Epigenome plasticity in plants

James P. B. Lloyd and Ryan Lister ✉

Abstract | Plant intra-individual and inter-individual variation can be determined by the epigenome, a set of covalent modifications of DNA and chromatin that can alter genome structure and activity without changes to the genome sequence. The epigenome of plant cells is plastic, that is, it can change in response to internal or external cues, such as during development or due to environmental changes, to create a memory of such events. Ongoing advances in technologies to read and write epigenomic patterns with increasing resolution, scale and precision are enabling the extent of plant epigenome variation to be more extensively characterized and functionally interrogated. In this Review, we discuss epigenome dynamics and variation within plants during development and in response to environmental changes, including stress, as well as between plants. We review known or potential functions of such plasticity and emphasize the importance of investigating the causality of epigenomic changes. Finally, we discuss emerging technologies that may underpin future research into plant epigenome plasticity.

Memory
A recording of past events or actions that is 'stored' at the epigenetic level.

Epimutation
The process of generating a heritable phenotypic change without change in the genome sequence, which creates an epiallele.

DNA methylation
Covalent addition of a methyl group to the fifth carbon of cytosine bases in DNA.

Histone post-translational modifications
(Histone PTMs). Covalent modification of the tails of histone proteins, around which DNA is wrapped, that can affect gene expression and DNA accessibility.

Australian Research Council Centre of Excellence in Plant Energy Biology, School of Molecular Sciences, The University of Western Australia, Perth, Western Australia, Australia.

✉*e-mail: ryan.lister@uwa.edu.au*

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Plant development is not as deterministic as animal development, with plant organ number and growth timing being influenced by the environment. This plastic development can be in response to anticipated changes in the environment, such as the changing of the seasons, but also from rare stressful events, such as high salt exposure. In recent years, the role of epigenetic mechanisms in how plants develop in an ever-changing environment has garnered great interest.

The word epigenetics has had a long history, being used to describe many phenomena — from how developmental decisions are made, to cellular memory such as the clonal inheritance of silencing, to more broadly describing any gene expression changes not resulting from a mutation[1,2]. Here, we define epigenetics as changes in phenotype that are stable through cell division and not the result of a genetic variant. This definition includes both stable changes induced in normal development and random variation in gene expression that is heritable but has no underlying genetic mutation, and is thus an epimutation.

Many epigenetic phenomena are underpinned by non-sequence changes to the DNA or chromatin, including DNA methylation, histone post-translational modifications (PTMs) or the position of histone variants throughout the genome. Mammalian genomes are methylated genome-wide, that is, broadly decorated with DNA methylation except for specific localized regions[3]. This is also true for plant genomes highly enriched in transposable elements, such as maize and tomato, whereas other plants, including flowering plants such as *Arabidopsis thaliana* or mosses such as *Physcomitrium patens*, exhibit a mosaic pattern, with long stretches of little to no DNA methylation punctuated with sites of dense DNA methylation[3,4].

Rapid advances in genomics technologies in recent years have underpinned the discovery of various cell-specific and tissue-specific epigenomic patterns in plants[5–8]. DNA methylation patterns are frequently maintained through cell divisions in plants[9] (FIG. 1), and evidence for the stability of histone modifications in various eukaryotes is being established (BOX 1). Moreover, there are numerous examples of DNA methylation or histone PTM variation during development or in response to environmental changes within a plant, and between plants in the form of epialleles (BOX 2). However, it is often difficult to establish causation between the presence of an epigenetic variant and a change in gene expression or plant phenotype. This can be due to the presence of underlying genetic differences that could cause epigenome variation, or the potential for an epigenetic mark to correlate with, and be the downstream result of, changes in expression[10].

A deeper understanding of how plants store information about their developmental past and their environment will contribute substantially to our ability to breed and engineer the crops of tomorrow. Understanding natural epigenome variation will yield insights into how gene regulation is orchestrated in plants, and how it might be manipulated in the future. The generation of artificial epigenetic variation offers a new tool to introduce stable novel phenotypes into crops or reduce adverse effects of tissue culture[11–15].

Here, we discuss how the epigenome varies within and between cell types during development and its known and potential roles in controlling cell fate decisions.

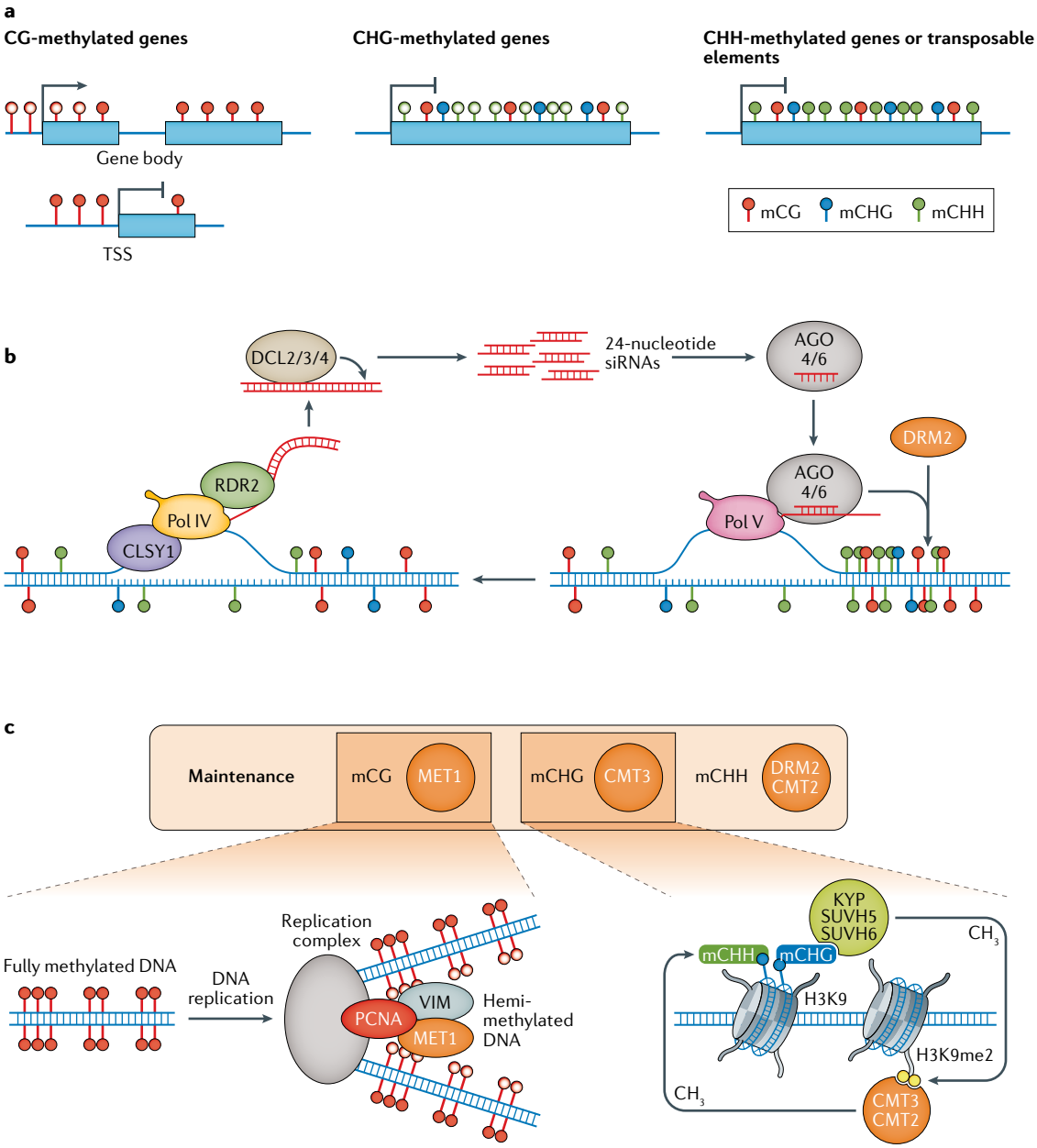

Fig. 1 | **The landscape of DNA methylation in plants. a** | DNA methylation is found in symmetrical (CG and CHG) and asymmetrical (CHH) sequence contexts in plants (H is any nucleotide other than G). In flowering plants, genes with DNA methylation can be broadly categorized into four groups[114]: gene-body DNA methylation (gbM), enriched for methylated CG (mCG) within the transcribed region of the gene and depleted from the transcriptional start site (TSS); TSS methylation, with mCG around the TSS, leading to repression of gene expression; generally repressed CHG-methylated genes, which are enriched for mCHG methylation, depleted in mCHH methylation and may have mCG; and CHH-methylated genes, which are enriched for mCHH, may have methylation in the CG and CHG contexts, and are generally silenced, exhibiting a state similar to transposable element-associated DNA methylation, which is usually found in all three contexts (mCG, mCHG and mCHH). **b** | Establishment of DNA methylation in all three contexts can result from RNA-directed DNA methylation (RdDM) via the generation of small RNAs targeting specific loci for methylation[115]. In brief, RNA polymerase IV (Pol IV) and RDR2, guided by proteins such as classy 1 (CLSY1), produce double-stranded RNA that is cleaved into small interfering RNAs (siRNAs) by DCL2–4. These siRNAs direct AGO4 or AGO6 to chromatin-bound transcripts produced by Pol V, leading to the recruitment and activity of the DNA methyltransferase domains rearranged methyltransferase 2 (DRM2)[31,115], a homologue of the animal DNA methyltransferase 3 (DNMT3) protein, with rearrangement of the motifs within the methyltransferase domain[116]. **c** | Maintenance of mCG occurs via methyltransferase 1 (MET1), the plant orthologue of the animal DNMT1, which methylates hemi-methylated DNA after DNA replication[117–119], although, at some loci, MET1 is required for the correct inheritance of both CG and non-CG methylation[120]. In flowering plants, mCHG is maintained by chromomethylase 3 (CMT3), which binds to histone H3 dimethylated at lysine 9 (H3K9me2) histones and methylates CHG (mCHG) sites[115,121,122]. Kryptonite (KYP; also known as SUVH4), SUVH5 and SUVH6 are histone methyltransferases which, in turn, bind to mCHG and mCHH and methylate H3K9 (REFS[115,123–126]). This positive feedback loop maintains mCHG at many sites in the genome[115]. mCHH is often associated with the continuous re-establishment by the RdDM pathway, but many mCHH sites in flowering plants are maintained by CMT2 in a similar positive feedback loop[127–129].

Transposable elements
Selfish genetic elements that can expand in copy number within a genome, often at a fitness cost to the host.

Epialleles
Heritable phenotypic changes that are not the result of a change in the genome sequence, often encoded by stable changes in DNA methylation or histone post-translational modifications (PTMs).

Epigenome
The map of epigenetic marks decorating the genome, which can be informative about how epigenetic information can produce a particular phenotype.

RNA-directed DNA methylation
(RdDM). A molecular pathway in plants, in which small RNAs target de novo DNA methylation and silence the locus.

We discuss the potential role for epigenomic variation in response to stressful conditions, and evidence for whether stress has a role in altering the epigenome and shaping the plant's response to future stress exposures. Furthermore, we examine the presence of epigenomic differences between individual plants in the form of epialleles and their contribution to phenotypic variation. Finally, we discuss the importance of testing causal relationships between epigenetic marks and suspected functional consequences, and the emerging epigenome editing tools that could facilitate this.

Within-plant epigenomic variation

Specific cell fates are established and stabilized during the process of plant development through numerous mechanisms that regulate gene expression. For example, specific factors that maintain cell identity can be continuously expressed, as with the transcription factor WUSCHEL within the shoot apical meristem, whose expression is reinforced in the stem cell niche to maintain the niche[16]. Gene expression and cell fate can also be determined by cell position, in response to signals from neighbouring cells, as occurs within the shoot apical meristem[16]. Thus, the epigenome can be altered by signals that the cell is continuously exposed to, rather than through memory of the cell's lineage. Alternatively, a transcription factor or non-coding RNA can target the deposition of a self-reinforcing epigenetic mark that alters expression, thus locking the cell's phenotype into a stable state[17–19] (BOX 1; FIG. 1).

Although epigenomic variation in development and between cell types and tissues has been documented, it is currently unclear how frequently these differences reflect the activity of regulatory processes that are required to control normal plant development. Determining whether epigenetic marks regulate plant cell fate decisions is complicated by the possibility that some developmental pathways may be sensitive to normal epigenetic processes but not regulated by them. In these cases, a mutant that disables an epigenetic mark might lead to a developmental defect not because the mark regulates that process but as a secondary consequence of its loss. For example, *Arabidopsis* mutants unable to remove DNA methylation from the gene body of *ERECTA* and the promoter of epidermal patterning factor 2 (*EPF2*) exhibit silencing of these genes and altered stomatal development[20,21] (FIG. 2a). There is no evidence that DNA methylation at these genes changes during development to regulate their expression, but rather these mutants exhibit a failure in processes evolved to prevent transposable element silencing from targeting active genes[20,21].

Here, we explore what is currently known about the extent of epigenomic plasticity during development and examples of when it is involved in regulating development.

Epigenome differences between cells and tissues

To determine whether epigenetic marks vary within a plant, and whether this variation is involved in cell fate decisions, several studies have looked at cell type-specific and tissue-specific differences in the epigenomes of various plants. Base-resolution mapping of DNA methylation patterns of several different soybean tissues has revealed tissue-specific DNA methylation patterns, some of which correlated with tissue-specific differential gene expression[7]. In the shoot apical meristem of *Arabidopsis*[22] and rice[23], an increase in non-CG DNA context methylation (CHG in *Arabidopsis*, CHH in rice) was observed after the transition from vegetative to reproductive growth, possibly reflecting changes that reinforce transposable element silencing before production of the germ line. In rice, genome-wide increases in methylated CHH (mCHH) levels seem to result from activation of the RNA-directed DNA methylation (RdDM) pathway[23] (FIG. 1b), which suggests that some changes in DNA methylation reinforce silencing patterns of transposable elements rather than being related to cell fate determination. A comparison of DNA methylomes of several different *Arabidopsis* root cell types revealed that the methylomes of multiple different cell types were broadly similar, and the few differences around coding genes were not associated with variation in gene expression[8]. However, the columella was found to have a dramatically different genome-wide methylome compared with all other analysed root cell types, with hypermethylation in the mCHH context, mostly in transposable elements. This observation suggests an upregulation of DNA methylation via RdDM within this cell type, perhaps to reinforce silencing in neighbouring stem cells[8]. That said, the mechanisms underlying cell-specific or tissue-specific changes in the DNA methylome often remain unclear.

Classy 1–4 (CLSY1–4) proteins are chromatin remodellers that act to control RdDM activity at specific loci

Box 1 | Histone marks and models of their inheritance

A wide array of histone post-translational modifications (PTMs) decorate chromatin[100]. Many marks have been implicated in regulating gene expression, including the repressive modifications histone H3 di/trimethylated at lysine 9 (H3K9me2/3) and H3 trimethylated at lysine 27 (H3K27me3)[100]. By contrast, other marks such as histone acetylation, H3 trimethylated at lysine 36 (H3K36me3) and H3 trimethylated at lysine 4 (H3K4me3) have been associated with expressed genes[100]. For a histone PTM to be considered truly epigenetic in nature, it should be stable through cell divisions. Heritability of some histone PTMs after cell division has been described: repressive H3K9me3 and H3K27me3, but not activating marks, can be transmitted through the process of DNA replication in animals and fungi[138]. During DNA replication, a mixture of old and newly synthesized histones are placed on the daughter strands[138,139]. Histones with PTMs are diluted on the newly synthesized DNA and need to be fully re-established, necessitating a cluster of modified histones in the parental cell for each daughter strand to gain one. Re-establishment happens through the action of a read–write enzyme that both recognizes and catalyses the mark[138]. In the case of H3K9me3 and H3K27me3, the read–write module has been discovered, making these marks truly epigenetic[138]. In animals, factors are required to recruit enzymes for the re-establishment of H3K9me3 to the DNA replication fork, allowing for normal maintenance of H3K9me3 profiles after cell division[140–142]. The CAF-1 histone chaperone complex is required for normal deposition of new histones, including the variant H3.1, and mutations disabling the CAF-1 subunit FASCIATA2 in *Arabidopsis* lead to a depletion of H3K9me2 in heterochromatin, suggesting that DNA replication-dependent re-establishment of H3K9me2 is important in plants[143]. Incorporation of the histone variant H3.1 during DNA replication allows for rapid deposition of H3K27me3 after S phase is complete[137]. Further work is required to catalogue the histone PTMs that are maintained after cell division and whether these processes function during meiosis to allow for trans-generational inheritance, although the male germ line of *Arabidopsis* undergoes a complete resetting of H3K27me3, which allows for the expression of genes involved with sperm differentiation[144].

by aiding RNA polymerase IV (Pol IV) recruitment and small RNA production[24] (FIG. 1b). *CLSY* genes in *Arabidopsis* exhibit some level of tissue-specific expression[25,26], and examination of leaves, rosettes, flowers and ovules revealed striking differences in DNA methylation in the CHH context, which correlated with small RNA abundance at these sites[25]. In *clsy* mutants, tissue-specific changes in DNA methylation were observed, with most changes in mCHH sites in leaves and rosettes occurring in *clsy1*, whereas mutations in *CLSY3–CLSY4* had a profound impact on the methylomes of ovules[25]. Therefore, CLSY proteins play a major role in determining tissue-specific profiles of RdDM-deposited DNA methylation, but the function of this variation is unclear. Together, these studies have charted cell-specific and tissue-specific differences in DNA methylation within plants, but the functional consequence of these changes remain to be determined, and may be related to maintenance of transposable element repression.

DNA methylome differences driving development

Some changes in the epigenome have been linked to developmental changes, although it is often difficult to demonstrate that such changes are required for normal developmental processes. Comparison of the DNA methylomes of *Arabidopsis* male sex cells with somatic tissues revealed several regions that become methylated in the sex cells in the CHH context via RdDM[27]. Four such regions overlapped genes that increased in expression in an RdDM mutant in meiocytes, but not in leaves, suggesting that RdDM is required for specific silencing of these genes in the male sex lineage, which is unusual given that RdDM generally targets transposable elements[26,27]. One region was a pre-tRNA gene located within the last intron of the meiotic factor multipolar spindle 1 (*MPS1*)[27,28], where loss of methylation led to an increase in intron retention[27] (FIG. 2b). Defects in meiosis were then observed in RdDM mutants; importantly, transformation of *MPS1* lacking the final intron into the RdDM mutant reduced the meiotic defects, suggesting that mis-splicing of *MPS1* was responsible for these defects[27]. Moreover, the nurse cells that surround the meiocytes produce transposable element-derived small RNAs that can travel through plasmodesmata into meiocytes and establish transposable element silencing[26]. Intriguingly, these small RNAs seem to also target non-cognate sites (with up to three mismatches) in meiocytes, establishing the genic DNA methylation via RdDM[26]; however, they do not induce RdDM at these genic sites within the nurse cells, suggesting a unique environment in meiocytes that facilitates this methylation[26]. Furthermore, *CLSY3* was specifically induced in nurse cells and essential for the expression of small RNAs for these transposable elements that target non-cognate genic sites[26]. This work[26,27] provides a fascinating example of how cell type-specific DNA methylation patterns can arise[26] and how they can be required for cell type-specific functions[27].

In tomato fruit ripening, DNA demethylation occurs at tens of thousands of genomic loci through the activity of demeter-like protein 2 (DML2) (FIG. 2c), a homologue of the *Arabidopsis* DNA demethylase repressor of silencing 1 (*ROS1*)[29,30]. Interestingly, only approximately half of the genes responsive to DML2-mediated active DNA demethylation increase in expression when demethylated[30]; thus, the simple model that promoter methylation is repressive does not hold true at all genomic sites. Methylation-sensitive transcriptional repressors or transcriptional activators that preferentially bind to methylated DNA could potentially play a role in activating these genes[30]. Mutations in factors responsible for the deposition of DNA methylation often have mild developmental phenotypes[31], suggesting that the role of this process in controlling development is often limited. However, some key studies, as discussed above, have shown that DNA methylation patterns can change with important developmental consequences. How many of these methylome changes are targeted during development is not yet clear, including the ~30,000 regions that change during tomato fruit ripening[30]. Taken together, these studies show that DNA methylation changes can be used to regulate some developmental changes in plants, but more research is required to determine the sequence-specific factors and chromatin contexts that contribute to the targeting of these changes.

Histone PTMs in cell fate decisions

Some repressive histone marks can be maintained through cell division, and thus have the potential to stably control plant gene expression and development (BOX 1). The Polycomb group (PcG) chromatin remodelling complex is responsible for histone H3 trimethylated at lysine 27 (H3K27me3), a conserved repressive epigenetic mark[32] (BOX 1). *Arabidopsis* contains three homologues of the catalytic core of the Polycomb repressive complex 2: curly leaf (CLF), swinger (SWN) and medea (MEA)[33]. The *clf swn* double mutant displays extremely abnormal development, with callus-like growths and shoot-like structures emerging from roots[34,35], and H3K27me3 differentially marks genes during cell type transitions[5,6], suggesting that PcG proteins and H3K27me3 play major roles in maintaining cell fate decisions throughout the plant. Comparison of *Arabidopsis* root hair cells versus

Box 2 | Epialleles and epimutations

Epialleles are the product of epimutations, such as the deposition of meiotically stable DNA methylation that alters development[71–74]. Epialleles reflect a more stable form of epigenome plasticity than discussed in the context of development and, therefore, can be important in populations that could be selected for during evolution. An early example of a plant epiallele is from *Linaria vulgaris*, with a natural mutation first described by Linnaeus, in which the floral symmetry was changed from bilateral to radial[71]. No underlying sequence change was discovered, but DNA methylation causing transcriptional silencing of a floral development gene was identified[71]. This epiallele was stable through meiosis, but occasional revertants were observed in which DNA methylation was lost and floral shape returned to wild type[71], indicating that epimutations can have major phenotypic consequences in plants. Moreover, this was the first natural 'mutant' to have its causative epimutation identified, suggesting that epialleles occur in natural populations[71].

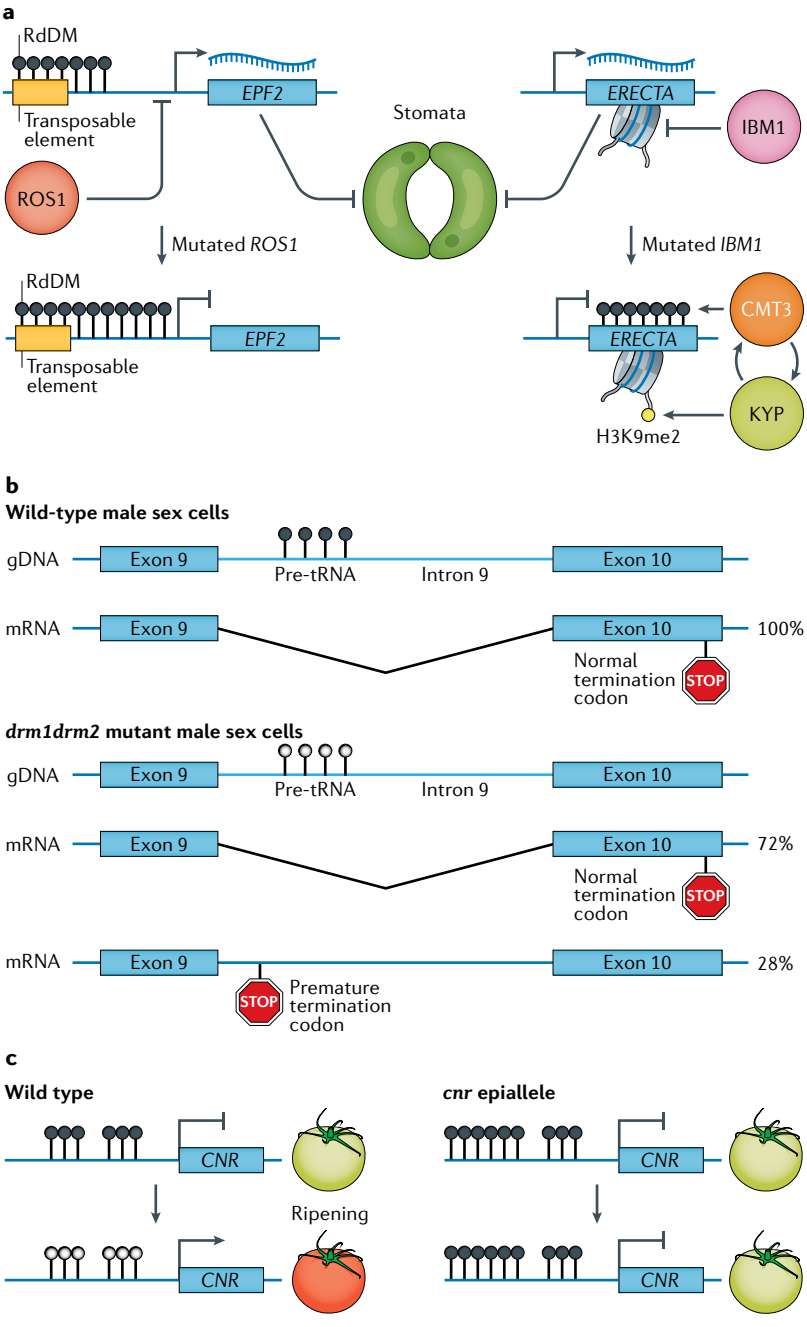

Fig. 2 | **Roles of DNA methylation in plant development.** **a** | Maintenance of normal DNA methylation patterns is required for normal plant growth and development. A mutation disabling the DNA demethylase repressor of silencing 1 (ROS1) in *Arabidopsis* causes an overproduction of stomata and epidermal patterning factor 2 (*EPF2*) repression[20]. ROS1 normally acts to prevent the spread of DNA methylation deposited at a nearby transposable element by RNA-directed DNA methylation (RdDM), allowing *EPF2* to be expressed[20]. Mutations in the histone H3 dimethylated at lysine 9 (H3K9me2) demethylases *IBM2* and *EDM2* in *Arabidopsis* cause the *ERECTA* gene to become repressed and stomata to become overproduced[21]. Accumulation of H3K9me2 leads to methylation of CHG sites in the gene body, and *ERECTA* is silenced. Both *EPF2* and *ERECTA* do not seem to be regulated by DNA methylation during development, but both genes are silenced by different DNA methylation pathways when normal protections to the epigenome are absent. **b** | During development of the male sex lineage of *Arabidopsis*, the pre-tRNA gene located with intron 9 of multipolar spindle 1 (*MPS1*) becomes methylated by the RdDM pathway[27]. In wild-type plants, all *MPS1* transcripts splice out intron 9, but in mutants lacking RdDM, methylation is lost and intron 9 is retained[27]. **c** | DNA methylation is dynamically regulated in the development of tomato fruit. Many genes in tomato undergo active demethylation via demeter-like protein 2 (DML2) to induce ripening, including at the colourless non-ripening (*CNR*) locus[29,30]. The *CNR* locus is also the site of a naturally occurring and stable epiallele[74]; the promoter is hypermethylated in non-fruit tissue and DNA demethylation at this site is blocked, meaning that plants with the *cnr* epimutation do not ripen[30,74]. CMT3, chromomethylase 3; drm2, domains rearranged methyltransferase 2; gDNA, genomic DNA; KYP, kryptonite (also known as SUVH4).

non-hair cells revealed that many transcriptionally inactive genes in each cell type harbour H3K27me3 (REF.[5]). In maize, H3K27me3 varies considerably between tissues but not between different inbred lines of maize[36]. By contrast, the DNA methylome of maize is highly stable between tissues, varying more between inbred lines[37], suggesting that H3K27me3 plays a larger role in controlling cell fate decisions compared with DNA methylation. Wound induced dedifferentiation 3 (WIND3) is a key developmental regulatory transcription factor that is normally repressed by H3K27me3 in *Arabidopsis*[35]. Overexpression of *WIND3* phenocopies PcG mutants by converting root hairs to callus[35], identifying a key target whereby H3K27me3 can drive premature transdifferentiation between cell types. In the distantly related land plant, the moss *P. patens*, the deletion of PcG components causes the premature generation of post-fertilized structures[38,39]. This observation suggests that PcG and H3K27me3 have conserved roles in the control of cell fate that were present in the last common ancestor of all land plants. Taken together, DNA methylation and histone PTMs can play major roles in cell fate decisions, but it is not always clear how these modifications are targeted to specific loci during development, and careful experimental design is needed to determine causality of a mark within a cell type.

Transdifferentiation
The process of a differentiated cell adopting the cell type identity of another cell type without going through a dedifferentiation process.

Environmentally responsive epigenome plasticity

Anticipated environment-induced development

Plants need to align their development to their surrounding environment, for example, to flower at the correct time of year. Thus, the ability of plant cells to sense and respond to, and even remember, environmental cues is essential to their survival. In *Arabidopsis*, the transcription factor flowering locus C (FLC) represses genes important for the transition to flowering; however, exposure to the cold (winter) progressively silences *FLC*, allowing plants to flower in the spring[18]. At the organismal level, this silencing is gradual, but at a gene level it is a binary switch from on to off at each locus[18]. This switch occurs over three stages through conversion of *FLC* from an active state exhibiting H3 trimethylated at lysine 36 (H3K36me3) to a repressed state harbouring H3K27me3 (REF.[18]); this occurs via expression of an antisense RNA

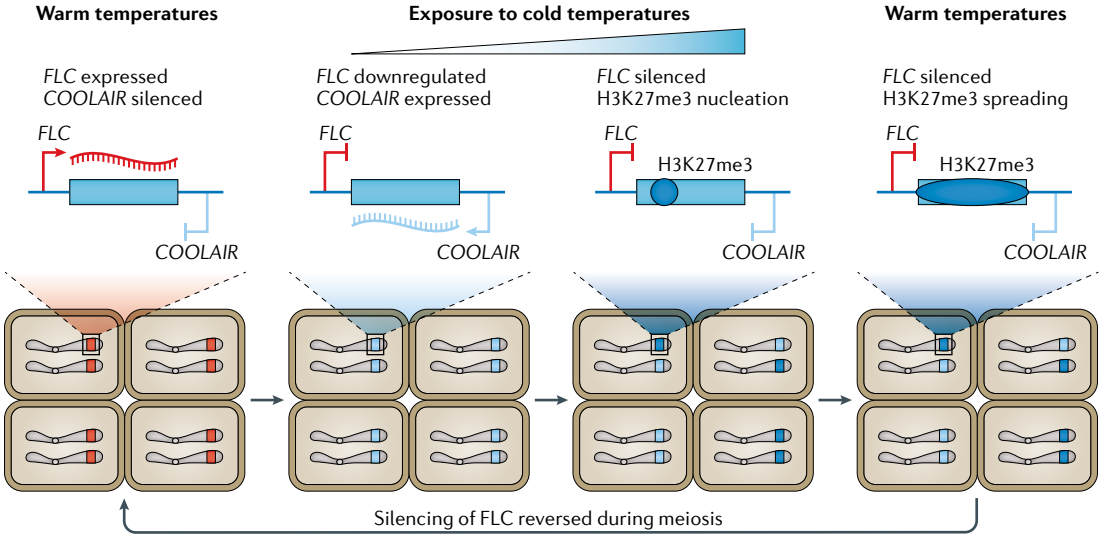

Fig. 3 | **Epigenome plasticity in development in response to environmental cues.** Seasonal changes in temperature epigenetically reprogramme the *Arabidopsis FLC* locus, ensuring appropriate timing of flowering. In initial warm temperatures, *FLC* is expressed in each cell, and the antisense transcript *COOLAIR* is repressed. Upon transition to colder temperatures, an unknown signal induces the expression of *COOLAIR*[130], which downregulates *FLC* as the expression of *COOLAIR* and *FLC* are mutually exclusive[131]. Each copy of *FLC* in a cell is regulated independently[131], therefore local chromatin changes must be controlling this switch between sense and antisense transcription[18]. A cloud of *COOLAIR* transcripts surround the *FLC* locus in *cis*[131]. The 3′ end of *COOLAIR* is processed by an FCA-containing complex, which interacts with a chromatin remodelling complex and eventually leads to the reduction of histone H3 trimethylated at lysine 36 (H3K36me3)[132]. H3K36me3 is antagonistic with H3 trimethylated at lysine 27 (H3K27me3)[133]; thus, termination of *COOLAIR* may pave the way for H3K27me3 acquisition[132]. As the cold temperatures continue, in a stochastic process that occurs independently at each gene copy in each cell, *FLC* is silenced by Polycomb group (PcG) proteins depositing H3K27me3 at a nucleation site consisting of three nucleosomes at the first exon and the start of the first intron[133–135]. The switching of a single *FLC* allele in a single cell in response to cold is infrequent, meaning that it takes weeks to convert all cells to a silenced state[18]. This turns a binary switch at each copy of the gene across the plant into a quantitative measure of cold exposure across the whole plant[18]. Finally, upon return to warmer conditions, H3K27me3 spreads across the whole of *FLC*, in a DNA replication-dependent process, leading to the stable silencing of *FLC*, until it is reset before the next generation of plants[136,137].

that represses *FLC* expression and DNA-binding proteins that recruit PcG proteins to the repressed locus upon cold exposure (FIG. 3). The epigenetic silencing at the *FLC* locus provides an interesting model to understand how perception of the environment can gradually be translated into a change in a developmental programme through stochastic downregulation of individual *FLC* genes and establishment of stable silencing. Although many mysteries remain to be addressed, this model system demonstrates that epigenetic mechanisms can be employed to control plant development, but also highlights the sophistication of analysis required to understand the highly complex mechanisms underlying such a phenomenon.

Other less well-studied examples of the environment modulating plant development through the epigenome have been reported. For example, legumes produce root nodules to house symbiotic nitrogen-fixing bacteria, and the DNA demethylase-encoding demeter (*DME*) of *Medicago truncatula* is upregulated and required for normal nodule development, suggesting that reprogramming of DNA methylation is required for nodule development[40]. As another example, epigenetic marks have been suggested to play a role in bud dormancy of some perennial plants[41], which is a state used to shut down growth during cold periods. Poplar trees show reduced genome-wide DNA methylation before dormancy is broken, and knockdown of a DNA demethylase extends the length of time for buds to break dormancy after winter[42]. In tree peony, delivery of a drug that reduces DNA methylation levels helps to break bud dormancy[43], and in peaches, dormancy-related genes are marked by H3K27me3 (REF.[44]), suggesting that the dormancy control in peach might function using the same epigenetic mark used by *Arabidopsis* to control flowering timing[18]. However, caution should be taken not to over-interpret correlation of marks with developmental changes, or the consequences of drug treatments, which likely have pleiotropic effects. In conclusion, plants use stable epigenetic marks in developmental programmes, but outside well-studied examples such as that of *FLC* in vernalization, more work to establish causal relationships is required.

Stress-induced epigenome plasticity

Given that the epigenome exhibits plasticity in response to development and environmental cues, it is exciting to consider the prospect that the epigenome could respond to stress and possibly even store a memory of stresses to either prime the plant to withstand a second exposure (within-generation priming) or to pass this primed state to progeny (trans-generational inheritance of primed state).

Biotic
A term to denote a living factor that can influence plant growth, such as a bacterial pathogen.

Abiotic
A term to denote a non-living factor that can have an impact on plant growth, such as salt or heat.

There are three active ways in which stress could induce epigenetic changes (FIG. 4). Firstly, the stressor could induce a specific epigenetic change that alters the transcriptional profile of the plant to better respond to the stress (FIG. 4a). This would require the evolution of a sequence-specific factor to induce this change at the modified loci that confers increased robustness. Secondly, the stressor could induce random variation in epigenetic marks within the plant (FIG. 4b) that, if passed on to the next generation, could be selected for, similar to a genetic variant. Several studies have identified some stress conditions that can apparently prime plants, within one generation or trans-generationally (reviewed previously[45]). However, it is unclear whether these examples of priming occur via epigenetic changes or other mechanisms, such as induced expression of a transcription factor that creates a positive feedback loop to maintain its expression and a particular transcriptional profile, or direct maternal effects. Lastly, a third model of adaptive epigenome change in response to stress is conceivable through reinforcement of the normal silencing of transposable elements, which might otherwise be reactivated by the stress response (FIG. 4c).

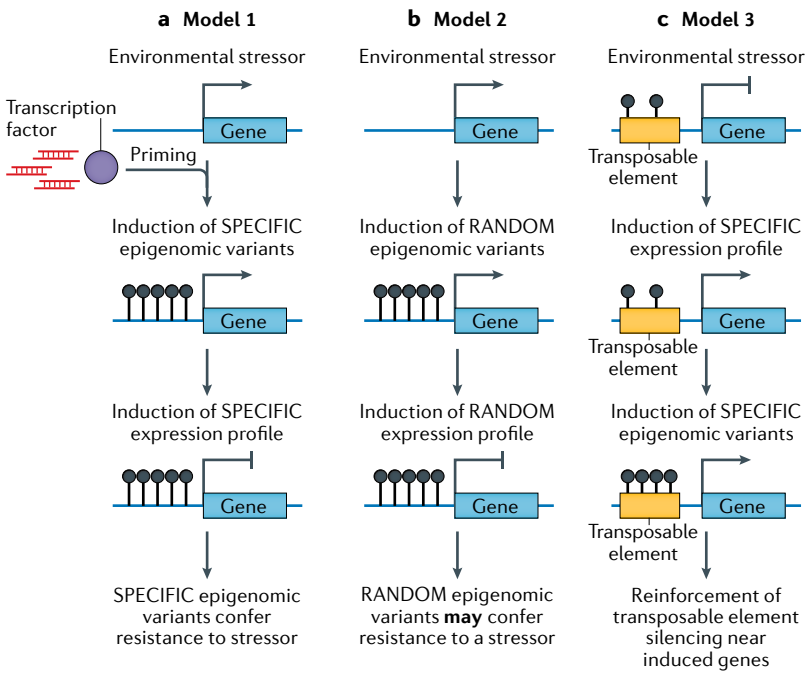

Fig. 4 | **Models for epigenomic plasticity induced by stress. a** | Model 1: stress may induce a programmed but stable change in the epigenome that then alters the expression of the target gene, leading to an increased response to the stress, as observed in *Arabidopsis* salt stress treatment over multiple generations[52]. These specific changes would need a sequence-specific mechanism such as transcription factors or small RNAs to guide these changes when the stress exposure occurred. **b** | Model 2: stress-induced increase in the rate of epimutation leading to the generation of widespread epigenomic and transcriptional changes at random target genes that could, due to random change, lead to an increased resistance to a particular stress, as suggested in *Arabidopsis* salt stress[51]. **c** | Model 3: stress experience by the plant can induce a stress-responsive transcriptional programme. After this new transcriptional programme is initiated, epigenomic changes can be induced at neighbouring transposable element sites to ensure that they remain repressed, reversing the causality from the epigenomic change being the initiator of the transcriptional change to the other way around, as seen in rice plants when undergoing phosphate starvation[10].

Epigenomic changes in response to abiotic stresses. A range of studies have examined the epigenomes of plants after various biotic and abiotic stress treatments[10,46–62]. However, the observed changes might be non-acclimative by-products of the stress or reinforce pre-existing gene expression profiles (FIG. 4c). For example, analysis of rice plants subjected to phosphate starvation identified many changes in the DNA methylome, in particular hypermethylation, some of which were partially stable through mitosis[10]. These regions of hypermethylation were proximal to genes that were transcriptionally induced by phosphate starvation[10]. Examination of the timing and location of these events revealed that transcriptional activation preceded DNA methylation gain, and the hypermethylation was localized at transposable elements upstream of the activated genes[10]. This is likely a protective response to local transcriptional activation of genes close to transposable elements, whereby a local increase in transcription may lead to reactivation of the transposable element, triggering the plant to respond by methylating the transposable elements to ensure silencing. Here, DNA methylation seems to be acting in homeostasis, ensuring the integrity of the genome during the stress, rather than acting to respond to the stress per se. However, it is unclear how widespread this mechanism is in plant stress responses or whether it is well conserved between plants. This study[10] highlights the careful investigation that is needed when considering the causality of epigenomic changes in relation to stress treatments.

Nonetheless, cases of specific epigenomic changes that seem to aid in stress response have been identified (FIG. 5a). For example, *Arabidopsis* seedling exposure to salt stress was found to induce increased survival of re-exposure of adult plants (priming) relative to control plants[46]. A decrease of H3K27me3 across the genome was observed, whereas H3 dimethylated at lysine 9 (H3K9me2) was stable[46]. The decrease of H3K27me3 at high-affinity K^+ transporter 1 (*HKT1*), a Na^+ transporter with a known role in salt stress tolerance, was accompanied by an increase in *HKT1* transcript abundance in primed plants compared with control[46], suggesting that the removal of H3K27me3 from *HKT1* may be causative for this improved stress response in exposed plants. In rice, a recent study identified a co-chaperone, BCL-2-associated athanogene 4 (BAG4), as a regulator of salt stress sensitivity[47]. Of the dysregulated genes in *bag4* mutants, the Na^+ transporter encoding *HKT1;5* was reduced in the roots of rice plants[47]. BAG4 was found to interact with the transcription factor myeloblastosis 106 (MYB106) and the DNA methylation binding protein SUVH7, and both of these factors bind to the *HKT1;5* promoter[47]. SUVH7 binds to a *MITE*-type transposable element rich in mCHG or mCHH, and when this element is deleted, the response of *HKT1;5* salt is reduced and less MYB106 is recruited to the promoter, suggesting that this methylated *MITE* acts as an enhancer[47]. The methylation level of the *MITE* increases during salt stress response, suggesting that it may be a factor in regulating *HKT1;5* during salt stress[47]. However, it is worth noting that other factors involved with *HKT1;5* regulation are induced during salt stress, including *BAG4* and *MYB106*,

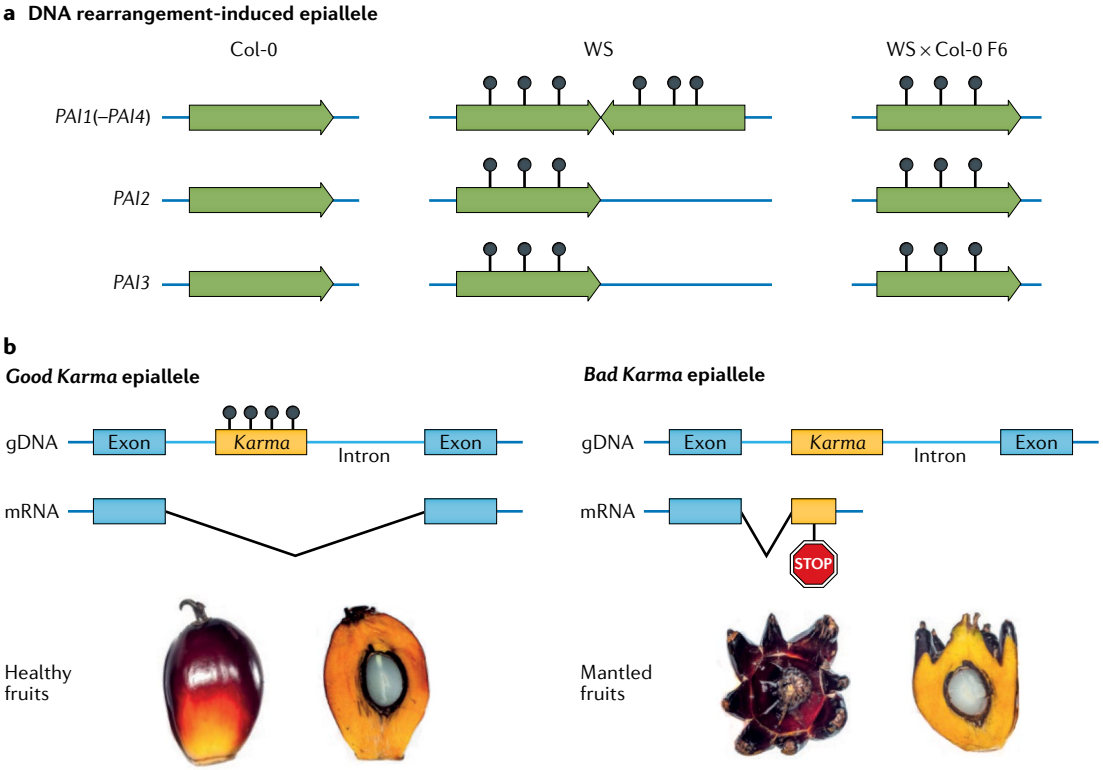

Fig. 5 | **Epialleles are heritable changes in the epigenome that can confer a phenotype on the plant. a** | Paramutation in the phosphoribosylanthranilate isomerase (*PAI*) gene family in *Arabidopsis*[72]. Some *Arabidopsis* strains, such as Wassilewskija (WS), have an inverted repeat of *PAI1* (*PAI1–PAI4*), causing all four *PAI* genes in the genome to gain DNA methylation[72]. When crossed with the Columbia (Col-0) strain, which harbours three unmethylated *PAI* genes, *PAI1–PAI3* gain methylation, which is stable over multiple generations even when the inverted repeat has not been inherited[72]. **b** | In palm oil plants, a highly productive hybrid is often reproduced through tissue culture rather than crossing of the parental strains. This tissue culturing can produce sporadic generation of the *Bad Karma* epiallele[13]. In wild-type plants with the *Good Karma* version of the epiallele, the Karma-type transposable element located within the intron of the oil palm *DEFICIENS* gene is heavily methylated[13]. In the *Bad Karma* epiallele resulting from tissue culture propagation, this transposable element loses DNA methylation, causing transcriptional termination within the transposable element after which the splicing machinery uses a 3′ splice site in the transposable element to produce a truncated transcript encoding a premature stop codon, resulting in abnormal plant development[13]. gDNA, genomic DNA. Images in part **b** are reprinted from REF.[13], Springer Nature Limited.

and it is unclear how DNA methylation levels during salt stress are controlled[47]. It also remains to be determined whether DNA methylation of the *MITE* enhancer directly increases expression of *HKT1;5*[47] or whether increased DNA methylation is a secondary response, as seen in rice response to phosphate starvation[10].

Effects of salt treatment can even prime plants through meiosis[52]. One study that examined the effects of salt exposure on *Arabidopsis* plants over five generations found that the progeny of the salt-exposed plants, even after one generation of no salt exposure, were more salt-tolerant than control plants[52]. However, a generation of no salt exposure was enough to erase this advantage, revealing that this is a short-lived stress memory. Mutations in the DNA methylation machinery abolished this priming, and memory was only heritable through the maternal line of plants[52]. The activity of the DNA demethylase DME was required to prevent paternal plants passing the primed state to their progeny[52]. Some consistently differentially methylated sites were identified (FIG. 5a), and were located in transposable elements[52]. This included one transposable element located downstream of the carbon/nitrogen insensitive 1 (*CNI1*) gene that controls expression of an antisense transcript where, during salt stress, DNA methylation is removed from the transposable element, the antisense transcript abundance is increased, and *CNI1* expression is reduced[52]. However, it is still unclear how differential methylation at these control sites is directed, why inheritance is only permitted via the maternal germ line, and the contribution to salt tolerance from *CNI1* versus other targets. By contrast, prolonged exposure to salt stress increases the rate of random epigenomic changes in plants[51]. Both genetic and DNA methylation changes were observed after ten generations of exposure to high salt conditions, and these variants differed between individual plants, indicating that the changes were the result of an increased rate of random variation, rather than the induction of a specific salt-responsive pathway[51] (FIG. 4b). Stresses can also have an impact on the epigenetic marks of transposable elements. For example, heat stress can decrease DNA methylation and increase chromatin accessibility at some transposable elements[63], and some transposable elements can increase expression

during heat stress, independent of DNA methylation changes, due to the acquisition of a heat-shock promoter element[64,65]. The activation and transposition of transposable elements can have profound effects on the epigenome of plants[66–69], so this factor needs to be considered when interpreting stress-induced changes.

Other studies have failed to identify trans-generational stress tolerance. One example is in *Arabidopsis* drought response, where exposing multiple generations of plants to drought resulted in only one detectable difference (in seed dormancy length) and few DNA methylation changes[53]. In fact, more differences in DNA methylation were observed between individual lineages of plants within the treatment condition than between treatments, due to a founder effect[53], highlighting the stability of the DNA methylome in response to drought. In another case, within-generation priming was observed, but no link to an epigenomic change has yet been found: excess light-stressed plants have a much greater response to a second exposure, but their DNA methylomes are highly stable[48,49], and mutants deficient in DNA (de)methylation are primed similarly to wild type[50]. These data suggest that the excess light stress memory is not stored in DNA methylation but do not rule out other histone marks or variants being involved. Such studies that rule out epigenetic mechanisms of priming are important to prevent bias in the literature from only reporting observations of positive relationships.

Epigenomic changes in response to biotic stresses. Epigenome plasticity has also been detected in response to biotic stresses, where DNA methylation changes have been reported upon plant exposure to pathogens, and mutations in some DNA methylation factors cause changes in plant–pathogen interactions[54–59,61]. For example, *Arabidopsis* infected with the fungal pathogen *Fusarium oxysporum* requires active DNA demethylation for normal defence, suggesting that either DNA demethylation is needed for an active defence gene expression programme or that active demethylation is required to maintain the methylome state for normal function[58]. A single exposure of *Arabidopsis* to the bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000 (Pst DC3000) improved resistance in progeny to both Pst DC3000 and the oomycete pathogen *Hyaloperonospora arabidopsidis* (Hpa)[62]. However, as this effect was not passed down to subsequent generations it is not a trans-generational effect[62]. One challenge in the investigation of trans-generational effects is that priming in the progeny of an exposed plant can be the result of a direct parental effect, that is, the cells that become the progeny could have been directly exposed or affected in the parental plants. By contrast, repeated exposures of *Arabidopsis* to Pst DC3000 led to improved resistance to both Pst DC3000 and Hpa even after one stress-free generation[70], and promoters of candidate stress-responsive genes had increased H3K9 acetylation[70], suggesting erasure of repressive heterochromatin of these genes. Supporting this observation, the *drm1drm2cmt3* triple mutant was already highly resistant to pathogen treatment, and no trans-generational priming was observed[70], which indicates that removal of repressive marks is important for trans-generational priming. Further work is required to understand whether demethylation of specific pathogen-related genes is causative of priming, how this demethylation programme is activated and targeted, and the range of plant species and their pathogens in which such epigenomic programmes function.

Taken together, these studies suggest that stress-induced priming of plants via epigenome modifications is likely rare and stress-specific, and can be challenging to identify confidently; how important this phenomenon is in natural populations remains unclear.

Between-plant epigenomic variation

Diversity between individual plants can arise from either genetic mutations or stable non-genetic changes in the form of epimutations[71] (BOX 2).

Natural epialleles and their role and origin

A major issue with identifying epialleles within natural populations of plants is that genetic variation can underlie the epigenetic differences. In some cases, genetic alterations can induce epigenetic changes that are maintained even after the initiating genetic change has been removed, for example, through crossing[72,73] (FIG. 5a) or transposable element mobilization[66]. Large-scale epigenetic changes can result from such genetic alterations, but may also arise from the dysfunction of normal (de) methylation pathways. The colourless non-ripening (*cnr*) epimutation is an example of a natural epiallele discovered in tomato that prevents normal ripening of fruits[74] (FIG. 2c). Less than 0.1% of independent plants bore fruit through reversion to the wildtype DNA methylation state, highlighting the stability of this epiallele[74]. The exact origin of the *cnr* epiallele is unknown, but epimutation could have occurred via breakdown of the normal demethylation pathway that is activated during development (FIG. 2c). The *cnr* epiallele is maintained partly by the RdDM pathway and methyltransferase 1 (MET1) activity[75] (FIG. 1); however, tomato homologues of the *Arabidopsis* chromomethylase 3 (CMT3) DNA methyltransferase seem to have the primary role in maintaining the stability of this epiallele, with the homologue of *Arabidopsis* CMT2 DNA methyltransferase not involved[75], indicating that maintenance of mCHG by the CMT3–kryptonite (KYP; also known as SUVH4) positive feedback loop (FIG. 1c) plays a central role in the stability of this epiallele.

Across *Arabidopsis* strains, genomic and epigenomic variation is common[76]. Whereas the DNA methylation variation at individual bases and mCG variation in gene bodies seems to be unlinked to DNA sequence variation, much of the variation involving non-CG DNA methylation is linked to genetic variation[76,77] (FIG. 1a). Variation involving non-CG DNA methylation is very frequently located near sites of transposable element presence or absence variation between strains, as observed in *Arabidopsis*, rice and maize, indicating a strong genetic determinant for natural DNA methylation variation[66,68,69]. As described above, large-scale epigenetic changes can result from genetic changes leading to silencing that is maintained after the sequence change is lost[72,73] (FIG. 5a) or from the mis-targeting of epigenetic modifiers[78],

Epigenetic recombinant inbred lines
(epiRILs). Plants derived from a cross of genetically identical plants, except for one parent harbouring a mutation that disrupts a certain epigenetic mark. Over subsequent generations, the lines become homozygous for the normal or disrupted epigenetic states at particular genomic regions, with each line harbouring normal or altered modification states at different regions in the genome.

Epigenome-wide association study
A study design that aims to link the presence or absence of an epigenetic mark, such as DNA methylation, at different genomic positions, with a phenotypic trait.

such as the acquisition of gene-body DNA methylation (gbM) (FIG. 1a). Polymorphisms at individual cytosines arise at a rate even higher than the genetic mutation rate in *Arabidopsis*, although the reversion rate is also high[76,78–80], and their contribution to phenotypic variation is unclear. Examination of a *Populus trichocarpa* tree >300 years old revealed that its per-year epimutation rate of DNA was slightly lower than that of the annual rate in *Arabidopsis*, but the distribution across genomic features is similar between the two species[81]. Given the accumulation of epimutations over the age of the tree, this implies that epimutations accumulate through mitosis, rather than occurring during meiosis[81]. Between populations of *Arabidopsis* plants, genes are typically unmethylated, have gbM or exhibit transposable element-like methylation[77,82], and the fluctuations of epialleles between these three states likely reflects an ongoing antagonism between CG and non-CG methylation in transcribed regions. It has been speculated that gbM may have a function, such as in controlling splicing patterns of flowering plants[83]. However, analysis of intron retention in epigenetic recombinant inbred lines (epiRILs) at sites of the genome with loss of gbM did not reveal any differences in splicing patterns[84]. Instead, gbM in the CG context seems to be a by-product of CMT3. Natural losses of *CMT3* in *Eutrema salsugineum* and *Conringia planisiliqua* eliminate gbM in these two species[84]. Sporadic inclusion of H3K9 monomethylated histones into transcribed regions can recruit CMT3, but not CMT2, and could lead to low levels of H3K9me2 (REF.[85]). Thus, gbM may be an epigenetic scar of CMT3 activity, although why it is targeted to some genes and not others is still unclear. Taken together, gbM is a common site of epimutations, relative to transposable elements, and can have an impact on the expression of an epimutated gene[79].

Another interesting subclass of natural epialleles are paramutations, which are able to convert the wild-type (paramutable) allele to a paramutagenic allele, which can in turn convert other paramutable alleles to the paramutagenic state[86]. Paramutations were first discovered in maize and tomato, and have been characterized in other plant species and even in animals in some rare cases[86]. The mechanisms underlying each paramutation are likely diverse, but many seem to rely on small RNAs[86]. It is likely that small RNAs that function in the RdDM pathway (FIG. 1b) are generated from paramutagenic alleles and convert the other allele to a repressed state, which then starts to produce small RNAs as part of a positive feedback loop from the recently converted allele, allowing it to become paramutagenic itself when it next comes in contact with a paramutable allele[86].

Artificial epialleles and their potential in agriculture

Clonal plant propagation has the potential to induce epialleles. Whereas growing a new plant from a cutting in tissue culture creates a genetically identical plant, forcing the regeneration of a whole plant pushes it through an abnormal developmental process that seems to be epimutagenic[11–15]. This process is a source of epialleles that allows their investigation between genetically identical plants. In maize, post-tissue culture plants had differentially methylated regions at many stress-responsive sites, thus tissue culture may act to replicate an extreme natural stress[12], with common differential regions shared between individual regenerants[14]. Tissue culturing of elite hybrid oil palm can induce the 'mantled' phenotype, where the plants develop abnormally and exhibit homeotic floral phenotypes (FIG. 5b), reminiscent of B-class floral mutations, that result in abortive fruit and very poor oil yield, which is economically and environmentally costly[13]. Due to the non-Mendelian nature of this variation, including the existence of revertants to normal development, it was suspected that an epigenetic mechanism was responsible[13,87,88]. An epigenome-wide association study was performed, tracking DNA methylation in regenerated clones with and without the mantled phenotype, enabling mapping to a B-class (*DEFICIENS*) gene involved in floral development[13]. The loss of methylation (predominantly mCHG) was mostly within a transposable element of the Karma family located with an intron of the *DEFICIENS* gene[13] (FIG. 5b). Small RNAs targeting this transposable element are highly expressed in the shoot apical meristem relative to the leaves, which is where the cuttings for tissue culture are taken from, suggesting that stochastic loss of small RNAs in regenerating plants is responsible for the epiallele[13].

epiRILs have proven highly valuable for studying epigenetic contributions to phenotypes as well as a source of novel phenotypic variation. epiRILs have been developed by crossing the decreased DNA methylation 1 (*ddm1*) or *met1* mutant with wild-type plants and then selfing the resulting progeny repeatedly, so that each epiRIL will be homozygous for a different pattern of DNA methylation[89,90]. Thus, the between-line differences in hypomethylation have created a valuable library of epialleles to study. Variation in epiRIL populations included flowering time variation due to flowering Wageningen (*FWA*) promoter demethylation, as with the *fwa* epiallele[89,90], which was originally discovered from a forward-mutant screen[91], as well as improved pathogen resistance[90,92]. Such work could offer potential ways to introduce disease resistance into plants without editing the genome, and it is possible that such sites might be regulated during infection as part of a plant's natural defence mechanism. However, further investigation is needed to establish whether these artificial epialleles are sites of epigenomic regulation during normal growth and defence. By comparing epiRILs with recombinant inbred lines, which vary in natural genetic diversity between *Arabidopsis* strains, it was estimated that epigenetic variation had the same phenotypic potential as genetic variation, although this is a challenging comparison to make as epiRILs harbour some genetic variation, including from transposable element mobilization, and recombinant inbred lines harbour some epigenomic variation[93]. Thus, generation of novel epigenomic diversity in plants has the potential to benefit crop breeding programmes, including crops low in natural genetic diversity. Beyond epiRILs, overexpression of the human DNA demethylase ten–eleven translocation 3 (*TET3*) in *Arabidopsis* and tomato has been used to generate new epialleles, thus offering an exciting technique to create new epigenomic variation[94,95]. Although this approach

will generate epigenome variation widely throughout the genome, targeted epigenome editing tools to change marks at specific loci are being developed (see below), allowing more precise artificial induction of epialleles.

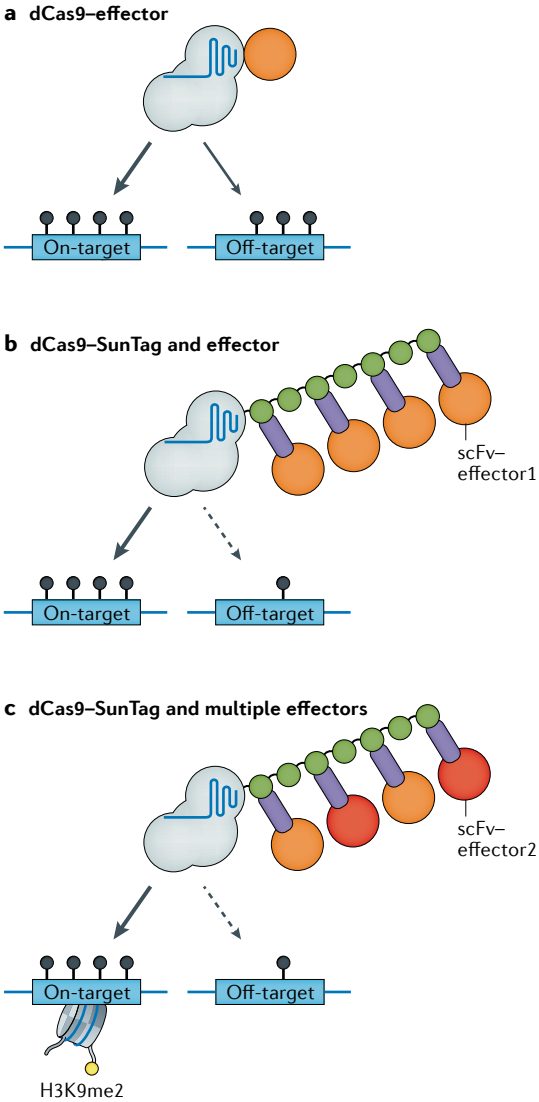

Fig. 6 | **Genome editing tools can be modified to edit the epigenome. a** | Direct fusions of epigenome modifying effector domains to programmable DNA-binding proteins have been able to edit the epigenome in animals and plants, although this can result in both transient and off-target changes. **b** | Separation of epigenome modifying effector from the targeting module (for example, nuclease-dead CRISPR-associated protein 9 (dCas9)) can lead to higher specificity of editing (less off-target editing). By expressing dCas9 fused to the SunTag, multiple effectors can be recruited at a single locus via fusion of the effector to the single-chain variable fragment (scFv) antibody that binds to corresponding epitopes on the SunTag. By still expressing dCas9 at high levels, but the effector at lower levels, less free modifying enzyme is expressed in the cell, so editing is more specific. **c** | To recreate the stable epigenetic silencing of some endogenous pathways, multiple epigenome modifying effectors may need to be expressed simultaneously, and systems such as the dCas9–SunTag offers such potential. H3K9me2, histone H3 dimethylated at lysine 9.

Future directions

As we have reviewed here, the epigenome can vary during development, in response to stress, or between individuals. However, it is often hard to prove that the epigenetic variant is causative of the change in gene expression or plant phenotype. Therefore, a critical aspect in future investigations will be establishing the causal relationships between observable epigenetic variation and associated changes in cell or plant activities. Key factors to consider in analyses include: establishing the temporal ordering of events, such as whether the epigenomic change occurs before or after associated transcriptional or other cellular changes[10]; assessment of whether the epigenetic change is related to maintenance of other genomic features such as transposable element silencing or the centromere, rather than regulation of gene expression; investigating whether the effect can be replicated in an experimental system, such as the rescue of a specific phenotype in a mutant[27]; determining whether underlying genetic variation is driving the epigenetic changes; and accounting for founder variation between plants when establishing the study[48,53], as well as spontaneous epialleles[51].

Emerging technological advances in single-cell genomics and targeted epigenome editing are providing new tools enabling researchers both to characterize epigenome plasticity at unprecedented resolution and scale, and to directly functionally interrogate the causal roles of these changes. The transcriptomes of single cells of some plant tissues have been revealed, indicating where and when different genes are expressed during plant development[96–99], but our understanding of epigenomic determinants of many of these changes is still non-existent. The range of different marks identified and profiled in animals is greater than has been confirmed and studied in plants[100]. Current technologies to study DNA methylation[101] and histone marks[102–104] at single-cell resolution and their application to plants will allow for a deeper understanding of the range of epigenome marks and their dynamics during plant development and response to the environment. While these approaches will enable increased accuracy and precision in detection of correlations between epigenome variation and plant cellular activities, determining whether the epigenome changes are causative will be greatly advanced by new capabilities in the deliberate targeted editing of different epigenetic modifications precisely where desired in the genome. Epigenome editing at specific sites in the genome, using nuclease-deficient versions of genome editing tools linked to domains that can change epigenetic modifications, has the potential to make desired localized changes to the epigenome straightforward. However, this technology will benefit from further refinement before being widely applied in animals and plants. Typically, epigenome modifying domains are directly fused to a programmable DNA-targeting protein, such as a zinc-finger (ZF) array or nuclease-dead CRISPR-associated protein 9 (dCas9) (FIG. 6a). Alternatively, the DNA-targeting protein can recruit the epimodifier via a binding platform such as the SunTag (FIG. 6b), which has the potential to recruit multiple modifiers at once (FIG. 6c). In animals, a range

of editing tools have been developed, but some issues requiring ongoing improvement have been identified, including modification stability[105,106], specificity[107,108] and efficacy in altering transcriptional activity[109].

Targeted epimodifiers have been tested in *Arabidopsis*, often using the *FWA* locus as a target[110–113]. The *FWA* promoter has a known propensity to be demethylated, generating *fwa* epialleles, and *fwa* can be re-methylated to restore a wild-type phenotype[110–113]. The catalytic domain of the human TET1 DNA demethylase has been targeted via ZF and dCas9 to demethylate *FWA*[111], whereas the catalytic domain of a tobacco DRM was able to restore DNA methylation in the *fwa* epiallele[112,113]. Notably, induced methylation at the *FWA* promoter and early flowering across two generations has been reported, even after loss of transgene encoding the epigenome editing system, suggesting stable induction of DNA methylation[112]. The continued production of small RNAs targeting the *FWA* promoter could aid the effectiveness and heritability of re-methylation at this locus[110]. The same DRM protein was also able to silence the *fwa* epiallele when directly fused to a ZF, but not when some RdDM factors were mutated[113], suggesting that silencing was at least in part due to the pre-existing chromatin state and sequence characteristics of this locus. Of the range of epimodifiers targeted to the *FWA* promoter in the *fwa* background after fusion to a ZF for targeting[110,113], many factors involved in RdDM were able to induce early flowering. However, when multiple factors from different stages of RdDM were simultaneously recruited, repression was enhanced[113], likely reflecting the difficulty of a single factor achieving establishment of a stable and potent change to the epigenome. Off-target effects for ZF–DMS3 (defective in meristem silencing 3) and ZF–NRPD1 (nuclear RNA polymerase D 1) were identified and examined, identifying methylated and silenced sites across the genome[113]. Interestingly, the frequency of silencing increased when both factors were expressed in the same plant[113], supporting the notion that multiple silencing factors recruited to the same site can improve the forced epigenetic change, and providing hope that a potent combination of epimodifiers for generalized targeted gene silencing or activation in plants can be discovered[105,106].

Although targeting of a limited number of loci has been reported, the generalizability of such systems for inducing targeted epigenetic changes and modulation of transcription at any locus is still to be determined. Clearly, an improved understanding of the molecular mechanisms that establish and maintain the diverse epigenetic marks is important for advancing the development of targeted epigenome editing tools. Combining such tools with an understanding of epigenomic plasticity at single-cell resolution and new synthetic biology technologies that enable precise spatio-temporal control of their activity will be indispensable for establishing the causal roles of observed epigenetic variation as well as providing new approaches for artificial epiallele induction to generate phenotypic diversity.

Conclusions

The epigenome can change during plant development, due to random epimutations within a population or in response to stress, but establishing whether a specific epigenetic change is inert, functions in maintenance of the genome or controls plant fitness can be difficult to establish. The emergence of single-cell epigenome profiling methods is poised to greatly advance our ability to characterize epigenomic variation. Furthermore, although still in their infancy, the development of highly effective locus-specific epimodifiers could revolutionize our ability to study natural epigenomic changes in development and stress as well as allowing us to develop novel epialleles for agriculture.

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