Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Epigenetic processes in plant stress priming: Open questions and new approaches

C. Jake Harris[1], Anna Amtmann[2] and Jurriaan Ton[3]

Abstract

Priming reflects the capacity of plants to memorise environmental stress experience and improve their response to recurring stress. Epigenetic modifications in DNA and associated histone proteins may carry short-term and long-term memory in the same plant or mediate transgenerational effects, but the evidence is still largely circumstantial. New experimental tools now enable scientists to perform targeted manipulations that either prevent or generate a particular epigenetic modification in a particular location of the genome. Such 'reverse epigenetics' approaches allow for the interrogation of causality between individual priming-induced modifications and their role for altering gene expression and plant performance under recurring stress. Furthermore, combining site-directed epigenetic manipulation with conditional and cell-type specific promoters creates novel opportunities to test and engineer spatiotemporal patterns of priming.

Addresses

[1] Department of Plant Sciences, University of Cambridge, Cambridge, CB2 3EA, UK
[2] School of Molecular Biosciences, University of Glasgow, Glasgow, G128QQ, UK
[3] School of Biosciences, University of Sheffield, Sheffield, S10 2TN, UK

Corresponding author: Amtmann, Anna (anna.amtmann@glasgow.ac.uk)

Current Opinion in Plant Biology 2023, **75:**102432

This review comes from a themed issue on **Epigenetics and gene regulation**

Edited by **Nathan Springer** and **Hidetoshi Saze**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.pbi.2023.102432

Introduction

Environmental stress such as drought, cold, pests or pathogens, triggers responses in plants that enable adaptation and protection. Previous exposure to a transient, non-lethal stress event often improves plant performance under recurring stress; a phenomenon called priming (also acclimation, hardening) [1]. Terminology in this research field is yet to be harmonised and some definitions are offered in Box 1. Priming has many facets, but it generally includes at least two stress events separated by a period of no stress (Figure 1a). The critical observation is that a plant that has experienced the initial event, the primed plant, shows a different response to the later event compared to a plant that has not experienced the stress before (naïve plant). It implies that there is a biological entity in the primed plant which persists during the stress-free period and alters the second response — in short, a memory. As the performance of the primed plant during the stress-free period is not impeded, it is assumed that the memory is dormant, not imposing a metabolic cost. Memory in plants is a hot topic; what is its nature and how is it established, maintained, used, and erased? Chromatin-based biology (epigenetics) offers compelling candidates, but it is important not to neglect other possibilities such as stable metabolites and protein modifications, or stored hormone derivatives and signal precursors [1]. Very likely several different mechanisms co-exist and collaborate, and the effectiveness of a particular process will depend on the exact priming scenario and whether it requires short-term or long-term memory (Figure 1b). In this review we will focus on epigenetic processes (Figure 1c) and summarise the current state of mechanistic insights as well as highlight open questions and opportunities to address them through novel technology.

Epigenetic priming mechanisms

There is convincing evidence that transcriptional responses to recurring stress differ between primed and naïve plants [2]. Although many of the reported differences may be a mere reflection of different stress tolerance rather than its cause, transcripts are a good readout of priming because they are fast, easily tractable, and quantitative. Important mechanistic insight has been obtained from studying the chromatin context of so-called 'memory' genes, which include genes that show transcript changes that persist during the recovery period (type I) and genes that return to control expression levels during recovery but show faster and/or stronger responses when the stress re-occurs (type II) [2]. Some emerging trends from this research are described below with example references. For a more comprehensive list of reported observations see Table 1.

Box1. *What's in a name? That which we call* priming

Definitions of priming and memory vary between authors and disciplines within plant science. The biotic stress field mostly refers to **priming** as a sensitisation of inducible defence mechanisms, which develops after exposure to an (a)biotic stimulus and causes a faster and/or stronger defence reaction to recurrent stress. The abiotic stress field uses a broader definition of priming, also referred to as acclimation or hardening, whereby priming either leads to a sustained up-regulation (Type-I), or a sensitisation (Type-II) of adaptive responses. Adopting this broader definition of stress priming, we can then define **stress memory** as the partitioning of information that is maintained over cell division and interacts with regulatory networks between the genome, epigenome, proteome, and metabolome to influence the whole-organism phenotype. In this context, **memory genes** are defined as genes displaying type-I or type-II expression profiles. Finally, the terms **induced resistance** (against pests and diseases) and **acquired tolerance** (to abiotic stress) are used to describe the adaptive consequence of memory and priming, providing the plant with improved fitness under recurrent stress. In summary, **stress memory** is the blueprint, **priming** is the pathway, and **induced resistance/acquired tolerance** is the end result.

Memory genes involved in short-term (<1 week) priming for dehydration, heat, pathogens or herbivory are characterised by several changes; *(a)* an increase of active transcriptional marks, such as H3K4me2/3 [3–5] or H3K9Ac [6,7], and/or a reduction in repressive marks, such as H3K27me3 [41], *(b)* chromatin remodelling around the promoters resulting in a more open, accessible structure [8,9], and *(c)* persistence of stalled polymerase and pre-initiation complex during recovery periods [3,5]. The findings suggest that short-term priming relies on facilitating active transcription of memory genes and on maintaining the transcriptional machinery in a 'ready-to-go' state to enable faster and/or stronger re-induction.

By contrast, long-term and trans-generational priming has been associated with the release of gene repression or TE silencing, apparent in H3K27me3 or DNA methylation profiles. The role of H3K27me3 in long-term somatic temperature memory is well established for vernalisation [10], but its role in long-term stress priming remains unclear. Gaps in H3K27me3 islands caused by osmotic/salt priming were reported to persist for at least 10 days in unstressed conditions, offering candidate sites for somatic long-term memory [11]. Upon heat treatment, the H3K27me3 demethylase REF6 is directly activated by the transcription factor HSFA2 which leads to inhibition of *tasi*RNA biosynthesis and transgenerational effects on flowering time [12].

Environmental stress has been reported to cause both hyper- and hypo-DNA methylation genome-wide [13,14]. In some studies, these changes were linked to improved stress tolerance, stronger transcriptional responses, and TE activation. *Cis*-regulation is the prevailing model, whereby the epigenetic status of a TE determines the expression of the nearby gene. For instance, enhanced susceptibility of the Arabidopsis *ros1* mutant to *Pseudomonas syringae* can be explained by hyper-methylated TE loci in the promoters of two defence regulatory genes [15], and increased CHG methylation in the first intron of the *RPP7* gene ensures race-specific resistance against downy mildew [16,17]. However, an increasing number of reports highlight the potential for *trans*-regulation of memory genes by priming-associated hypomethylation of TEs implicating mechanisms such as non-coding RNAs [18,19] and long-range interactions between TEs and memory genes [20].

Transmission of priming to the progeny requires escape from the endogenous machinery that resets the epigenetic marks in the germline. Experimentally, this can be achieved through mutants in critical players such as ROS1, DDM1, and REF6/ELF6 [15,20,21], but natural environmental stress can also interfere with epigenetic resetting. For example, severe heat stress in the parent plant inhibits the biosynthesis of *si*RNAs, which play a critical role in correct DNA methylation in embryos, and improves heat responses in the progeny [22]. Plants also possess dedicated epigenetic feedback loops to maintain global homeostasis of particular marks [23–26], and these are likely to play a role in the erasure of accumulated epigenetic memory.

Open questions

Chromatin research has achieved important insights into the epigenetic modifications occurring in primed plants. Nevertheless, some fundamental questions remain open and need to be addressed urgently to achieve a breakthrough.

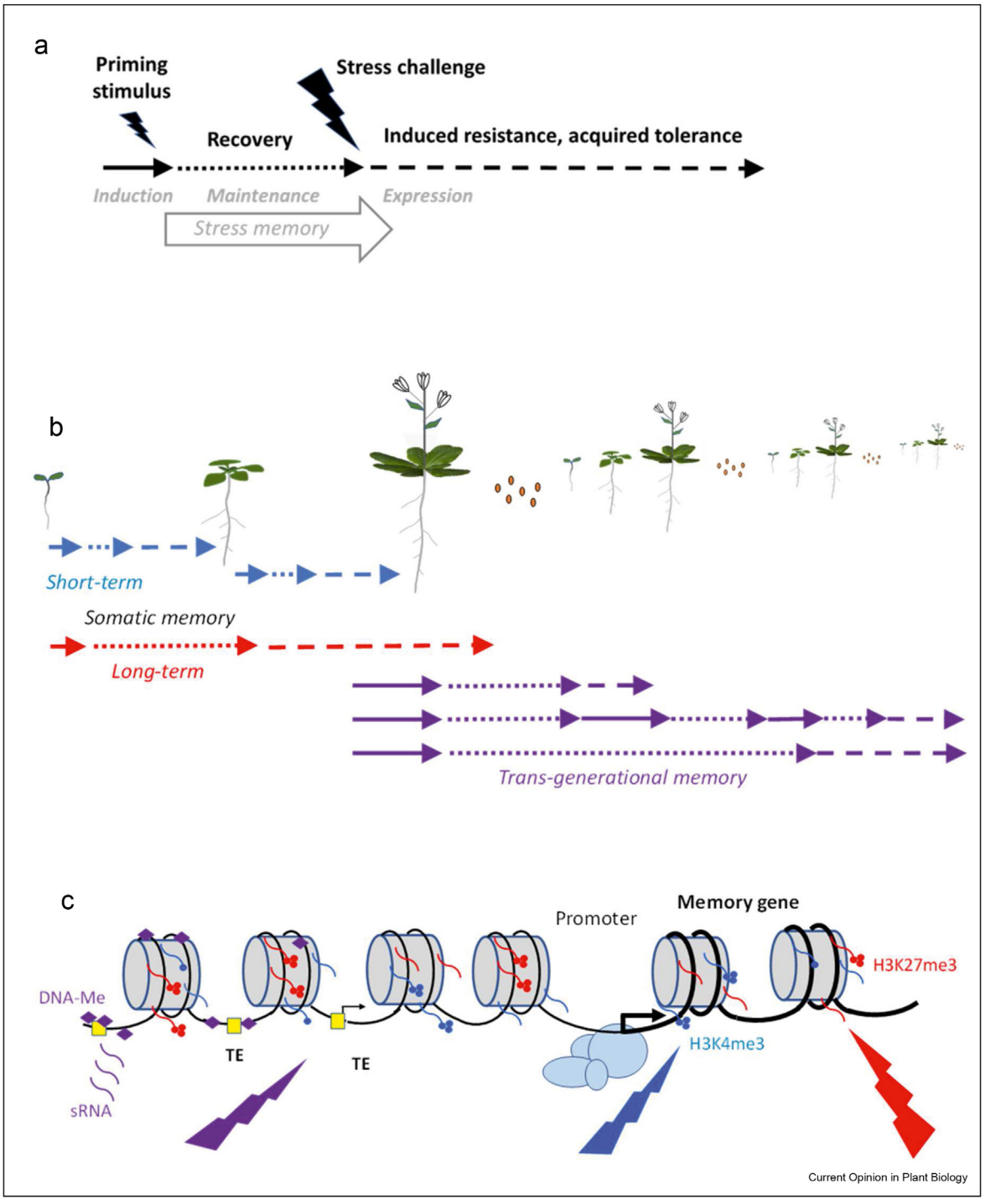
Which of the observed epigenetic changes are causal?

While reports of chromatin modifications in primed plants are mounting, the evidence for their function in priming to date remains largely correlative and circumstantial. Mutants and suppressor screens have shown that several key components of the transcriptional and epigenetic machinery are required for priming, but they do not test whether a change observed in a particular locus is essential for priming. This question can only be answered by targeted, site-specific manipulations that either prevent or generate a particular modification in a particular location. Furthermore, the exact temporal sequence of events upon stress application and release still needs to be unravelled, which would require such manipulations to be inducible and conditional.

Which mechanisms confer specificity?

The effectiveness of epigenetic stress memory depends on the eliciting stress and the targeted processes, yet the specificity-determining components remain unclear.

Figure 1



Experimental protocols and evidence for priming a: Basic priming scheme. Priming involves an initial priming stimulus (solid arrow) and a later stress challenge (dashed arrow) separated by a recovery period of no stress (dotted arrow). The priming stimulus induces a stress memory, which is maintained over the recovery period and leads to improved performance when the stress reoccurs. **b: Examples of priming protocols used to interrogate priming mechanisms**. Different protocols are applied to study short term (blue arrows) and long-term (red arrows) somatic memory in the same plant, or transgenerational memory in the next and following generations (purple arrows). Short-term memory is studied by assessing priming effects within a few days after the initial stimulus (see for example [3–5]). Treatments can be applied at early or later stages of plant growth (first and second set of blue arrows) depending on suitability for abiotic or biotic treatments. To test more long-term somatic memory (red arrows) the recovery periods should be longer to eliminate effects of short-lived changes (e.g. 10 days in Ref. [11]). Effects of priming lasting into the next generation are often only seen in the immediate progeny (top set of purple arrows, e.g. Ref. [22]) and sometimes require repetitive treatments in every generation (middle set of purple arrows [27]). Only a few studies have found evidence for long-lasting transgenerational priming effects. In this case the 'recovery' period needs to

Stress-inducible transcription factors can bring some stress-specificity to the chromatin remodelling of memory-related genes, but it is unclear what distinguishes memory genes from non-memory genes induced by the same stress and TF. Epigenetic changes like DNA demethylation and euchromatisation are common to different stresses, raising questions about how genome-wide mechanisms confer stress-specific memory. The spatiotemporal patterns of stress may entail specificity, and future research should measure these patterns and their relation to stress responses. Single-cell approaches could also shed light on how stress-induced chromatin changes are maintained and proliferated throughout plant growth and development.

Novel approaches

The advent of new technologies opens the door to addressing questions over causality and the spatial organisation of epigenetic effects in the context of priming (Figure 2).

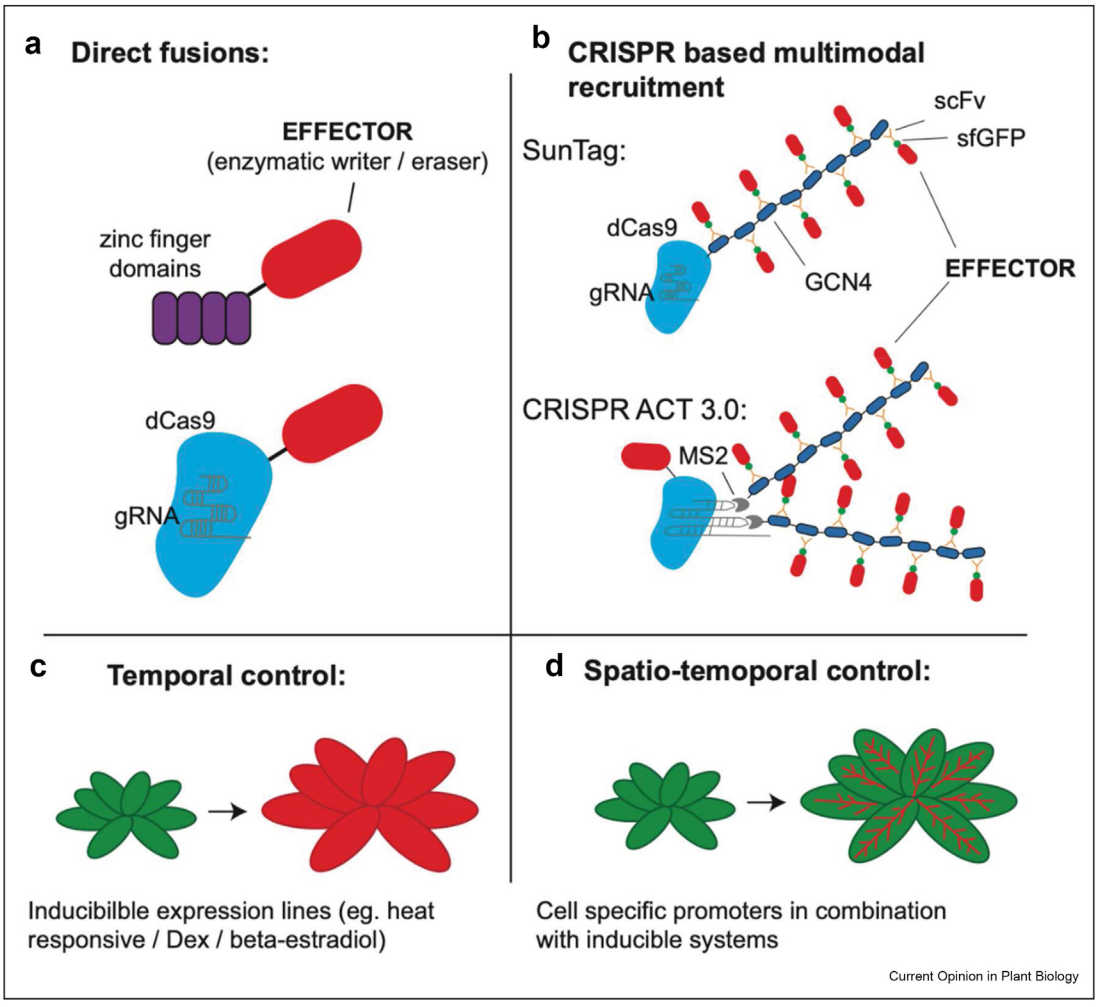
Testing causality and separating direct versus indirect effects

There is no shortage of reports describing genome-wide epigenetic and chromatin alterations in response to stress [45,46]. However, only a subset of chromatin changes are likely to impact future performance at the transcriptional or physiological levels. The remainder are likely inert passive consequences of transcription rewiring or may reflect downstream events that occur long after the initial priming event. The challenge is then to identify which changes are important for priming. One way to begin separating direct from indirect effects is through precise temporal control of the chromatin modifiers using ectopic expression systems [76]. For instance, a recent study used an estradiol-inducible expression system to demonstrate that functional complementation of the H3K27me3 demethylase JMJ30 prior to heat acclimation is necessary for type-2 priming of HSP genes [41]. In another study, a Dex-inducible system was used to demonstrate the succession of chromatin rearrangements after binding of the pioneer transcription factor, LFY [47]. It was shown that binding of LFY to its target motif leads to displacement of the H1 linker histone and recruitment of the chromatin remodelling factor SWI3B within the first 24 h, which preceded chromatin relaxation over the course of 5 days. This temporal delay between pioneering TF binding and subsequent accessibility to non-pioneering TFs has implications in both developmental and priming contexts.

Another way to determine which chromatin changes are causal for priming is to use a 'reverse' epigenetics approach, whereby individual epigenetic modifications are introduced/removed at precise locations. The technology to do this has only recently been developed [48]. Most emerging tools are based around catalytically deactivated CAS9-CRISPR systems that recruit chromatin modifiers to site-specific regions of the genome [49–51]. For instance, the SunTag system has been used to both deposit and remove DNA methylation at precise regions using DRM [52] and TET1 [53] effectors, respectively. TET1 is a mammalian derived protein that does not exist in plants, yet is highly effective *in planta* for DNA methylation removal. The numerous effectors described in mammals [77] therefore provide a reservoir of potential to generate chromatin targeting tools with novel capabilities. Direct fusion of the bacterial derived MQ1 effector [54] to dCAS9 can also target methylation - particularly effective in the CG context - and viral systems can be used to deliver guide RNAs [55]. Synthetic zinc fingers can also guide epigenome modifiers, and have been used to enhance

include at least one stress-free generation (bottom set of purple arrows, [31,35]). A detailed description of the experimental protocol is important to allow correct interpretation of the results. What is the precise nature of the priming stimulus, when and for how long is it applied, and how long is the recovery period? While many proteins and metabolites can remain elevated for several days after stress relief, a memory over longer periods, especially if the plant grows, needs to either involve stable molecules that can be stored in strategic tissue locations, or epigenetic modifications that 'self-replicate' during mitosis. If the two stress treatments are applied across generations, the memory-holding entities need to be able to pass through the germline. Effects from seed quality should be exhausted in the first generation, while epigenetic changes could potentially extend to several generations. Another consideration is the strength and duration of the priming stimulus; it needs to be an effective trigger without causing damage and growth/development retardation that may confound the interpretation of the response to subsequent stress. Finally, what constitutes an improved response? Stronger and faster, or more focussed? More stress-specific or broader and cross-protective? Performance measurements are often time consuming and context dependent. Transcripts are excellent reporters of altered responses, but do not necessarily cause the acquired tolerance. A critical approach is also important when interpreting evidence on potential memory carriers from different experimental protocols. The priming stimulus induces many changes and some of them may persist, but this alone does not prove a causative role in the acquired resistance/tolerance. **c: Proposed epigenetic mechanisms underpinning stress memory in primed plants**. DNA (black line) is wrapped around nucleosomes (grey spheres) composed of histones. Epigenetic processes observed within or in the vicinity of so-call memory genes include enhanced and persisting methylation of lysine 4 in histone 3 (H3K4me2/3, blue tails with circles indicating methyl groups) and other active marks (e.g. H3K9Ac, not shown here), as well as recruitment of chromatin remodelers reducing nucleosome occupancy around promoters, and maintenance of the transcription initiating complex during recovery periods (light-blue spheres). Other observed changes are a decrease of repressive/silencing marks such as tri-methylation of lysine 27 in histone 3 (H3K27me3, red tails) and DNA methylation (purple rhombus). The latter can be mediated by siRNAs and can lead to the activation of transposable elements (yellow squares), with effects on nearby (cis) or distant genes (trans). The different processes have been linked to short-term (blue) and long-term (red) somatic memory, and to transgenerational memory (purple), although this distinction is not strict. Note that not all of the modifications shown occur within the same gene and they will differ depending on priming status and transcriptional activity. For details, see text and Table 1.

Figure 2



'Reverse Epigenetics' methods for precise modulation of epigenetic marks in the genome. a. Direct fusions. The upper panel depicts a zinc-finger fused to an 'effector' domain. In the epigenome engineering context, 'effectors' are typically catalytically active enzymatic proteins/domains that can add or remove (writer/eraser, respectively) particular epigenetic marks such as DNA and histone modifications. Zinc fingers can be designed to target particular regions of the genome, with each zinc finger domain recognising a particular trinucleotide context. The lower panel depicts a catalytically deactivated CAS9 (dCAS9) fused to an effector in complex with a guide RNA that directs the complex to complementary regions of the genome through Watson-Crick base pairing. **b. CRISPR-based multimodal recruitment**. To improve efficiency, researchers have turned to techniques that allow multiple effector copies to be recruited to a particular locus while benefitting from the ease of targeting afforded by CRISPR dCAS9. Upper panel: In the SunTag system, dCAS9 is fused to a tail containing 10–20 copies of the GCN4 epitope, separated by flexible linker regions. GCN4s are recognised by a single chain variable fragment (scFv) antibody, which is fused to superfolder GFP (for subcellular visualisation), and the effector protein of interest. Lower panel: In the ACT 3.0 activation system, the SunTag epitope tail system is fused to an MS2 protein, which recognises a modified hairpin portion of the guide RNA, allowing multiple epitope tails to bind to a single dCAS9 complex, in addition, ACT 3.0 directly fuses VP64 to the dCAS9 to further assist transcriptional activation. In principle, ACT 3.0 can be modified to direct any effector proteins of interest. **c. Temporal control**. By combining the targeting approaches with inducibility researchers can control the timing of epigenetic mark manipulation. This is achieved through driving epigenome-engineering components by inducible promoters, such as heat/Dex or beta-estradiol systems. **d. Spatiotemporal control**. Inducible control of epigenetic modifications can be further refined by specifying particular cell types, through the use of cell-type specific promoters.

cassava's resistance to blight by hypermethylating the meSWEET10a promoter [56]. This research exemplifies that precise epigenome modification can be a viable strategy for crop improvement.

Histones can also be modified using these epigenome engineering strategies. To mimic the natural state, targeted modifications might need to span several nucleosomes to achieve the desired effect. Nevertheless, CRISPR directed histone acetylation at the ATREB promoter was sufficient to increase expression and improve drought resistance of *Arabidopsis thaliana* [57], and SunTag systems that recruit histone modifiers can institute gene silencing [58]. Another recent study showed that H3K4me3 accumulation and expression of the heat responsive memory gene locus, APX2, could be reduced by fusing an inducible dCAS9 to the catalytic domain of the H3K4me3 demethylase JMJ18, and

Table 1

Proposed epigenetic mechanisms underpinning priming.

Molecular observation	Molecular machinery	Priming stimulus	Effect	Memory	Gene targets	Ref.
High H3K4me3		Dehydration	Reduced water loss	Short-term somatic	Type-II memory genes (*Rd29B*, *Rab18*)	[3,5]
Poised pre-initiation complex	Pol-II stalled (Ser5P), *MYC2*, *TPB* (*Med25*)	Dehydration	Reduced water loss	Short-term somatic	Type-II memory genes (*Rd29B*, *Rab18*)	[3,5]
High H3K4me2/3	*HSFA2*	Heat	Thermotolerance	Short-term somatic	Type-I memory gene (*APX2*)	[4]
Low nucleosome occupancy	*FGT1*, *BRM*, *CHR11/17*	Heat	Thermotolerance	Short-term somatic	Type-I memory gene (*HSA32*)	[8]
Nucleosome replication	*BRU1/TSK/MGO3*	Heat	Thermotolerance	Short-term somatic	Type-I memory genes	[28]
Low H2K27me3	JUMONJI (JMJ) proteins	Heat	Thermotolerance	Short-term somatic	Type-II memory genes (HSP22) and HSP17.6C)	[41]
High H3K4me3, H3K9Ac, open chromatin	*NPR1*, *SNI1*, W- and P-box motifs.	*P. syrinage*	Enhanced disease resistance	Short-term somatic	Nearby type-II memory genes (e.g. *WRKY, PR* genes).	[7,9,29·30]
DNA hypo-methylation, siRNAs	*MOM1*	*P. syrinage*	Enhanced disease resistance	Short-term somatic	Distant type-I memory genes (e.g. PRR/NLR genes)	[18]
Low nucleosome occupancy	*CAF1*	*fas2* mutant	Enhanced disease resistance	Short-term somatic	Nearby Type-I memory genes (*WRKYs*, *PRs*)	[33]
H3K9/14Ac, H3K4me2,	*PAD4*, *SID2*,	BABA	Enhanced disease resistance	Short-term somatic,	Nearby type-II memory genes (e.g. *PTI* and *PR*)	[34]
H3K9me2 H3K27me3, non-CG DNA hypo-methylation	*NPR1*, *SNI1*, *SUVH4*	BABA	Enhanced disease resistance	Long-term somatic, trans-generational	Nearby and distant type-II memory genes	[35–37]
Altered nucleosome occupancy	*EDA16* (negative regulation)	Flg22	Enhanced disease resistance	Short-term somatic	Nearby Type-I memory genes	[38]
Altered non-CG DNA methylation, low H3K56ac	H1, H3K56ac	Flg22	Enhanced disease resistance	Short-term somatic	Nearby type-I and type-2 memory genes	[39]
DNA hypo-methylation	*ROS1*, *AGO1*, RdDM	JA	Enhanced herbivore resistance	Long-term somatic	REP2 transposons and distant type-I and type-II memory genes	[19]
Low H3K27me3		Hyperosmotic (salt)	Reduced Na uptake	Long-term somatic	Type-II memory gene (HKT1)	[11]
DNA hyper-methylation	Long non-coding RNAs	Hyperosmotic (salt)	Enhanced survival	Transgenerational	Type-I memoryCNI1/ATL31	[27]
Low H3K27me3, inhibition of siRNA	*REF6*, *HSFA2*, *SGIP3*, *DSS3*	Heat		Transgenerational	HTTP	[12]
Inhibition of siRNA synthesis	*RDR6/SGS3*	Heat		Transgenerational		[22]
TE activation (if siRNA defective)	*ONSEN*	Heat	Acquired heat sensitivity	Transgenerational	Nearby genes	[40]
H3K9Ac, DNA hypo-methylation	*NPR1*, *ROS1*, RdDM	*P. syringae*	Enhanced disease resistance	Transgenerational	Nearby and distant type-II memory genes (*WRKYs*, *PRs*)	[6,31,32]
Release of silencing		UV-C, flagellin, heat/cold	Enhanced HR	Transgenerational	Transgenes	[42–44]
DNA hypo-methylation	*DDM1*	*ddm1*-induced epi-RILs	Enhanced disease resistance	Transgenerational	Distant type-1 and type-2 memory genes	[20]

targeting the construct to the promoter of APX2 [59]. Importantly, some H3K4me3 reduction at APX2 still occurred with a catalytically dead version of JMJ18, demonstrating that either JMJ18 and its associated binding partners, or dCAS9 itself, is sufficient to reduce H3K4me3. This critical catalytically dead control underscores the importance of careful experimental design and interpretation for researchers using chromatin engineering to investigate priming.

Monitoring spatial organisation and cell-type specific effects

Another key outstanding question in priming concerns the spatial organisation and distribution of cellular memory in the plant. Spatiotemporal patterns might link stress and target specificity of priming with tissue context. Do all cells that experience a particular priming event behave similarly? Is the enhanced response observed in primed plants due to more cells becoming responsive, or because the same cells react more strongly? Researchers are beginning to parse these questions using cell-type specific and single-cell profiling approaches.

Using manual dissection and transgenic techniques, scientists have been able to gain a deeper understanding of epigenetic variation within and between plant tissues. For example, the INTACT and FACS methods have allowed researchers to analyse individual cell types, revealing significant variations in levels of methylation [60], chromatin accessibility [61], histone modifications [62] and variants [61,63], particularly in gamete and meristematic tissues. However, a major challenge with these approaches is their low-input nature, which can make traditional chromatin profiling difficult. To overcome this challenge, researchers are increasingly turning to transposase-based approaches that enable direct integration of NGS-compatible primers [63]. Although ChIP alternatives such as CUT&Run [78–80] and CUT&Tag [64–66] show promise, they have not yet been widely adopted in plant research.

Single-cell approaches provide an avenue to gain an unbiased view of cell-type specific responses, and of heterogeneity within populations of isolated cells [67]. Many initial studies in *A. thaliana* have focused on single-cell transcriptomics in root tissue [68–71]. Researchers have also used single-cell ATAC-sequencing to profile chromatin accessibility across a range of maize tissues [72]. This work demonstrated that chromatin features can be used to parse cell identity at high resolution and provided evidence for non-cell autonomous transcription factor action, which has important potential implications in the context of priming.

Recently, there has been a surge of pre-print manuscripts documenting single-cell approaches in various contexts, including pathogen infection and abiotic stresses. A single-cell transcriptomic analysis of *A. thaliana* leaf tissue during *P. syringae* infection used pseudotime inference in combination with information from reporter lines, and provided evidence that virulent bacteria reprogram large sections of the leaf towards susceptibility along a spatial and temporal continuum [75]. Developing technologies such as spatial transcriptomics directly combine single-cell resolution genomics with retention of spatial information from the tissue [73]. Furthermore, multi-omics approaches that allow the simultaneous profiling of multiple chromatin marks within the same cell [74] could help to unravel the relationships between marks and their dynamics during transition states, such as priming.

Conclusions

The biology underpinning priming is complex and variable, depending on many factors, and cause-effect relationships are difficult to extract. The study of chromatin-based processes has generated novel mechanistic insight into potential molecular memory carriers, but open questions remain concerning causality, specificity and location. New technologies create exciting opportunities to address these questions. By using precise temporal and locus control of epigenetic modifiers, as well as cell type-specific and single-cell profiling approaches, researchers will be able to gain a deeper understanding of the complex molecular mechanisms that underlie priming. This knowledge will be essential for developing new strategies for crop improvement and stress adaptation, as well as for advancing our fundamental understanding of epigenetic regulation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

Funding: This work was supported by a Royal Society University Research Fellowship Grant (URF\R1\201016) and an ERC Starting Grant funded by UK Research and Innovation, UKRI (EP/X025306/1) to CJH, and by grants from the Biotechnology and Biological Sciences Research Council, BBSRC, to AA (BB/R019894/1, BB/X002721/1) and to JT (BB/W015250/1).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Hilker M, Schwachtje J, Baier M, Balazadeh S, Bäurle I, Geiselhardt S, Hincha DK, Kunze R, Mueller-Roeber B, Rillig MC, *et al.*: **Priming and memory of stress responses in organisms lacking a nervous system**. *Biol Rev* 2015, **91**:1118–1133.

2. Oberkofler V, Pratx L, Bäurle I: **Epigenetic regulation of abiotic stress memory: maintaining the good things while they last**. *Curr Opin Plant Biol* 2021, **61**, 102007.

3. Ding Y, Fromm M, Avramova Z: **Multiple exposures to drought "train" transcriptional responses in Arabidopsis**. *Nat Commun* 2012, **3**:740–749.

4. Lämke J, Brzezinka K, Altmann S, Bäurle I: **A hit-and-run heat shock factor governs sustained histone methylation and transcriptional stress memory**. *EMBO J* 2016, **35**:162–175.

5. Liu N, Avramova Z: **Molecular mechanism of the priming by jasmonic acid of specific dehydration stress response genes in Arabidopsis**. *Epigenet Chromatin* 2016, **9**:1–23.

6. Luna E, Bruce TJ a, Roberts MR, Flors V, Ton J: **Next-generation systemic acquired resistance**. *Plant Physiol* 2012, **158**: 844–853.

7. Schillheim B, Jansen I, Baum S, Beesley A, Bolm C, Conrath U: **Sulforaphane modifies histone H3, unpacks chromatin, and primes defense**. *Plant Physiol* 2018, **176**:2395–2405.

8. Brzezinka K, Altmann S, Czesnick H, Nicolas P, Gorka M, Benke E, Kabelitz T, Jähne F, Graf A, Kappel C, *et al.*: **Arabidopsis FORGETTER1 mediates stress-induced chromatin memory through nucleosome remodeling**. *Elife* 2016, **5**:1–23.

9. Baum S, Reimer-Michalski EM, Bolger A, Mantai AJ, Benes V, Usadel B, Conrath U: **Isolation of open chromatin identifies regulators of systemic acquired Resistance**. *Plant Physiol* 2019, **181**:817–833.

10. Costa S, Dean C: **Storing memories: the distinct phases of Polycomb-mediated silencing of Arabidopsis FLC**. *Biochem Soc Trans* 2019, **47**:1187–1196.

11. Sani E, Herzyk P, Perrella G, Colot V, Amtmann A: **Hyperosmotic priming of Arabidopsis seedlings establishes a long-term somatic memory accompanied by specific changes of the epigenome**. *Genome Biol* 2013, **14**, R59.

12. Liu J, Feng L, Gu X, Deng X, Qiu Q, Li Q, Zhang Y, Wang M, Deng Y, Wang E, *et al.*: **An H3K27me3 demethylase-HSFA2 regulatory loop orchestrates transgenerational thermomemory in Arabidopsis**. *Cell Res* 2019, **29**:379–390.

13. * Hannan Parker A, Wilkinson SW, Ton J: **Epigenetics: a catalyst of plant immunity against pathogens**. *New Phytol* 2021, **233**: 66–83.

This is a comprehensive review about the impacts of disease-induced epigenetic changes on the plant immune system. The paper reviews cis- and trans-acting mechanisms by which epigenetic changes at transposons prime defence gene expression and covers emerging evidence that stress-induced epigenetic changes influence the rate of evolution of the plant immune system.

14. Lloyd JPB, Lister R: **Epigenome plasticity in plants**. *Nat Rev Genet* 2021. 0123456789.

15. ** Halter T, Wang J, Amesefe D, Lastrucci E, Charvin M, Rastogi MS, Navarro L: **The arabidopsis active demethylase ros1 cis-regulates defense genes by erasing dna methylation at promoter-regulatory regions**. *Elife* 2021, **10**:1–62.

The authors provide compelling evidence that enhanced disease susceptibility of the DNA-hypermethylated*ros1* mutant of Arabidopsis is caused by increased DNA methylation in promoters of two defence-regulatory genes, *RMG1* and *RLP43.* The study furthermore demonstrates that WRKY transcription factor-binding sequences (W-box elements) in these promoters are selectively targeted by ROS1 to antagonise RNA-directed DNA methylation and so regulate basal resistance.

16. Lai Y, Cuzick A, Lu XM, Wang J, Katiyar N, Tsuchiya T, Le Roch K, McDowell JM, Holub E, Eulgem T: **The Arabidopsis RRM domain protein EDM3 mediates race-specific disease resistance by controlling H3K9me2-dependent alternative polyadenylation of RPP7 immune receptor transcripts**. *Plant J* 2019, **97**:646–660.

17. Lai Y, Lu XM, Daron J, Pan S, Wang J, Wang W, Tsuchiya T, Holub E, McDowell JM, Keith Slotkin R, *et al.*: **The Arabidopsis PHD-finger protein EDM2 has multiple roles in balancing NLR immune receptor gene expression**. *PLoS Genet* 2020, **16**:1–31.

18. Cambiagno DA, Nota F, Zavallo D, Rius S, Casati P, Asurmendi S, Alvarez ME: **Immune receptor genes and pericentromeric transposons as targets of common epigenetic regulatory elements**. *Plant J* 2018, https://doi.org/10.1111/tpj.14098.

19. ** Wilkinson SW, Parker AH, Muench A, Wilson RS, Hooshmand K, Henderson MA, Moffat EK, Rocha PSCF, Hipperson H, Stassen JHM, *et al.*: **Long-lasting memory of jasmonic acid-dependent immunity requires DNA demethylation and ARGONAUTE1**. *Nature Plants* 2023, https://doi.org/10.1038/s41477-022-01313-9.

The authors show that seedling treatment with the stress hormone jasmonic acid (JA) induces resistance against caterpillars that lingers for several weeks, which is associated with type-I and type-II priming of MYC-dependent defence genes. The work furthermore provides independent lines of evidence that this JA-induced immune memory is dependent on ROS1-dependent DNA demethylation of AtREP2 transposons, which generate small interfering RNAs that bind to AGO1 to *trans*-regulate priming of distant defence genes.

20. Furci L, Jain R, Stassen J, Berkowitz O, Whelan J, Roquis D, Baillet V, Colot V, Johannes F, Ton J: **Identification and characterisation of hypomethylated DNA loci controlling quantitative resistance in Arabidopsis**. *Elife* 2019, **8**, e40655.

21. Antunez-Sanchez J, Naish M, Ramirez-Prado JS, Ohno S, Huang Y, Dawson A, Manza-Mianza D, Ariel F, Raynaud C, Wilbowo A, *et al.*: **A new role for histone demethylases in the maintenance of plant genome integrity**. *Elife* 2020, **9**, e58533.

22. Zhong SH, Liu JZ, Jin H, Lin L, Li Q, Chen Y, Yuan YX, Wang ZY, Huang H, Qi YJ, *et al.*: **Warm temperatures induce transgenerational epigenetic release of RNA silencing by inhibiting siRNA biogenesis in Arabidopsis**. *Proc Natl Acad Sci U S A* 2013, **110**:9171–9176.

23. Williams BP, Pignatta D, Henikoff S, Gehring M: **Methylation-sensitive expression of a DNA demethylase gene serves as an epigenetic rheostat**. *PLoS Genet* 2015, **11**:1–18.

24. Lei M, Zhang H, Julian R, Tang K, Xie S, Zhu JK: **Regulatory link between DNA methylation and active demethylation in Arabidopsis**. *Proc Natl Acad Sci U S A* 2015, **112**:3553–3557.

25. Rigal M, Kevei Z, Pélissier T, Mathieu O: **DNA methylation in an intron of the IBM1 histone demethylase gene stabilizes chromatin modification patterns**. *EMBO J* 2012, **31**: 2981–2993.

26. Williams BP, Gehring M: **Principles of epigenetic homeostasis shared between flowering plants and mammals**. *Trends Genet* 2020, **6**:751–763.

27. Wibowo A, Becker C, Marconi G, Durr J, Price J, Hagmann J, Papareddy R, Putra H, Kageyama J, Becker J, *et al.*: **Hyperosmotic stress memory in Arabidopsis is mediated by distinct epigenetically labile sites in the genome and is restricted in the male germline by DNA glycosylase activity**. *Elife* 2016, **5**:1–27.

28. Brzezinka K, Altmann S, Bäurle I: **BRUSHY1/TONSOKU/MGOUN3 is required for heat stress memory**. *Plant Cell Environ* 2019, **42**:771–781.

29. Mosher RA, Durrant WE, Wang D, Song J, Dong X: **A comprehensive structure-function analysis of Arabidopsis SNI1 defines essential regions and transcriptional repressor activity**. *Plant Cell* 2006, **18**:1750–1765.

30. Jaskiewicz M, Conrath U, Peterhälnsel C: **Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response**. *EMBO Rep* 2011, **12**:50–55.

31. López Sánchez A, Stassen JHM, Furci L, Smith LM, Ton J: **The role of DNA (de)methylation in immune responsiveness of Arabidopsis**. *Plant J* 2016, **88**:361–374.

32. Stassen JHM, López A, Jain R, Pascual-Pardo D, Luna E, Smith LM, Ton J: **The relationship between transgenerational acquired resistance and global DNA methylation in Arabidopsis**. *Sci Rep* 2018, **8**:1–13.

33. Mozgová I, Wildhaber T, Liu Q, Abou-mansour E, Haridon FL, Métraux J, Gruissem W, Ho D, Hennig L: **Chromatin assembly**

factor CAF-1 represses priming of plant defence response genes. *Native Plants* 2015, **1**, 15127.

34. Po-Wen C, Singh P, Zimmerli L: **Priming of the Arabidopsis pattern-triggered immunity response upon infection by necrotrophic Pectobacterium carotovorum bacteria**. *Mol Plant Pathol* 2013, **14**:58–70.

35. Luna E, López A, Kooiman J, Ton J: **Role of NPR1 and KYP in long-lasting induced resistance by β-aminobutyric acid**. *Front Plant Sci* 2014, **5**:1–8.

36. Meller B, Kuźnicki D, Arasimowicz-Jelonek M, Deckert J, Floryszak-Wieczorek J: **BABA-primed histone modifications in potato for intergenerational resistance to phytophthora infestans**. *Front Plant Sci* 2018, **9**:1–17.

37. * Catoni M, Alvarez-Venegas R, Worrall D, Holroyd G, Barraza A, Luna E, Ton J, Roberts MR: **Long-lasting defence priming by β-aminobutyric acid in tomato is marked by genome-wide changes in DNA methylation**. *Front Plant Sci* 2022, **13**:1–13.
This study shows that tomato undergoes wide-spread DNA hypomethylation at CHH context, which is associated with type-II priming of defence genes against the necrotrophic fungus*Botrytis cinerea*. While there was some enrichment of differentially methylated regions in the promoters of fungus-responsive genes, the majority of primed gene promoters were not targeted for CHH hypomethylation, amounting to a growing body of evidence that defence gene priming by DNA hypomethylation involves *trans*-regulating mechanisms.

38. * Pardal AJ, Piquerez SJM, Dominguez-Ferreras A, Frungillo L, Mastorakis E, Reilly E, Latrasse D, Concia L, Gimenez-Ibanez S, Spoel SH, *et al.*: **Immunity onset alters plant chromatin and utilizes EDA16 to regulate oxidative homeostasis**. *PLoS Pathog* 2021, **17**:1–25.
This work demonstrates that chromatin remodeling ATPase EDA16 acts as a negative regulator of Arabidopsis immunity. Transcriptomic analysis and global analysis of nucleosome occupancy (MNase-seq data) of wild-type,*eda16*, and *EDA16* overexpression lines revealed that *EDA16*selectively represses a specific subset of genes controlling redox homeostasis, which show type-I priming after treatment with the MAMP Flg22.

39. ** Sheikh AH, Nawaz K, Tabassum N, Almeida-Trapp M, Mariappan KG, Alhoraibi H, Rayapuram N, Aranda M, Groth M, Hirt H: **Linker histone H1 modulates defense priming and immunity in plants**. *Nucleic Acids Res* 2023, **51**:4252–4265.
This research focused on defence memory after treatment with the bacterial elicitor Flg22 and revealed a role for H1 linker histones. The*3h1* mutant, which is impaired in the deposition of three H1 histone variants, failed to develop Flg22-induced resistance against *Pseudomonas syringae*, which was associated with loss of memory-associated gene expression (Type-I and Type-II), altered patterns of non-CG DNA methylation, and reduced deposition of H3K56ac.

40. Ito H, Gaubert H, Bucher E, Mirouze M, Vaillant I, Paszkowski J: **An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress**. *Nature* 2011, **472**:115–120.

41. ** Yamaguchi N, Matsubara S, Yoshimizu K, Seki M, Hamada K, Kamitani M, Kurita Y, Nomura Y, Nagashima K, Inagaki S, *et al.*: **H3K27me3 demethylases alter HSP22 and HSP17.6C expression in response to recurring heat in Arabidopsis**. *Nat Commun* 2021, **12**:1–16.
This study shows that a reduction in the repressive H3K27me3 mark by JMJ proteins during acclimation to heat is necessary for type-2 priming of HSP genes, thereby ensuring increased expression upon recurrent heat exposure.

42. Molinier J, Ries G, Zipfel C, Hohn B: **Transgeneration memory of stress in plants**. *Nature* 2006, **442**:1046–1049.

43. Iwasaki M, Paszkowski J: **Identification of genes preventing transgenerational transmission of stress-induced epigenetic states**. *Proc Natl Acad Sci U S A* 2014, **111**:8547–8552.

44. Tittel-Elmer M, Bucher E, Broger L, Mathieu O, Paszkowski J, Vaillant I: **Stress-induced activation of heterochromatic transcription**. *PLoS Genet* 2010, **6**:1–11.

45. Probst AV, Mittelsten Scheid O: **Stress-induced structural changes in plant chromatin**. *Curr Opin Plant Biol* 2015, **27**:8–16.

46. Secco D, Whelan J, Rouached H, Lister R: **Nutrient stress-induced chromatin changes in plants**. *Curr Opin Plant Biol* 2017, **39**:1–7.

47. ** Jin R, Klasfeld S, Zhu Y, Konkol A, Wagner D: **LEAFY is a pioneer transcription factor and licenses cell reprogramming to floral fate**. *Nat Commun* 2021, **12**:626.
The authors demonstrate that the master TF, LFY, has true 'pioneering' capacity, binding to its cognate motif in the context of the nucleosome (in vitro and in vivo). They also place LFY under the control of an inducible promoter, showing that LFY binding to chromatin initiates H1 displacement and SWIR3 recruitment prior to reduced nucleosome occupancy.

48. Gallego-Bartolomé J: **DNA methylation in plants: mechanisms and tools for targeted manipulation**. *New Phytol* 2020, **227**:38–44.

49. Dubois A, Roudier F: **Deciphering plant chromatin regulation via CRISPR/dCas9-Based epigenome engineering**. *Epigenomes* 2021, **5**:17.

50. Jogam P, Sandhya D, Alok A, Peddaboina V, Allini VR, Zhang B: **A review on CRISPR/Cas-based epigenetic regulation in plants**. *Int J Biol Macromol* 2022, **219**:1261–1271.

51. Gardiner J, Ghoshal B, Wang M, Jacobsen SE: **CRISPR–Cas-mediated transcriptional control and epi-mutagenesis**. *Plant Physiol* 2022, **188**:1811–1824.

52. Papikian A, Liu W, Gallego-Bartolomé J, Jacobsen SE: **Site-specific manipulation of Arabidopsis loci using CRISPR-Cas9 SunTag systems**. *Nat Commun* 2019, **10**:729.

53. Gallego-Bartolomé J, Gardiner J, Liu W, Papikian A, Ghoshal B, Kuo HY, Zhao JM-C, Segal DJ, Jacobsen SE: **Targeted DNA demethylation of the *Arabidopsis* genome using the human TET1 catalytic domain**. *Proc Natl Acad Sci* 2018, **115**, 201716945.

54. Ghoshal B, Picard CL, Vong B, Feng S, Jacobsen SE. *CRISPR-based targeting of DNA methylation in Arabidopsis thaliana by a bacterial CG-specific DNA methyltransferase*, vol. 118; 2021, e2125016118.

55. Ghoshal B, Vong B, Picard CL, Feng S, Tam JM, Jacobsen SE: **A viral guide RNA delivery system for CRISPR-based transcriptional activation and heritable targeted DNA demethylation in Arabidopsis thaliana**. *PLoS Genet* 2020, **16**:1–19.

56. * Veley KM, Elliott K, Jensen G, Zhong Z, Feng S, Yoder M, Gilbert KB, Berry JC, Lin ZD, Ghoshal B, *et al.*: **Improving cassava bacterial blight resistance by editing the epigenome**. *Nat Commun* 2023, **14**:85.
Xanthomonas causes cassava bacterial blight (CBB) through TAL-mediated activation of the cassava endogenous gene meSWEET10. As TAL binding to meSWEET10 was found to be sensitive to methylation, the researchers used an RdDM component (DMS3) fused artificial zinc finger targeted meSWEET10a, to induce methylation at this locus. The epigenetically modified cassava plants displayed enhanced resistance to CBB

57. Felipe J, Paixão R, Gillet F, Ribeiro TP, Bournaud C, Lourenço-tess IT, Noriega DD, Melo BP De, Almeida-engler J De, Grossi-de-sa MF. *Improved drought stress tolerance in Arabidopsis by CRISPR/dCas9 fusion with a Histone AcetylTransferase*, vol. 9; 2019:8080.

58. Wang M, Zhong Z, Gallego-Bartolomé J, Li Z, Feng S, Kuo HY, Kan RL, Lam H, Richey JC, Tang L, *et al.*: **A gene silencing screen uncovers diverse tools for targeted gene repression in Arabidopsis**. *Native Plants* 2023, **9**:460–472.

59. ** Oberkofler V, Bäurle I: **Inducible epigenome editing probes for the role of histone H3K4 methylation in Arabidopsis heat stress memory**. *Plant Physiol* 2022, **189**:703–714.
Using a heat-inducible dCAS9 fused to an H3K4me3 demethylase (JMJ18), the authors directly test whether heat priming-induced enrichment of H3K4me3 at APX2 is required for transcriptional memory. Indeed dCAS9-JMJ18 leads to targeted reduction of H3K4me3 and transcriptional memory at APX2. Importantly, this also occurs to some extent when a catalytically deactivated version of JMJ18 was used, demonstrating that factors in addition to enzymatic H3K4me3 removal likely contribute to these effects.

60. Kawakatsu T, Stuart T, Valdes M, Breakfield N, Schmitz RJ, Nery JR, Urich MA, Han X, Lister R, Benfey PN, *et al.*: **Unique cell-type-specific patterns of DNA methylation in the root meristem**. *Native Plants* 2016, **2**, 16058.

61. Buttress T, He S, Wang L, Zhou S, Saalbach G, Vickers M, Li G, Li P, Feng X, Histone H2B: **8 compacts flowering plant sperm through chromatin phase separation**. *Nature* 2022, **611**:614–622.

62. You Y, Sawikowska A, Neumann M, Posé D, Capovilla G, Langenecker T, Neher RA, Krajewski P, Schmid M: **Temporal dynamics of gene expression and histone marks at the Arabidopsis shoot meristem during flowering**. *Nat Commun* 2017, **8**, 15120.

63. Zhu D, Wen Y, Yao W, Zheng H, Zhou S, Zhang Q, Qu LJ, Chen X, Wu Z: **Distinct chromatin signatures in the Arabidopsis male gametophyte**. *Nat Genet* 2023, **55**:706–720.

64. Ouyang W, Zhang X, Peng Y, Zhang Q, Cao Z, Li G, Li X: **Rapid and low-input profiling of histone marks in plants using nucleus CUT&tag**. *Front Plant Sci* 2021, **12**:1–11.

65. Ouyang W, Luan S, Xiang X, Guo M, Zhang Y, Li G, Li X: **Profiling plant histone modification at single-cell resolution using snCUT&Tag**. *Plant Biotechnol J* 2022, **20**:420–422.

66. Tao X, Feng S, Zhao T, Guan X: **Efficient chromatin profiling of H3K4me3 modification in cotton using CUT&Tag**. *Plant Methods* 2020, **16**:1–15.

67. Cole B, Bergmann D, Blaby-Haas CE, Blaby IK, Bouchard KE, Brady SM, Ciobanu D, Coleman-Derr D, Leiboff S, Mortimer JC, *et al.*: **Plant single-cell solutions for energy and the environment**. *Commun Biol* 2021, **4**:1–12.

68. Shahan R, Hsu CW, Nolan TM, Cole BJ, Taylor IW, Greenstreet L, Zhang S, Afanassiev A, Vlot AHC, Schiebinger G, *et al.*: **A single-cell Arabidopsis root atlas reveals developmental trajectories in wild-type and cell identity mutants**. *Dev Cell* 2022, **57**:543–560.e9.

69. Zhang TQ, Xu ZG, Shang GD, Wang JW: **A single-cell RNA sequencing profiles the developmental landscape of arabidopsis root**. *Mol Plant* 2019, **12**:648–660.

70. Shulse CN, Cole BJ, Ciobanu D, Lin J, Yoshinaga Y, Gouran M, Turco GM, Zhu Y, O'Malley RC, Brady SM, *et al.*: **High-throughput single-cell transcriptome profiling of plant cell types**. *Cell Rep* 2019, **27**:2241–2247.e4.

71. Nolan TM, Vukašinović N, Hsu C-W, Zhang J, Vanhoutte I, Shahan R, Taylor IW, Greenstreet L, Heitz M, Afanassiev A, *et al.*: **Brassinosteroid gene regulatory networks at cellular resolution**. *bioRxiv* 2022, **4721**:2022. 09.16.508001.

72. ** Marand AP, Chen Z, Gallavotti A, Schmitz RJ: **A cis -regulatory atlas in maize at single-cell resolution**. *Cell* 2021, **184**:3041–3055.

The authors concordantly assess the chromatin accessibility (snATAC-seq) and transcriptome (snRNA-seq) landscape at the single-cell level in six different maize organs. This reveals several insights, including the presence of tissue-specific cis-regulatory regions under selective pressure, the observation that silent accessible regions tend to be marked by H3K27me3, and the identification of putative TFs acting in a non-cell autonomous manner.

73. Cox KL, Ranjan Gurazada SG, Duncan KE, Czymmek KJ, Topp CN, Meyers BC: **Organizing your space: the potential for integrating spatial transcriptomics and 3D imaging data in plants**. *Plant Physiol* 2022, **188**:703–712.

74. Gopalan S, Wang Y, Harper NW, Garber M, Fazzio TG: **Simultaneous profiling of multiple chromatin proteins in the same cells**. *Mol Cell* 2021, **81**:4736–4746.e5.

75. Zhu J, Signe Lolle S, Andrea Tang A, Bella Guel B, Brian Kvitko B, Benjamin Cole B, Gitta Coaker G: **Single-cell profiling of Arabidopsis leaves to *Pseudomonas syringae* infection**. *Cell Rep* 2023, **42**, 112676.

76. Sun B, Looi LS, Guo S, He Z, Gan ES, Huang J, Xu Y, Wee WY, Ito T: **Timing mechanism dependent on cell division is invoked by Polycomb eviction in plant stem cells**. *Science* 2014, **343**, 1248559.

77. Policarpi C, Dabin J, Hackett JA: **Epigenetic editing: dissecting chromatin function in context**. *Bioessays* 2021 May, **43**, e2000316, https://doi.org/10.1002/bies.202000316. Epub 2021 Mar 16. PMID: 33724509.

78. Skene PJ, Henikoff S: **An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites**. *Elife* 2017, **6**, e21856. 2017.

79. Zheng XY, Gehring M: **Low-input chromatin profiling in Arabidopsis endosperm using CUT&RUN**. *Plant Reprod* 2019, **32**:63–75.

80. Simonini S, Bemer M, Bencivenga S, Gagliardini V, Pires ND, Desvoyes B, van der Graaff E, Gutierrez C, Grossniklaus U: **The Polycomb group protein MEDEA controls cell proliferation and embryonic patterning in Arabidopsis**. *Dev Cell* 2021, **56**:1945–1960.