB1. This mark is bound by the chromo domain of SUV39H1, which stimulates catalytic activity of the enzyme[152]. Furthermore, the same mark is bound by the HP1 protein, which can bridge adjacent nucleosomes[153]. Therefore, heterochromatin components can both write and read the H3K9me3 mark and compact their target chromatin. Heterochromatin factors also collaborate with RNAi in plants, yeast and some animals to convey epigenetic inheritance.

**Polycomb proteins**

Early genetic studies classified Polycomb (PcG) and Trithorax into two antagonistic groups that maintain the memory of spatial patterns of expression of homeotic genes throughout development. These complexes also have key roles in the maintenance of developmentally or environmentally programmed expression states, such as X-chromosome inactivation or cold-induced vernalization in plants[3]. PcG proteins are found in two main classes of complex—PRC2 and PRC1—that are responsible for deposition of the H3K27me3 and H2AK119Ub marks via EZH2 and RING1A/1B, respectively[3]. PcG proteins can be recruited to specific regions of the genome by DNA-binding proteins or noncoding RNAs[3]. PRC2 complexes contain a writer, the histone methyltransferase enzyme EZH2 (or its less efficient paralogue EZH1), and a reader, the EED subunit. Similar to HP1, CBX subunits of PRC1 complexes contain a chromodomain that specifically recognizes H3K27me3. Finally, another PRC1 subunit, PHC1-3, can oligomerize and induce 3D clustering in nuclear foci in vivo[3].

**Noncoding RNAs**

Noncoding RNAs (ncRNAs) belong to several classes, and neither their production nor their functions can be generalized. Many ncRNAs, such as microRNAs, regulate post-transcriptional processes, whereas others are involved in transcriptional regulation. Short noncoding RNAs, such as short interfering RNAs (siRNAs) and PIWI-interacting RNAs (piRNAs), are shorter than 30 nucleotides, whereas long noncoding RNAs (lncRNAs) vary in size (up to more than 100 kilobases). The best characterized of these is probably the X-inactive specific transcript (Xist)[154]. Many short ncRNAs act within or outside chromatin, and some, for example siRNAs and tRNA fragments, can diffuse extracellularly[14], whereas many nuclear lncRNAs are chromatin-associated. Enhancer RNAs can activate genes[155], but most short and long ncRNAs are repressive, act via chromatin (H3K9me3, Polycomb) or DNA methylation[154,156], and can induce epigenetic memory by building self-enforcing loops with heterochromatin or the RNAi machinery. They are also involved in the regulation of higher-order chromatin architecture.

**DNA methylation**

The mechanisms that allow DNA methylation to be copied during DNA replication represent one of the best-understood epigenetic systems, and involve specific proteins that recognize CpG hemi-methylated DNA and thereby redeposit DNA methylation on newly replicated DNA. DNA methylation is maintained by the DNA methyltransferase DNMT1 and its partner UHRF1 (also known as NP95), which specifically binds hemimethylated DNA and stimulates DNMT1 via its ubiquitin ligase activity (Fig. 2). Therefore, as recently reviewed in detail[157], a single complex contains both the 'writer' and the 'reader' of the epigenetic methyl CpG mark, and both moieties are essential for the maintenance of DNA methylation.

**Box 2**

## Novel approaches for epigenetics

The understanding of epigenetic inheritance requires the ability to tell whether progenies retain parental phenotypes. Early studies on mitotic inheritance were severely hampered by limitations in describing the molecular states of individual cells. However, modern low-cell and single-cell technologies are allowing high-throughput quantitative measurements of molecular species in a few or even single cells, and simultaneous measurement of multiple molecules, including proteins and RNAs, RNAs and DNA methylation or RNAs and chromatin accessibility[158,159]. These techniques are complemented by increasingly robust and predictive analytical tools[160]. Furthermore, techniques such as single-cell Hi-C can provide information on three-dimensional chromatin folding[161–164], which can be complemented by high-content super-resolution microscopy and molecular modelling approaches[165]. Low-cell and single-cell studies allow the investigation of individual germline cells, mature gametes, zygotes and early stages of embryonic development[166].

The study of epigenetic inheritance also requires the ability to follow molecular changes through time and cell division. Recent progress in lineage-tracing techniques has been instrumental in accomplishing this goal, as such techniques allows cell pedigrees to be established. Early tracing techniques led to the labelling of one or a small group of cells. However, more recent approaches allow prospective multiplex tracing by introducing barcodes of essentially unlimited complexity into dividing cells, as well as retrospective tracing by extensive DNA sequencing and reconstruction of the history of acquisition of spontaneous mutations. The division tree of large cell populations can thus be reconstructed[167]. Coupling lineage tracing with single-cell 'omic' technologies thus promises to make it possible to understand the gene expression histories of cell lineages.

These descriptive techniques can be complemented by the versatile toolbox of genome engineering technologies such as CRISPR–Cas. It is possible to mutate the genome precisely, reversibly and at multiple sites simultaneously[168]. Furthermore, one can tether proteins of interest to selected genomic positions in order to silence or activate genes, to induce reversible changes in 3D chromatin architecture and to visualize loci of interest in live imaging experiments[169,170]. This surge in approaches and technologies is revolutionizing the ways in which epigenetic processes can be studied, understood and harnessed to understand biological and pathological processes and to develop novel therapeutic strategies.

sequence polymorphisms can affect epigenomic landscapes. For example, analysis of chromatin accessibility and 'CCCTC-binding factor' (CTCF) DNA binding in parents and children from families with different ancestry found a substantial percentage of bound sites that were unique to each ancestry, with differential binding being explained mainly by genetic variation[56]. As CTCF can affect 3D genome architecture and gene expression, this finding suggests that rewiring of epigenomic landscapes might frequently occur as a consequence of mutations. On the other hand, mutations that affect histone and DNA methyltransferases, or demethylases (TET enzymes), chromatin remodellers and other chromatin factors including histones, are frequently found in disease[57] and their effects may be specifically targeted by therapeutic interventions[58]. The often cryptic relationship between DNA sequence and epigenetic changes can mean that mutations may be overlooked or mistaken for epimutations, leading to misconceptions about the driver versus passenger role for epigenetic changes[59]. This issue should be partly addressed by cheaper and faster sequencing methods, which will be able to produce genomic and epigenomic information from the same sample.

### Chromatin and DNA methylation in mutagenesis

Mutation rates vary in different parts of the genome, at different stages of the life cycle and in diseases such as cancer, where they depend on the cell of origin, environmental exposure and cancer type[60–62]. Mutation rates can be affected by DNA methylation[63] and nucleosome positioning[64]. Higher-order chromosome folding also influences mutagenicity. A large-scale survey of balanced chromosomal abnormalities in patients with congenital anomalies revealed disruption of TADs encompassing known syndrome-linked loci in 7.3% of cases[65], and a combination of Hi-C chromosome capture with whole-genome sequencing in multiple myeloma showed significant enrichment of copy number variation breakpoints at TAD boundaries[66] that are frequently bound by CTCF. Furthermore, CTCF is frequently mutated in human cancer[57]. Hypermutation of the heterochromatic inactive X chromosome has also been noted in cancer and may be due to DNA replication stress in aberrantly proliferating cells[67].

### The role of the repetitive genome

Transposable elements are intimate components of genomes, with gene regulatory potential that may lead to phenotypic diversity. Indeed, transposable elements and their relics constitute a major fraction of most eukaryotic genomes. McClintock proposed that transposons were turned on or off by environmental changes or during development, acting as 'control elements'. We now know that transposons can influence gene activity in multiple ways, acting as regulatory elements or interfering with transcription[68]. Genomes have evolved species-specific mechanisms to limit transposon activity, for example by targeting repressive heterochromatin machinery, either through specific RNAs or DNA binding factors. In *Drosophila*, heterochromatin-dependent mechanisms allow the expression of specific clusters of transposon relics in order to produce PIWI-interacting RNAs (piRNAs) that, in turn, inhibit transposition. piRNAs are maternally heritable and can be amplified via a ping-pong system, effectively allowing the organism to resist new invasions and adapt their genome to them[69]. *Caenorhabditis elegans* uses heterochromatin components to prevent illegitimate repetitive DNA transcription and genome instability[70]. Plants produce small RNAs derived from double-stranded precursors, which are synthesized by dedicated polymerases and target DNA methylation and the H3K9 methylation machinery[71]. Finally, numerous strategies are deployed in mammals, the most recently characterized being the repression of endogenous retroviruses (ERVs) by the KAP1 protein (also known as TRIM28), which co-recruits heterochromatin proteins such as SETDB1 by interacting with Krüppel-associated box (KRAB) domain-containing zinc-finger proteins (KZFPs). This strategy also enables the rapid evolution of gene regulation strategies via binding of KZFP to ERVs near genes[72], thus influencing gene expression dynamics and levels.

### Environmental epigenetics

Recently the influence of the environment in development and physiology has been underlined. Gene × environment interactions determine how individuals with the same or different genotypes will respond to environmental variation. The importance of epigenetics in environmental responses is well-established in plants, particularly in Polycomb-based vernalization[73], but similar processes appear to take place in some animal species.

### Environmental epigenetic regulation in animals

In *Drosophila*, environmentally induced phenotypes that depend on epigenetic regulation involve transmission across several generations[74–76]. *C. elegans* has been shown to translate several environmental stimuli, such as viral infection, starvation or elevated temperatures, into modification of epigenetic components[77–79]. Whereas starvation and viral infection induce inheritance via the production of small RNAs[77,78],

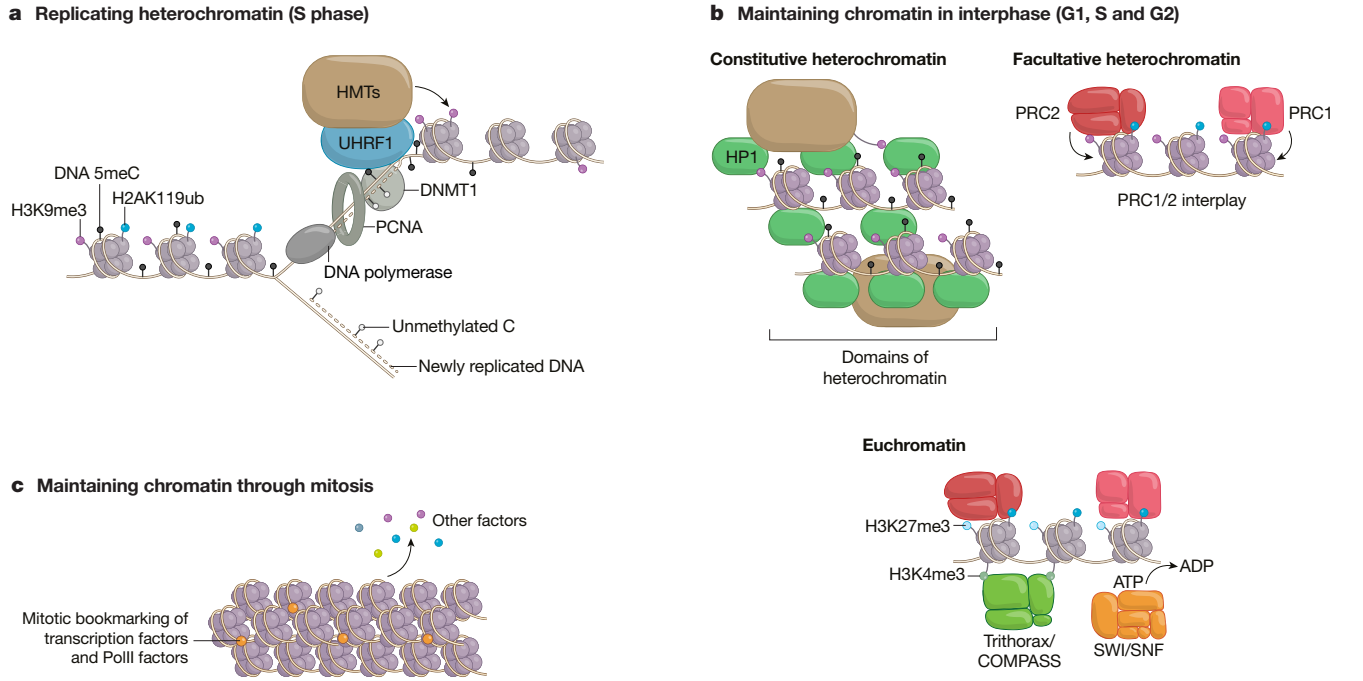


**Fig. 2 | Maintaining chromatin states through the cell cycle. a**, DNA replication during the S phase of the cell cycle is a challenge to the maintenance of nucleosome marks. Epigenetic components, such as HMTs and UHRF1, interact with components of the DNA replication machinery, such as the PCNA clamp, in order to reconstitute chromatin domains after the passage of the fork. The case of DNA methylation is depicted schematically. Newly replicated DNA is unmethylated (empty lollipops; the methylated template DNA strand is not shown here for simplicity). The UHRF1/DNMT1 complex associated with PCNA facilitates remethylation of hemimethylated DNA after DNA replication. **b**, Both constitutive (involving H3K9 methylases and HP1) and facultative (involving PRC1 and PRC2) heterochromatin, as well as euchromatic features (involving an interplay between PRC1, PRC2, Trithorax/COMPASS and ATP-dependent chromatin remodelling complexes), are stably maintained during interphase in order to prevent genes from inappropriately switching their functional states. SWI/SNF is a nucleosome remodelling complex. **c**, During mitosis, most chromosome-associated factors are evicted during chromosome condensation, but 'mitotic bookmarking' of genes is achieved by the maintenance of key components (such as certain transcription factors or RNA polymerase III) bound to their target loci.

apparently without involvement of chromatin, temperature-dependent epigenetic inheritance involves the H3K9 methylation machinery (SET-25)[79], without RNA interference (RNAi), suggesting that, depending on the type of stimulus, the RNAi machinery and chromatin regulators can act differently to drive inheritance.

Examples of environmental effects are by no means limited to model organisms. Temperature is a major sex-determining factor in many reptiles. In a turtle species in which sex is determined by temperature during egg incubation, the KDM6B H3K27me3-specific demethylase exhibits sexually dimorphic, temperature-dependent expression that regulates the sex-determining gene *Dmrt1*[80]. In Australian central bearded dragons, chromosomal sex determination is overridden by high temperatures to produce sex-reversed female offspring. Temperature induces alternative splicing of KDM6B and of JARID2, a PRC2-recruiting component[81]. It is intriguing that temperature affects PRC2 factors in diverse animal and plant species, suggesting that temperature sensing by PRC2 might be evolutionarily conserved, although this is not the only environmental effect that can stably modify chromatin. Another form of environmentally induced chromatin regulation is found in social insects such as the carpenter ant *Camponotus floridanus*, in which the balance between morphologically distinct worker castes depends on the levels of histone acetylation, which may be influenced by feeding behaviour[82].

## Metabolism and epigenetics in mammals

DNA and chromatin modifications use metabolic products. For example, *S*-adenosylmethyonine (SAM) is the methyl donor in DNA and histone methylation; folate and vitamins B6 and B12 induce SAM production; α-ketoglutarate (αKG) is required for DNA and histone demethylation; succinate and fumarate inhibit DNA and histone demethylases; acetyl-coenzyme A is the acetyl donor for histone acetylation; β-hydroxybutyrate inhibits class I histone deacetylases; and the NAD+/NADH ratio regulates the sirtuins (class III histone deacetylases). Therefore, metabolic alterations can induce global perturbations of the epigenome and mutant metabolic components represent potential therapeutic targets[83,84]. On the other hand, metabolic changes can affect specific loci and induce long-lasting epigenetic modifications, including intergenerational epigenetic inheritance[85–87]. The effectors of these perturbations are DNA methylation, Polycomb components, and transfer RNA (tRNA) fragments, which, among other effects, repress genes associated with endogenous retroelements and might thereby help to preserve genome integrity[87–89]. A protein restriction diet in mice can also induce DNA methylation and repression of a subset of ribosomal DNA (rDNA) genes[90], although the inducer and the roles of this rDNA 'epiallele' remain to be identified. In summary, there are compelling examples in which the environment is linked to epigenetic regulation. However, confounding effects, the impact of multifactorial exposure, access to appropriate tissues and assessment of causality for DNA sequence versus epigenetic variation remain major challenges, particularly in humans. Most importantly, there is an urgent need to identify direct links between environmental changes, metabolic changes and epigenetic components. The recent discovery that histone demethylases KDM5A and KDM6A (also known as UTX) can sense oxygen concentrations and thereby modulate H3K4me3 and H3K27me3 levels[91,92] is a first step in this direction.

## Transgenerational epigenetics

The modern evolutionary synthesis[93] postulates that evolution acts mainly via natural selection on phenotypes, ultimately affecting DNA sequences. The discovery that non-DNA sequence information, such as parental, ecological, behavioural and cultural information, can be heritable[94] has not broken the modern framework of evolutionary

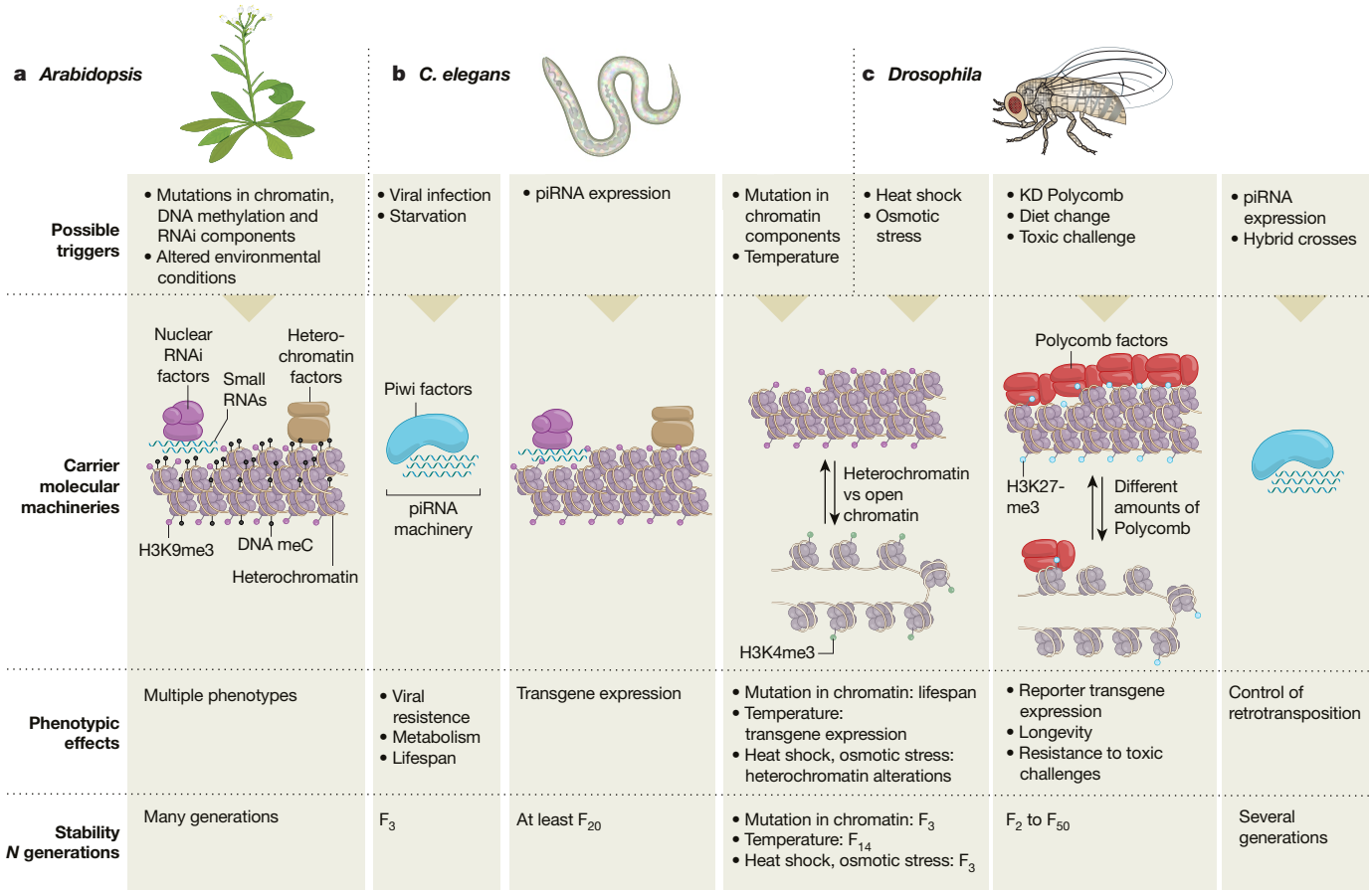


**Fig. 3 | Transgenerational epigenetic inheritance.** Hallmarks of TEI in plants (**a**), *C. elegans* (**b**) and flies (**c**). From top to bottom, the Figure shows the triggering mechanisms, the molecules involved in establishment and transmission of transgenerational memory (carrier molecular machinery), the phenotypic consequences of epigenetic changes and the stability of TEI phenomena in terms of the number of generations (*N*) in which inheritance has been reported.

synthesis. Indeed, one could postulate that complex chains of DNA-driven events ultimately drive parental and ecological behaviours and, therefore, DNA sequence alone would still explain these complex forms of inheritance. A direct demonstration that other molecules, in addition to DNA, carry substantial heritable information would represent an important conceptual change in evolutionary biology.

When adults are exposed to a stimulus or an intervention, their germline, as well as the germline of the fetus in pregnant females, is exposed. We thus distinguish intergenerational inheritance, in the $F_1$ of exposed males and up to the $F_2$ of exposed females, from transgenerational inheritance, starting from the $F_2$ of exposed males and the $F_3$ of exposed females[44,95]. There is abundant evidence for intergenerational inheritance in plants and some animals[86,95–100], suggesting that this phenomenon might be involved in establishing early developmental patterning. What about transgenerational epigenetic inheritance (TEI)[100]? In yeast, TEI has been well-documented[101] and is known to involve RNAi-dependent heterochromatin deposition, leading to Clr4-dependent H3K9me3 marking of silent heterochromatin[47,102]. By contrast, in the absence of RNAi-dependent amplification, H3K9me3 alone is insufficient to drive stable epigenetic memory, unless the histone demethylase Epe1 is mutated[47,102]. In *Tetrahymena*, TEI participates in the phenomenon called 'programmed DNA elimination' from the transcriptionally active somatic nucleus and, again, it involves small RNA-dependent formation of heterochromatin on the DNA elements to be eliminated[103]. In plants, TEI has also been well-described. Plant epialleles can be stable over many generations, and TEI is generally conveyed by RNA-directed DNA methylation (Fig. 3a), a mechanism that can also promote recovery from loss of DNA methylation in a subset of epialleles in *Arabidopsis*[95,104]. Chromatin components such as the histone chaperone CAF-1 also modulate DNA methylation-dependent TEI[105]. Future work is needed to elucidate the link between nucleosome dynamics and inheritance of DNA methylation.

Unlike plants, the germline is separated from the soma in most sexually reproducing organisms, and Weismann postulated that information can flow only from germ cells to the soma[106]. Furthermore, a large number of epigenome features are erased in germline cell chromatin before and during meiosis. An important open question, however, is how much of the epigenome resists erasure? Evidence for substantial epigenetic inheritance of molecules other than DNA through gametes would overturn a fundamental tenet of neo-Darwinism. *C. elegans* epialleles (epigenetically modified alleles that induce specific phenotypes and are heritable over many generations) involve heterochromatin components (Fig. 3b), which, depending on the induction paradigm, may or may not involve piRNAs[79,107]. In *Drosophila* (Fig. 3c), heterochromatin components can induce TEI upon heat shock or osmotic stress[108], whereas piRNAs produce TEI in response to transposable element activity[69]. A second mechanism that can lead to TEI in *Drosophila* relies on Polycomb proteins[109]. Post-eclosion dietary manipulation with a low-protein diet that resulted in elevation of the PRC2 enzymatic subunit E(z) or inhibition of PRC2 by RNAi or by an E(z) inhibitor induced a change in H3K27me3 and in longevity that could be inherited for at least two generations[110]. Furthermore, perturbation of chromosome architecture and of PRC2 function was shown to induce stable but reversible TEI in *Drosophila*[111]. Exposure of *C. elegans* to bisphenol A also induced alterations in the levels of H3K9me3 and H3K27me3 through five generations[112] and, in plants,

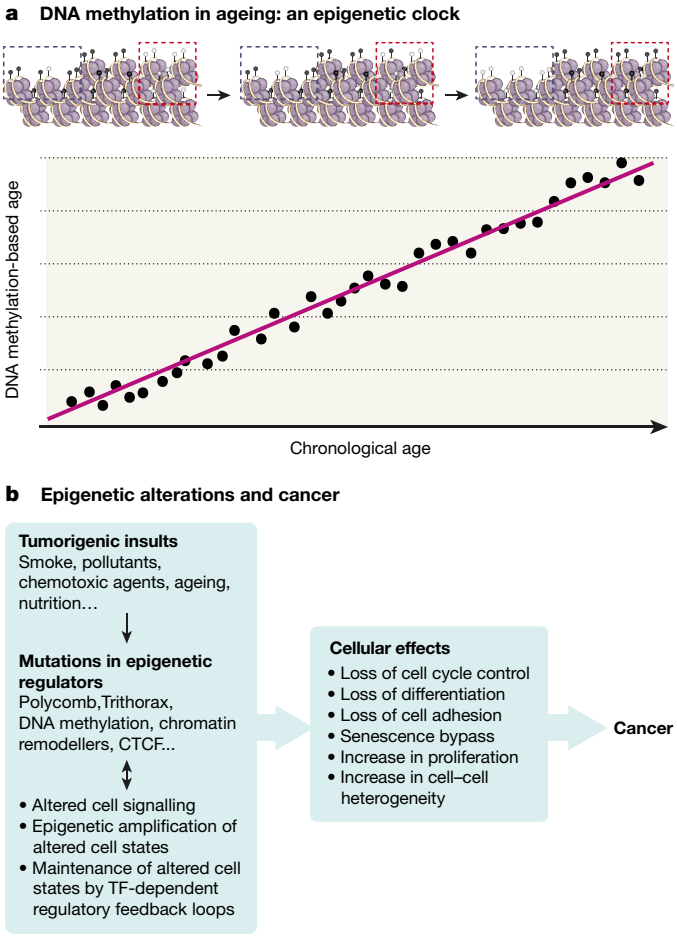


**Fig. 4 | Epigenetics and disease. a**, The 'epigenetic clock' consists of a specific set of genomic CpG sites whose levels of DNA methylation change progressively with age, leading to an estimate of age based on DNA methylation that correlates tightly with chronological age. Rather than a global change in methylation levels, some of the age-related CpG sites show increased methylation (black lollipops, red outline), whereas others show decreased methylation (white lollipops, blue outline). The relationship between changes in DNA methylation and chromatin architecture in ageing remains to be investigated, as well as the cause–consequence relationships between ageing, DNA methylation and gene expression changes. **b**, The genes encoding epigenetic components such as DNA methylases and demethylases, Polycomb, Trithorax, chromatin remodellers, DNA methylation components and CTCF are frequently mutated or dysregulated in cancer, often as a result of environmental insults or physiological changes such as ageing. These mutations alter cellular properties such as cell division, cell differentiation, adhesion and proliferation, and increase the heterogeneity of gene expression, thereby promoting tumorigenesis.

TEI of vernalization is prevented by the function of ELF6, which is a H3K27-specific demethylase[52]. These data suggest that both heterochromatin and Polycomb can induce TEI. Notably, the presence of a histone binding domain that recognizes the same mark as is deposited by the enzymatic moiety in both heterochromatin and PRC2 might provide these systems with amplification potential[28]. Differential levels of their marks might be reconstituted at each generation through differential affinity of PRC2 or other heterochromatin complexes to chromatin regions endowed with differential initial densities of marked nucleosomes (Fig. 3c).

## Transgenerational inheritance in mammals

In vertebrates, DNA methylation is globally reduced twice in each generation: immediately after fertilization and in developing primordial germ cells[113]. Histone marks and 3D genome organization are also reprogrammed in the germline and after fertilization[19]. Furthermore, it is difficult in mammals and virtually impossible in humans to exclude potential confounding elements, such as maternal contribution, components of seminal fluids, changes in utero or postnatal effects[114]. So, what is the evidence for mammalian TEI? A classic example of multigenerational inheritance, insertion of the IAP endogenous retrovirus at the mouse *agouti* coat-colour locus, depends on heritable, variable methylation of the IAP retrovirus on an alternative promoter for the *agouti* gene[115]. A recent systematic survey of murine IAP insertions has indicated that multigenerational inheritance is rare, however[116]. Several reports have attracted attention for their suggestion that diet or exposure to chemicals and behavioural stresses can be transmitted to the progeny for multiple generations[117–119], but some of these results have been criticized[120]. In humans, epidemiological evidence from the Överkalix population cohort established links between grandpaternal food supply at the beginning of the twentieth century and the mortality rate of subsequent generations[121], although molecular evidence is unavailable for this cohort. DNA methylation has been suggested as a potential mechanism for these effects, and retroelements and some genes involved in neurological and metabolic disorders remain methylated during the wave of DNA demethylation in human primordial germ cells[122]. However, a recent report in which a high-fat diet induced insulin resistance, obesity and addictive-like behaviours up to the third generation did not identify heritable changes in the DNA methylome[123]. Other chromatin components might also be involved. For example, transient overexpression of the H3K4-specific KDM1A histone demethylase in mouse spermatogenesis has been shown to induce TEI[124]. These studies suggest that TEI is limited but possible in humans. Future work should address the underlying mechanisms of TEI, and epigenome-wide association studies should complement genome-wide association studies in order to assess the relative contributions of DNA sequence and epigenome alterations in disease[59].

## Epigenetics, health and disease

Changes in the levels of DNA methylation, histone modifications and changes in non-coding RNA (ncRNA) function are common in disease, as are mutations in epigenetic components[57,125]. The ability to distinguish driver from passenger roles for epigenetic alterations will make it possible to identify diseases in which epigenetics might affect diagnosis, prognosis and therapy. Dissecting the interplay between epigenetic components and other disease pathways will also allow the development of combinatorial intervention approaches.

## The epigenetics of ageing

The application of machine learning to high-throughput DNA methylation data has identified indicators of chronological or biological age. One study found that changes in CpG methylation at 353 genomic sites produced a score that was highly correlated with age across tissues[126] (epigenetic clock; Fig. 4a). Strikingly, a comparison of different molecular predictors of age indicated that the epigenetic clock is the most highly correlated to biological age[127]. Furthermore, epigenetic age is adversely affected (accelerated) by a high body mass index, whereas it is reduced by high levels of education or physical activity, a low body mass index and consumption of fish, poultry, fruits and vegetables[128]. Many of the 353 CpGs investigated are located close to poised promoters of bivalent genes (marked by H3K4me3 and H3K27me3), or to active promoters[126], suggesting that ageing may correlate with reduced plasticity in the expression of some bivalent genes, which might resolve into repressed or active states, and with active genes changing their expression levels. More recently, integration with composite clinical measures of phenotypic age identified a set of CpG genomic sites that better predicts lifespan as well as healthspan[129]. Establishing the mechanistic links between the ageing process and variations in CpG methylation will be critical in order to identify the causes of ageing.

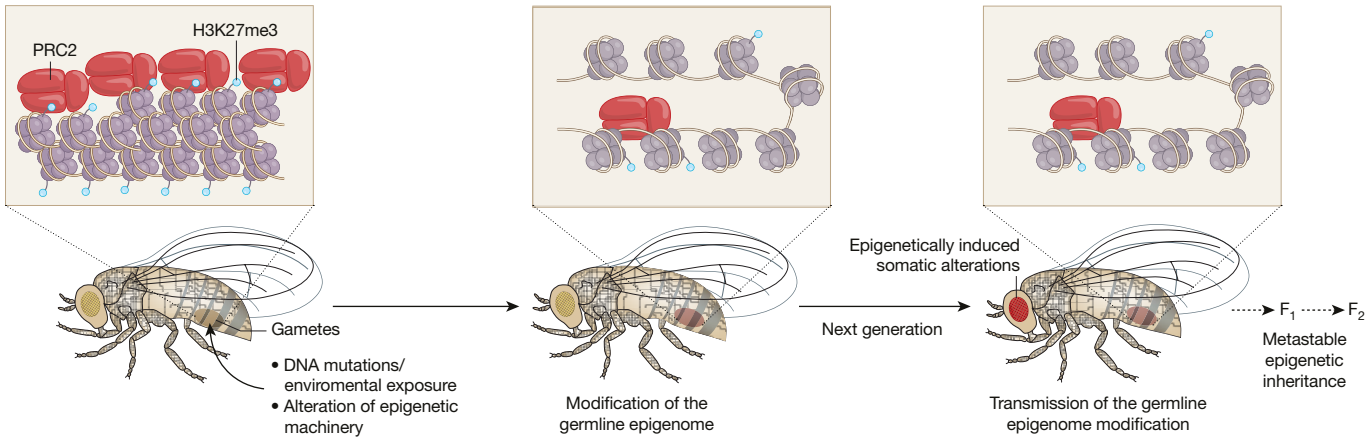


**Fig. 5 | Interactions between genome sequence, the environment and epigenetics in inheritance.** Environmental exposure can affect both the soma and the germline. When transient mutations or perturbations in epigenetic components occur (for example, PRC2 Polycomb components in *Drosophila*[111], as shown), the germline chromatin may acquire an alternative state that can be transmitted and produce a phenotype (here, a change in eye colour) in subsequent generations. The degree of epigenetic inheritance varies and depends on the molecular features of each system and species.

## Developmental epigenetics and disease

Drawing initially on epidemiological studies, Barker formulated the hypothesis of the fetal or developmental origin of health and disease (DOHaD)[130], which suggests that exposure to environmental factors such as chemicals, drugs, stress or infections during specific sensitive periods of intrauterine fetal development or early childhood might predispose an organism to diseases in adult life. Later work proposed that epigenetic components might mediate some of these effects[131,132]. Long-lasting changes to the epigenome that affect cancer susceptibility and biology have also been documented[133]. Other areas of intense study include obesity and diabetes[134], neurological disorders[125] and age-related conditions such as Parkinson's and Alzheimer's diseases[135,136]. Embryonic development and early life are two major susceptibility windows during which epigenetic programming is sensitive to environmental influences, such as diet, temperature, environmental toxins, maternal behaviour or childhood abuse[137]. Behavioural molecular genetics has identified a third susceptibility window, adolescence, during which adverse life experiences affect the risk of anxiety, depression and aggressive behaviour, associated with DNA methylation of specific genes[138] or with alterations in levels of HDAC1[139]. Furthermore, memory formation, a behavioural response to environmental stimuli, is associated with changes in histone and DNA modification at selected loci[140,141]. Future studies should establish whether any of these alterations are in fact causal. Interestingly, one study found that low maternal care in mice decreases DNMT3a and DNA methylation at the L1 promoter and simultaneously induces the mobilization of L1 elements in the hippocampus, suggesting that environmental variation can cause genetic and epigenetic changes simultaneously[142].

## Cancer epigenetics

Genome-wide association studies of specific types of cancer or from the cancer genome atlas project have identified frequent mutations in genes that encode epigenetic components[57,58,143]. These include mutations in DNA methylases and demethylases, histones[144] and histone modifiers, and genes involved in chromatin remodelling and chromosome architecture, but also metabolic genes such as *IDH1* and *IDH2* that affect histone and DNA methylation[57] and might perturb 3D genome architecture[145] (Fig. 4b). Repetitive DNA elements can also contribute to cancer. For instance, in Hodgkin lymphoma, transcription of the *IRF5* transcription factor gene is induced by DNA hypomethylation of a normally dormant endogenous retroviral long terminal repeat located upstream of the promoter, a phenomenon dubbed onco-exaptation[146], whereas in other tumours, DNA demethylating agents can have the opposite effect[147]. Although epigenetic perturbations are generally accompanied by mutations in cancer driver genes, sporadic cases in which cancer can be induced in the absence of obvious driver DNA mutations have also been reported in mice[148]. Furthermore, analysis of pancreatic cancer metastases did not uncover any obvious driver mutations; instead, large-scale chromatin reprogramming was observed, with changes in the level of H3K9me3 in many chromosomal domains[149]. These findings suggest that epigenetic changes can be major driver of oncogenic processes in certain circumstances.

## Concluding remarks

Epigenetic mechanisms buffer environmental variation while allowing plastic responses to the most extreme environmental conditions. In this sense, epigenetics is returning to and expanding the original Waddington definition. A frequently held misconception about epigenetics is that it is a carrier of freedom from a presumed DNA-encoded destiny. The great discoveries of the second part of the twentieth century have generated much excitement about the role of DNA in evolution, biology and medicine, which led to the view of DNA as the 'book of life'. The fact that the same DNA can correspond to different heritable phenotypes has now been portrayed as proof that 'DNA isn't your destiny', a statement which merely reflects the level of hype about epigenetics. Most organisms buffer environmental variation in physiology and inheritance, although buffering does not erase every bit of epigenetic information (Fig. 5). Phenotypes thus depend on specific combinations of genome composition, epigenetic components and environmental inputs. The advent of increasingly sophisticated and economically feasible approaches to genomics, biochemistry and genetics can at last clarify the extent to which epigenetic mechanisms influence life, inheritance and evolution. This will allow us to progress towards personalized precision medicine, as well as to investigate and clarify the effects that lifestyle and 'mind–body' interventions may have on health. It has been suggested that the extrapolation of epigenetic findings from mice before they are confirmed in humans may lead to 'serving epigenetics before its time'—that is, to rushed, unsupported conclusions that can cause harm and unnecessary anxiety[150]. We suggest that we are approaching 'the right time for serving epigenetics', for several reasons. The molecular machineries and mechanisms that enable states to be propagated are finally becoming clear; it is possible to test whether these mechanisms matter for biological processes, ageing or disease; and epigenetic alterations are more readily reversible than DNA mutations and can be targeted with increasing specificity. This allows the biomedical community to test the relevance of epigenetic components in specific diseases functionally, to exploit them as prognostic and diagnostic markers, and to use them as actionable targets for therapy. This path will deepen our knowledge and deliver benefits for human health. Therefore, the field of epigenetics is finally coming of age.

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**Additional information**
**Correspondence and requests for materials** should be addressed to G.C. and E.H.
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