Review

Molecular aspects of defence priming

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Plants can be primed for more rapid and robust activation of defence to biotic or abiotic stress. Priming follows perception of molecular patterns of microbes or plants, recognition of pathogen-derived effectors or colonisation by beneficial microbes. However the process can also be induced by treatment with some natural or synthetic compounds and wounding. The primed mobilization of defence is often associated with development of immunity and stress tolerance. Although the phenomenon has been known for decades, the molecular basis of priming is poorly understood. Here, I summarize recent progress made in unravelling molecular aspects of defence priming that is the accumulation of dormant mitogen-activated protein kinases, chromatin modifications and alterations of primary metabolism.

Defence priming

Besides structural barriers and constitutive secondary metabolites, plants evolved inducible defence mechanisms to ward off potentially dangerous microbes and insects. For example, pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs, respectively; see Glossary), damage-associated molecular patterns (DAMPs), pathogen effectors, or wound stimuli, can all initiate a stereotypic defence response [1] that involves the so-called priming of cells, both in tissue exposed to the PAMP, MAMP, DAMP, effector, or wound stimulus, and also in the systemic tissue [2–6]. Priming is the phenomenon that enables cells to respond to very low levels of a stimulus in a more rapid and robust manner than non-primed cells (Box 1) [2–4]. Thus, primed plants show faster and/or stronger, activation of defence responses when subsequently challenged by microbes, insects, or abiotic stress, and this is frequently linked to development of local and systemic immunity and stress tolerance [2–4].

As early as 1933 priming, called 'sensitization' at the time, was widely accepted to be the pivotal phenomenon in systemic plant immunity [7], although clear evidence for a critical role of priming in the systemic immune response was missing, and studies on priming that followed this early report were invariably descriptive. Thanks to the increasing number of molecular and genetic tools in the past decades, priming has now proven true as a critical process in various types of systemic plant immunity [2–5]. These include systemic acquired resistance (SAR) [5,8,9], induced systemic resistance (ISR) [2–4,10], the resistance provided by symbiotic fungi [11], β-aminobutyric acid-induced resistance (BABA-IR) [12] and wound-induced resistance [2–4,6]. Until fairly recently, however, molecular mechanisms of priming remained elusive. One hypothesis proposed that cell priming involves accumulation of inactive cellular proteins that play an important role in cellular signal amplification [3,13]. Subsequent exposure to biotic or abiotic stress could activate these dormant signalling proteins, thereby initiating signal amplification and lead to more rapid and robust activation of defence, immunity, and stress tolerance (Figure 1) [3]. The identity of these hypothetical signalling proteins, however, has just recently been disclosed.

Defence priming by accumulation of dormant mitogen-activated protein kinases

Mitogen-activated protein kinases (MPKs) are part of so-called MPK cascades. These signalling kinase modules can

Glossary

β-Aminobutyric acid (BABA): non-protein amino acid which can prime plant defence responses and confer resistance to biotic and abiotic stress.
Callose: plant polysaccharide made of β-1,3-linked glucose residues with some β-1,6 linkages. Callose is synthesised during plant development and in response to wounding and pathogen attack. Callose is considered to provide a physical entry barrier at bacterial and fungal penetration sites.
Chromatin: combination of DNA, RNA, histones, and non-histone proteins that makes up chromosomes. It packages DNA into a smaller volume to fit in the nucleus, to strengthen DNA during mitosis and meiosis, and to control gene expression and DNA replication.
Effector: weapon of a pathogen to manipulate its host; frequently a suppressor of PAMP- or MAMP-triggered immunity that, when recognised, induces hypersensitive cell death in the plant.
Histones: proteins associated with DNA in chromatin. They have a high content of basic amino acid residues such as lysine and arginine. There are five types of histones H1, H2A, H2B, H3, and H4.
Damage-associated molecular pattern (DAMP): signals arising from plants because of damage caused by microbes; originally referred to as 'endogenous elicitors'.
Induced systemic resistance (ISR): type of systemic, broad-spectrum immunity in plants. Induced systemic resistance is elicited by colonisation with selected strains of non-pathogenic, plant growth-promoting rhizobacteria and depends on the plant hormones ethylene and jasmonic acid.
Mitogen-activated protein kinase (MPK): enzyme which catalyses the transfer of a phosphoryl group from adenosine triphosphate to a protein substrate, such as WRKY transcription co-activators.
Microbe-associated molecular pattern (MAMP): molecular signatures typical of whole classes of microbes. Their recognition plays a key role in innate immunity in plants and animals.
Pathogen-associated molecular pattern (PAMP): molecular signatures typical for potential microbial pathogens of a given host organism.
Priming: physiological state that enables cells to respond to very low levels of a stimulus in a more rapid and robust manner than non-primed cells. In plants, priming plays a role in defence ('defence priming') and seed germination ('seed priming').
Systemic acquired resistance (SAR): type of systemic, broad-spectrum immunity in plants. Systemic acquired resistance is induced by local contact with a MAMP, PAMP, or effector and depends on the plant hormone salicylic acid.
Transcription co-activator: diverse array of cellular factors that connect sequence-specific DNA-binding activators to the general transcription machinery, or that help activators and the transcription apparatus to navigate through the constraints of chromatin.
WRKY transcription factor: member of a large family of transcription co-activator modulating many plant processes, which include responses to biotic and abiotic stress.

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Box 1. Defence priming and its potential in modern plant disease management

Defence priming is a unique physiological state that can be induced by molecular patterns of microbes or plants, pathogen-derived effectors, beneficial microbes, and treatment with some natural or synthetic compounds and wounding [2–4]. Primed plants show fast and/or strong, activation of defence responses when subsequently challenged by microbes, insects, or abiotic stress. This primed activation of defence is frequently linked to local and systemic immunity and stress tolerance [2–4]. While the phenomenon has been described copiously, the molecular mechanism(s) of defence priming are just beginning to be unravelled. Defence priming has now emerged as a promising means for sustainable modern pest management in the field, because some pesticides have been shown to exert their known plant health- and yield-increasing effects through priming [72].

be found in all eukaryotes [14]. MPK cascades function downstream of receptors and transmit extracellular stimuli (e.g. MAMPs) into intracellular responses, while at the same time amplifying the transducing signal [14,15]. Signal amplification is achieved by three hierarchically arranged, interacting types of kinases. MPK activity is induced upon phosphorylation by MPK kinases (MEKs or MPKKs); which are in turn activated, via phosphorylation, by MPKK kinases (MEKKs or MPKKKs). MPKKK activity is induced upon receptor–ligand binding.

For example, perception of the bacterial MAMP flagellin, or its conserved N-terminal 22-amino-acid epitope flg22, by the pattern recognition receptor (PRR) FLS2 stimulates a MPK cascade composed of MEKK1, MEK4/5, and MPK3/6 in leaf protoplasts of *Arabidopsis* (*Arabidopsis thaliana*) [16]. The cascade amplifies the transducing flagellin/flg22 signal and ultimately activates various defence genes including those encoding transcription co-activators WRKY22 and WRKY29, both of which play a role in plant immunity [17]. In *Arabidopsis*, there are 20 MPKs, 10 MEKs, and > 80 MEKKs [18]. Because of their key role in signal amplification, MPKs, MEKs, and MEKKs are excellent candidates for cellular signalling enzymes that mediate priming.

In 2009, a study with *Arabidopsis* reported the first identification of dormant MPKs that accumulate during priming. It was shown [8] that priming *Arabidopsis* by

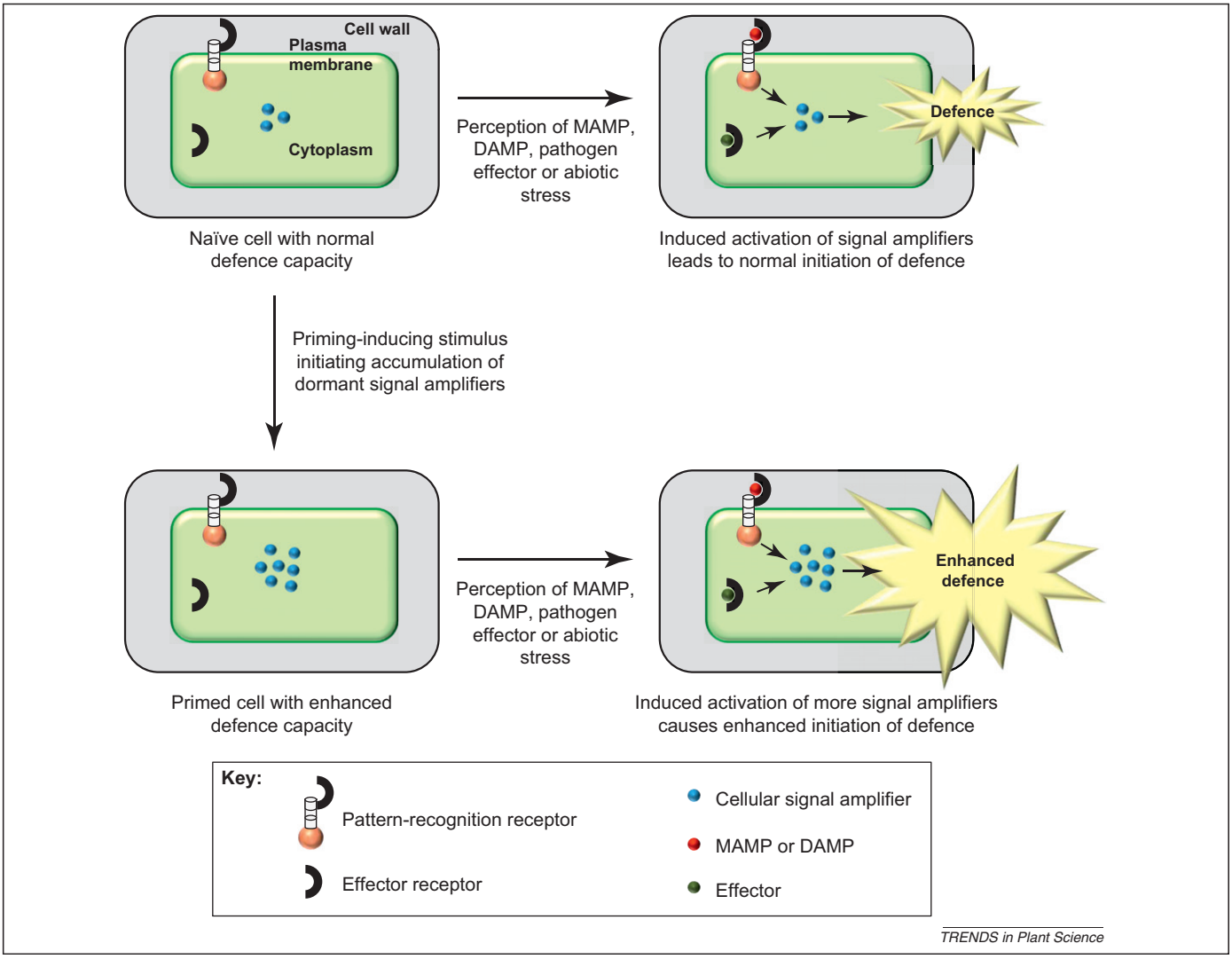

Figure 1. Accumulation of inactive cellular signal amplifiers is a likely mechanism of defence priming in plants. A priming-inducing stimulus seems to enhance the cellular level of inactive proteins with an important role in cellular signal amplification. Subsequent exposure to MAMPs, DAMPs, pathogen effectors, or abiotic stress activates more of these dormant signalling proteins in primed cells than in non-primed cells. It thereby initiates strong signal amplification which leads to more rapid and robust activation of defence associated with immunity and stress tolerance.

treatment with the SAR activator benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (subsequently referred to in this article as benzothiadiazole), a synthetic analogue of the plant hormone and defence-signal salicylic acid (SA), is associated with accumulation of mRNA and inactive proteins of MPK3 and MPK6. These enzymes were known to play a role in the direct defence response of *Arabidopsis* to microbial pathogens and various abiotic stresses [19–22], but until then they had not been associated with priming. Increased levels of MPK3 and MPK6 transcript and protein were also detected in upper *Arabidopsis* leaves after primary infection of lower leaves with the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) strain DC3000 expressing the *avrRpt2* avirulence gene and inducing SA biosynthesis throughout the plant. Upon challenge infection of upper leaves with bacteria, or after simply infiltrating water into the leaves, more of these proteins were activated in primed than in non-primed plants. The quantitatively greater activity of MPK3 and MPK6 in primed plants was linked to enhanced *PAL1* and *PR1* defence gene activation and to development of systemic immunity in these plants [8].

In the constitutively primed *edr1* mutant of *Arabidopsis* there was no need for a priming-inducing treatment to strongly activate MPK3 and MPK6 activity by water infiltration [8]. In addition, accumulation and enhanced activation of the two enzymes was attenuated in the priming-deficient *npr1* mutant [8]. Furthermore, primed *PAL1* and *PR1* activation and systemic immunity were lost in *mpk3* and reduced in *mpk6* mutants [8]. These findings argued that pre-stress accumulation of the signalling enzymes MPK3 and MPK 6 is a critical step in priming *Arabidopsis* for defence (Figure 1). This conclusion is supported by a very recent synthetic biology approach in which insulated mammalian MPK cascades were constructed in yeast (*Saccharomyces cerevisiae*) to explore how intrinsic and extrinsic perturbations affect the flexibility of these synthetic signalling modules [23]. The study revealed the critical importance of the relative kinase concentration in dictating the activation profile of MPKs. It also identified cascading itself as a mechanism for generating ultrasensitivity and low activation threshold [23].

In the study by Beckers *et al.* [8] priming and systemic immunity were absent in *mpk3* but not completely gone in *mpk6*. Thus, it was assumed that MPK3 is a major component in primed defence gene activation and systemic immunity in *Arabidopsis*, while MPK6 likely serves only a minor role [8]. This conclusion is supported by two findings, one demonstrating that the hyper-phosphorylation of MPK6 in the *mpk3* mutant did not cause resistance [8] and another showing that *MPK6*-silenced plants are not affected in their ability to express systemic immunity [24]. However, both MPK3 and MPK6 seem to be required for full priming and systemic immunity in *Arabidopsis,* because primed gene expression was somewhat affected in *mpk6* mutants and *MPK6*-silenced plants [8].

In *Arabidopsis*, overexpression of the *MPK3* gene under control of constitutive and inducible promoters failed. Yet overexpression of *MPK3* orthologues in other plants led to immunity. For example, overexpression of the gene for *Trichoderma*-induced protein kinase (TIPK), the cucumber (*Cucumis sativus*) orthologue to *Arabidopsis MPK3*, conferred immunity to *Ps* pv. *lachrymans* in cucumber leaves [25]. *TIPK* antisense cucumber plants were consistently more prone to pathogen attack, than the non-transformed control [25]. Similar to those results transformation of rice (*Oryza sativa*) with *MK1*, the pepper (*Capsicum annuum*) orthologue of *Arabidopsis MPK3*, resulted in constitutive expression of the transgene, permanent accumulation of the MK1 protein, and immunity to rice blast disease [26]. These findings demonstrate that, by constitutive expression of genes that are orthologous to *Arabidopsis MPK3*, priming and immunity can be conferred on plants that have not previously been primed.

The important role of MPK3 and MPK6 in plant immunity is further supported by the discovery that certain plant pathogens seem to use some of their effectors to dephosphorylate and thus inactivate MPK3 and MPK6. HopAl1, for example, is an effector that contributes to *Pst* virulence in plants. The effector acts as a phosphothreonine lyase that dephosphorylates, and therefore inactivates, MPKs and finally terminates PRR signalling [27]. HopAl1 directly interacts with *Arabidopsis* MPK3 and MPK6, suppresses MPK3 and MPK6 activation by flagellin, and dampens the associated immune response [28]. Thus, upregulation of MPK3 and MPK6 levels seems to enhance, and downregulation of their activity to reduce, immune responses in *Arabidopsis*.

Priming is tightly linked to chromatin modification

Chromatin state is pivotal to gene regulation in eukaryotes. The fundamental repeating unit and building block of chromatin is the nucleosome, in which 147 base pairs of genomic DNA are wrapped in one and three-quarters of a turn around an octamer core of two copies each of the histone proteins H2A, H2B, H3, and H4 [29] (Figure 2). RNA and non-histone proteins are further important components of chromatin. During gene regulation DNA and histones are subject to covalent modifications such as methylation, acetylation, ubiquitination, and poly-ADP-ribosylation [30]. Acetylation of lysine residues in the N-termini of histones H3 and H4 almost invariably comes with gene activity [31]. This covalent modification is assumed to mask the positive charge of lysine residues within histones and thus reduce the ionic interactions between histone proteins and DNA [32]. In addition, lysine acetylation on histones also provides docking sites for transcription co-activators containing bromodomains [33].

In contrast to acetylation, the role of histone methylation in chromatin regulation is highly complex and poorly understood. This is because both lysine and arginine residues can be methylated and up to three methyl groups can stick to the same lysine residue [34]. In addition, specific methylation patterns have been associated with activation as well as repression of genes. The closest correlation between histone methylation and gene activity was reported for tri-methylation of lysine 4 in histone H3 (H3K4me3) on promoters and coding regions of active genes [35]. By contrast, the role in gene regulation of di- and mono-methylation of the same lysine residues is less clear.

Another hypothesis on the molecular mechanism of priming suggested that chromatin modifications prime

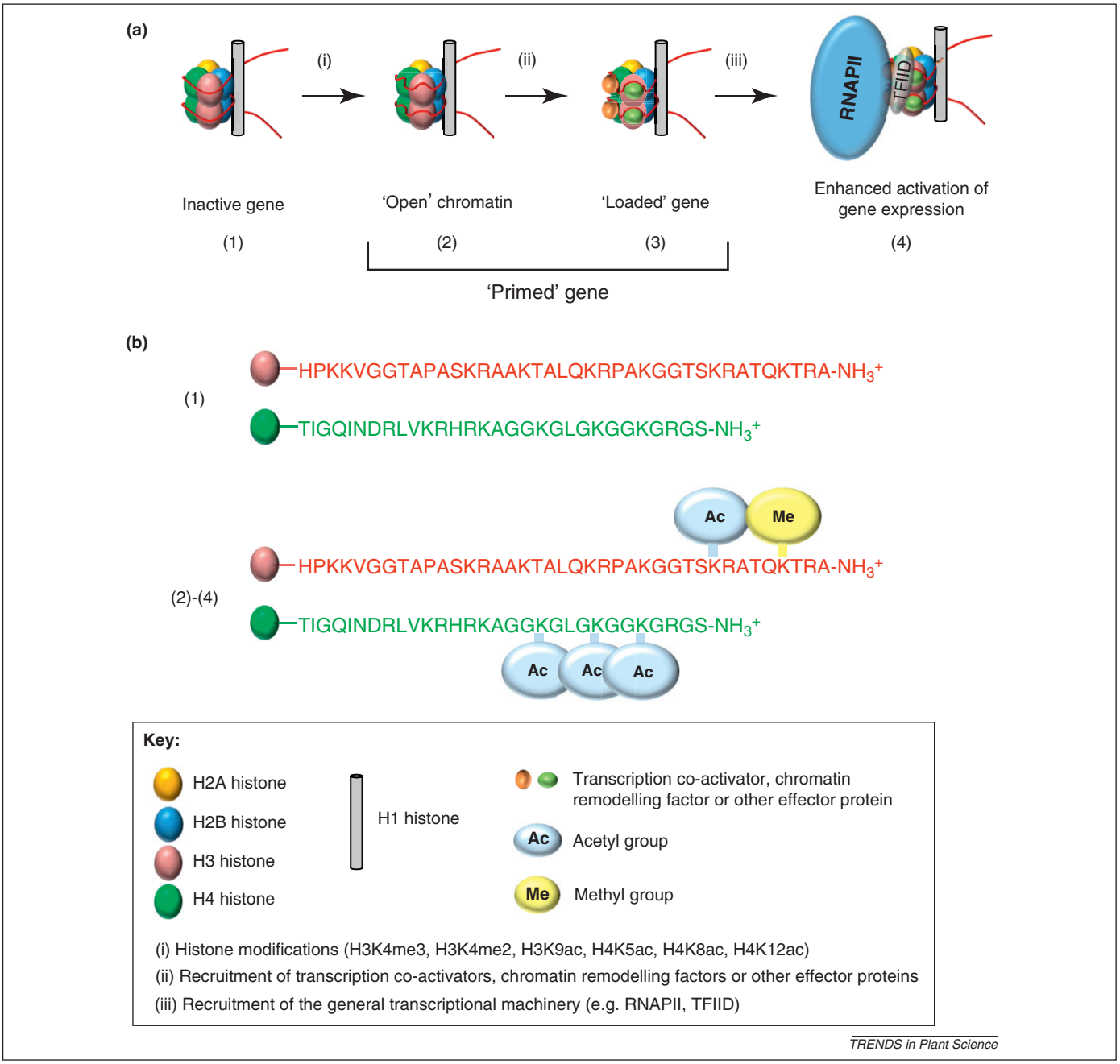

Figure 2. Chromatin modifications are likely involved in priming defence genes for faster and more robust activation of expression. **(a)** In nucleosomes, 147 base pairs of genomic DNA are wrapped in one and three-quarters of a turn around an octamer core of two copies each of histones H2A, H2B, H3, and H4. Histone H1 provides a higher order of chromatin packing. In this chromatin state, genes are transcriptionally inactive. A priming-inducing treatment causes covalent modifications of histones that reduce their ionic interaction with DNA and thus provide 'open chromatin' and/or docking sites for transcription co-activators, chromatin remodelling factors, or other effector proteins in chromatin. Binding of these effector proteins 'loads' the appropriate gene. Together, these processes could facilitate subsequent stress-induced recruitment of components of the general transcription machinery such as the RNA polymerase II (RNAPII) complex and transcription factor IID (TFIID), thereby enhancing transcription initiation and gene expression. Numbers (1–4) refer to the chromatin modification states described in **(b)**. **(b)** Inactive chromatin is characterised by ionic interactions of lysine residues in the N-termini of histone proteins H3 and H4 with genomic DNA. Methylation and acetylation of H3 lysine residues 4 and 9, respectively, and acetylation of H4 lysine residues 5, 8, and 12 are assumed to reduce this interaction and possibly also provide docking sites for transcription co-activators. Numbers (1–4) refer to the chromatin states described in **(a)**.

the defence genes for faster and more robust activation [13]. In fact, it was reported as early as 1993 that treatment of barley (*Hordeum vulgare*) plants with any of the three activators of plant immunity, N-methyl-nicotinic acid (Trigonelline), a culture filtrate of *Bacillus subtilis* (B50) or isonicotinic acid methyl ester (a synthetic SAR activator), causes faint, yet significant reduction in genomic DNA cytosine methylation associated with establishment of powdery mildew resistance [36]. This report linked changes in chromatin structure to induced plant immunity. More recent work demonstrated that replacement of histone H2A by its variant H2A.Z is important to the regulation of SA-responsive genes in *Arabidopsis* [37] and it has been speculated that H2A.Z deposition primes the SA-responsive genes for their activation by transcription co-activators [37,38]. Furthermore it was proposed

that chromatin modifications provide a memory for systemic immunity in *Arabidopsis* [38], but resilient experimental data supporting this assumption were missing.

In a study on the control of the *Arabidopsis WRKY70* locus, H3K4me3 has been uncoupled from expression of the *PR1* and *THI2.1* defence genes [39]. H3K4me3 has instead been associated with an 'actively' modified state of genes, and the authors hypothesized that this was in preparation for quick-changes in transcription during times of stress [39].

A very recent study with *Arabidopsis* demonstrated that priming the promoter of the transcription co-activator gene *WRKY29* by benzothiadiazole is associated with H3K4me3 and H3K4me2, as well as acetylation of H3K9, H4K5, H4K8, and H4K12 [40]. Yet these modifications did not activate *WRKY29* expression until the plants were challenged with an additional stress stimulus, such as the infiltration of water into leaves [40]. Thus, specific chromatin marks that for a long time have been associated with gene activity [39,41] are induced during priming before actual activation of defence genes. Similar observations were made for *WRKY6* and *WRKY53* when H3K4me3, H3K4me2, and activation of transcription were assayed [40]. Together these findings demonstrated that priming of certain *WRKY* promoters involves chromatin modifications that facilitate transcription activation of *WRKY* genes by subsequent stress, such as water infiltration. These chromatin modifications could either slack the interaction of histones and DNA, thus providing 'open chromatin' and/or docking sites for transcription co-activators, chromatin remodelling factors, or other effector proteins in chromatin [31,33,42,43]. Both these processes could facilitate recruitment of components of the general transcription machinery such as the RNA polymerase II (RNAPII) complex and transcription factor IID (TFIID), thus supporting transcription initiation and gene expression (Figure 2). Whether MPK3/6-mediated phosphorylation of protein substrates plays a role in this hypothetical scenario remains to be investigated. Nucleosome occupancy on the *WRKY* promoters was only slightly affected by benzothiadiazole [40], therefore removal of nucleosomes appears not to play a critical role in *WRKY6*, *WRKY29*, and *WRKY53* priming by this compound.

In a bona-fide SAR experiment, localised inoculation of *Arabidopsis* leaves with the bacterial pathogen *Ps* pv. *maculicola* in systemic leaves primed the *WRKY6*, *WRKY29*, and *WRKY53* defence genes for enhanced expression by a subsequent stress stimulus [40]. The systemic *WRKY6*, *WRKY29*, and *WRKY53* priming was associated with enhanced H3K4me3 and H3K4me2 on the *WRKY6*, *WRKY29*, and *WRKY53* promoters, and with augmented H4K5ac, H4K8ac, and H4K12ac on *WRKY29*. These histone modifications were induced on the *WRKY* promoters in systemic leaves after the primary *Ps* pv. *maculicola* infection of local leaves and before challenge with biotic or abiotic stress [40]. Thus, it seems that local infection by *Ps* pv. *maculicola* induces one or more endogenous signals, which are converted and stored in systemic leaves as specific histone modifications on the promoters of defence genes. Hence, as assumed previously [38], chromatin modifications indeed seem to serve as a memory for priming in systemic plant immunity.

Further analysis of H3K4me3 in known priming mutants revealed that this modification strongly correlates with the presence of priming. In the priming-deficient *npr1* mutant of *Arabidopsis* [8,9], *WRKY29* was activated after infiltration of water into leaves [40] but, in contrast to wild-type plants, this response was not primed after previous benzothiadiazole treatment of *npr1*. In a converse manner, in the constitutively primed *cpr1* and *edr1* mutants and the permanent immunity mutant *sni1* [8,9,40] benzothiadiazole treatment was not required for strong *WRKY29* activation by water infiltration [40]. In fact, the extent of *WRKY6*, *WRKY29*, and *WRKY53* activation in these mutants was similar to those observed in the benzothiadiazole-primed wild-type after water infiltration of leaves [40]. This finding revealed that the three *WRKY* genes were constitutively primed in *cpr1*, *edr1*, and *sni1*. Consistent with the *WRKY29* transcription response, benzothiadiazole elicited H3K4me3 on the *WRKY29* promoter in the wild-type plant, but not the priming-deficient *npr1* mutant plant whereas in *sni1* and *cpr1*, H3K4me3 levels were permanently enhanced. However, this strong correlation of H3K4me3 and *WRKY* priming was not evident for *edr1* in which H3K4me3 on the *WRKY29* and *WRKY53* promoters was low [40]. Thus a mechanism of priming other that H3K4me3 has been postulated for these two genes in *edr1* [40]. It is currently unclear whether the enhanced level of MPK3 seen in *edr1* is exclusively responsible for priming in this mutant.

So far, only few studies provide convincing evidence for the induction of a primed state of transcription by chromatin modifications in the context of plant immunity. A previous study revealed that in the permanently primed *sni1* mutant with constitutively enhanced immunity, the *PR1* defence gene promoter was associated with high levels of H3ac and H3K4me before *PR1* gene activation [44]. This constitutive histone modification in *sni1* has been ascribed to the loss of SNI1 which is believed to be a nuclear transcription repressor of *PR* genes [44] and a co-regulator of DNA recombination during the systemic immunity response [45]. In support of this, viral infection of tobacco (*Nicotiana tabacum*) led to systemic enhancement of DNA recombination associated with the development of systemic immunity [46].

Other studies that linked primed activation of transcription to chromatin modifications mostly addressed open questions in plant development. For example, in maize (*Zea mays*) cell type-specific stimulation of H3K4me3 was reported to prime the C_4 photosynthesis genes encoding phosphoenolpyruvate carboxylase in mesophyll, and decarboxylase malic enzyme in bundle sheet cells for enhanced activation by light [47]. Priming by histone modifications was also described for the vernalisation response in *Arabidopsis* [48]. Genetic and molecular studies showed that vernalisation, the autonomous pathway, and the frigida protein all can induce H3K4me3 and histone acetylation on the *FLOWERING LOCUS C* (*FLC*), which encodes a MADS-box transcription factor that blocks floral transition and enhances *FLC* activation by other transcription co-activators. Induction of histone deacetylation, H3K9me2, and H3K27me2 had the opposite effect. These chromatin modifications thus provide an additional level of regulation of *FLC* expression [48].

Another prominent example for primed gene activation by chromatin modification is the hormonal regulation of the bean (*Phaseolus vulgaris*) *PHASEOLIN* (*PHAS*) gene which encodes the major seed storage protein phaseolin in this plant. The *PHAS* promoter is not inducible by the plant hormone abscisic acid (ABA) in vegetative tissue, but it is inducible in intact embryos [49,50]. The tissue-specific response to ABA is caused by differential chromatin architecture in these two tissues. The *PHAS* promoter has a repressive chromatin configuration in leaf tissue that is disrupted by increased acetylation of H3K9 and H4K12 concomitant with an enhanced capacity for being transcribed in developing seeds [51]. This architectural change is mediated by *Phaseolus vulgaris* ABI3-like factor (ALF), a seed-specific transcription co-activator [52]. Ectopic expression of ALF can accordingly modify the chromatin structure of the *PHAS* promoter in vegetative tissue, rendering the promoter accessible to DNAse I [53]. Although the acetylation of H3K9 and H4K12 does not activate the *PHAS* promoter, it seems to prime it, thereby facilitating binding of one or more ABA-activated transcription co-activator(s) to their binding site. Together, these findings linked histone modification-induced priming of gene regulatory elements to key phenomena in plant development.

Defence priming by molecular modifications of primary metabolism

Mainly using reverse genetics, it has been reported over the past ten years that primary metabolism and retrograde signalling from organelles play important roles in the overall defence capacity of plants [54,55]. In 2002, it was reported that tubers of transgenic potato (*Solanum tuberosum)* plants constitutively expressing an antisense copy of the plastid ATP/ADP antiporter AATP1 strongly activated the oxidative burst and expression of the *PAL* and *PR* defence genes after exposure to fugal or bacterial MAMPS [56]. Primed activation of defence in AATP1 tubers was associated with the establishment of immunity against *Pectobacterium carotovorum* and *Alternaria solani* in tubers and to *Phytophthora infestans* in the leaves [56,57]. Similar results were made with the *Arabidopsis ntt1-2* double mutant that has T-DNA insertions in the two genes encoding plastid ATP/ADP antiporters. This mutant showed constitutive or primed activation of defence genes after inoculation with *Hyaloperonospora arabidopsidis*, *Botrytis cinerea*, and *Pst* DC3000, and it had induced immunity to these pathogens but only when grown in short days [58]. These findings implied that nocturnal ATP transport into chloroplasts is crucial to keep *Arabidopsis* from runaway activation of plant immunity, and that altered ATP/ADP antiport across the plastid envelope can prime plant defence and immunity. However, the mechanism(s) by which alterations in ATP/ADP antiport across the plastid envelope cause this metabolic priming are still unknown.

Other known molecular components of defence priming

It has been shown that the priming of *PAL1* activation and callose deposition in *Arabidopsis* by benzothiadiazole requires a functional *NPR1* gene [9]. The encoded NPR1 protein is a key transcription co-activator of SAR [59] whose overexpression primes *Arabidopsis* and rice for enhanced *PR* gene activation and immunity [60,61]. In *Arabidopsis* NPR1 also is important to the primed defence response to *H. arabidopsidis* during BABA-IR and the ISR response induced by *Pseudomonas fluorescens* WCS417r [62]. The WCS417r-induced ISR also requires the plant hormones ethylene and jasmonic acid and the transcription co-activators MYB72 and MYC2. MYC2 seems rather to play a crucial role in the systemic tissue during ISR induction [63], whereas *MYB72* expression is specifically activated in the WCS417r-colonised roots and thus seems to be involved in early steps of ISR establishment [64]. The molecular mechanism(s) by which MYB72 and MYC2 exert their specific role in the primed ISR response of *Arabidopsis*, however, are unknown.

The primed deposition of callose associated with BABA-IR and ISR helps *Arabidopsis* to ward off, for example, *H. arabidopsidis* and seems to require *SAC1b* and *ABA1*/*NPQ2*. These genes encode a polyphophoinositide phosphatase and zeaxanthin epoxidase [65,66], respectively. Zeaxanthin epoxidase plays a key role in the synthesis of ABA. Thus, BABA- and ISR-associated priming of callose deposition seems to be regulated by a phosphoinositide- and ABA-regulated signalling pathway whose identity is still unknown [62].

Over the past 20 years a wealth of papers confirmed that the systemic accumulation of SA, whether derived from methyl-SA [67] or *de novo* synthesis [68], is a key event in the SAR type of systemic immunity in plants. It was shown also that SA primes plants for enhanced induction of defence [69] and directly activates the expression of genes encoding PR proteins with anti-microbial activity [70]. However, the establishment of SAR is not always correlated with systemic accumulation of SA [71]. A recent publication [5] revealed that cell priming during SAR in *Arabidopsis* involves accumulation of the secondary metabolite azelaic acid (AZA). Upon localised bacterial infection of lower leaves this mobile nine-carbon dicarboxylic acid is transported throughout the plant and confers local and systemic immunity to *Ps* pv. *maculicola*. While doing so, AZA primes the plant to accumulate higher SA levels upon challenge infection than non-primed plants. Mutation of the AZA-responsive *AZI1* gene leads to the specific loss of pathogen- or AZA-induced priming of SA accumulation and attenuates systemic immunity. Thus, SA and AZA are plant secondary metabolites that after localised infection accumulate throughout the plant to prime defence and enhance immunity. AZI1 is a likely component to be involved in AZA-induced priming and systemic immunity in *Arabidopsis* but the biochemical mechanism(s) of AZI1 action remain elusive.

Conclusions and outlook

It has been known for a long time that priming is a part of systemic immunity responses in plants, but little is known about the molecular mechanism(s) of priming. Recently it has been demonstrated that specific chromatin modifications on defence gene promoters in primed leaves and the elevated accumulation of inactive MPK3 and MPK6 signalling proteins, in addition to the enhanced level of SA and AZA, are potentially crucial steps in the priming

mechanism that sensitises *Arabidopsis* for more rapid and robust activation of defence and immunity. It will be interesting to see whether one or more factor(s) upstream of MPK3 or MPK6 in the MPK cascade, such as members of the MEK and MEKK families of kinases and/or sensors for MAMPs, PAMPs, DAMPs, bacterial effectors, or abiotic stress stimuli do also play a role in priming. In addition, it will be interesting to identify further histone modifications associated with priming and elucidate whether the histone modifications observed during gene priming indeed provide docking sites for transcription co-activators. If so, the co-activators' identity needs to be disclosed in future work. It will also be interesting to learn whether there is a direct link between accumulation of dormant MPK3/6 signalling proteins and the priming-associated histone modifications. Furthermore, confirmation is needed whether there is a direct functional link between histone modifications and the primed state in plants. Finally, the role of AZI1 in priming needs to be elucidated.

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