Multiple chromatin-associated modules regulate expression of an intracellular immune receptor gene in Arabidopsis

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Summary

- The expression of an intracellular immune receptor gene *SNC1* (*SUPPRESSOR OF npr1*, *CONSTITUTIVE 1*) is regulated by multiple chromatin-associated proteins for tuning immunity and growth in Arabidopsis. Whether and how these regulators coordinate to regulate *SNC1* expression under varying environmental conditions is not clear.
- Here we identified two activation and one repression regulatory modules based on genetic and molecular characterizations of five chromatin-associated regulators of *SNC1*.
- Modifier of *snc1* (MOS1) constitutes the first module and is required for the interdependent functions of ARABIDOPSIS TRITHORAX-RELATED 7 (ATXR7) and HISTONE MONO-UBIQUITINATION 1 (HUB1) to deposit H3K4me3 and H2Bub1 at the *SNC1* locus. CHROMATIN REMODELING 5 (CHR5) constitutes a second module and works independently of ATXR7 and HUB1 in the MOS1 module. HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 15 (HOS15) constitutes a third module responsible for removing H3K9ac to repress *SNC1* expression under non-pathogenic conditions. The upregulation of *SNC1* resulting from removing the HOS15 repression module is partially dependent on the function of the CHR5 module and the MOS1 module.
- Together, this study reveals both the distinct and interdependent regulatory mechanisms at the chromatin level for *SNC1* expression regulation and highlights the intricacy of regulatory mechanisms of NLR expression under different environment.

Key words: chromatin, histone modification, NLR genes, *SNC1*, transcriptional regulation

Introduction

Intracellular Nucleotide-binding Leucine-rich Repeat (NLR) receptors play critical role in plant innate immunity for plants to defend various pathogens (Jones & Dangl, 2006; Ngou *et al*., 2022). They directly or indirectly detect intracellular effector proteins secreted from pathogens to initiate Effector-Triggered Immunity and also enhance Pattern-Triggered Immunity initiated by cell surface immune receptors (Pruitt *et al*., 2021; Tian *et al*., 2021). NLR genes are tightly regulated not only for a timely and effective immune response but also for a growth-defense balance. Under nonpathogenic condition, high expression of NLR genes often leads to plant dwarfism and even lethality (Gou & Hua, 2012; van Wersch *et al*., 2016). Most NLR genes are expressed at low levels and often with tissue specificities (Tan *et al*., 2007). For instance, NLR genes are preferentially expressed in shoots in *Arabidopsis thaliana* (hereafter Arabidopsis) but preferentially in roots in lotus (Munch *et al*., 2017). NLR genes often have a higher expression under pathogen attack (Mohr *et al*., 2010; Yang *et al*., 2021). About 2/3 of total NLR genes in Arabidopsis Col-0 accession are induced by various pathogens and immune elicitors (Yang *et al*., 2021). The upregulation of NLR genes during defense response has also been observed in other plant species such as rice (Gu *et al*., 2005), cabbage (Chen *et al*., 2016) and walnut (Chakraborty *et al*., 2016). This suggests transcriptional regulation of NLR genes is broadly present in plants.

The transcript-level expression control of NLR genes can occur through multiple mechanisms such as transcription factor regulation, chromatin modification, alternative splicing and alternative polyadenylation (Lai & Eulgem, 2018; Zhang *et al*., 2018; Yang *et al*., 2020). Histone post-translational modifications (hereafter, histone modifications) and ATP-dependent chromatin remodeling (hereafter, chromatin remodeling) constitute chromatin modification based transcriptional control by shaping chromatin structure to permissive or repressive states for transcription (Bannister & Kouzarides, 2011; Clapier *et al*., 2017), or they can affect RNA processes such as alternative polyadenylation to regulate gene expression (Lai & Eulgem, 2018; Lai et al., 2019) Histones have various covalent modifications on a number of residues including methylation, acetylation and ubiquitination. Some are associated with transcription activation such as trimethylation of histone H3 on lysine 4 or lysine 36 (H3K4me3 or H3K36me3), ubiquitination on histone 2B (H2Bub) and histone acetylation while others are associated with transcription repression such as H3K27me3 and H3K9me2 (Pfluger & Wagner,

2007). For instance, Arabidopsis SDG8 (SET DOMAIN GROUP 8), a histone lysine methyltransferase, deposits H3K36me3 at the *RESISTANT TO P. SYRINGAE 4* (*RPS4*) -like NLR gene *LAZARUS 5* (*LAZ5*) locus and activates *LAZ5* transcription to enhance plant disease resistance to various pathogens (Palma *et al*., 2010). In addition, Histone modifications can crosstalk with chromatin remodelers for gene regulation. For instance, the human Chd1 (Chromodomain Helicase DNA-binding 1), a chromatin remodeler, directly binds to methylated H3K4 (Sims *et al*., 2005), and its yeast ortholog affects the spatial distribution of H3K4me3 and H3K36me3 (Lee *et al*., 2017). A chromatin remodeler protein SWP73A directly binds to and represses several NLR genes through H3K9me2 (Huang *et al*., 2021). Therefore, chromatin modification based transcriptional control of NLR genes plays a key role in plant immunity.

Like many other NLR genes, the transcription of *SUPPRESSOR OF npr1*, *CONSTITUTIVE 1* (*SNC1*) is intricately controlled. A small change of *SNC1* transcripts could have discernible effects on Arabidopsis growth and immunity (Zou *et al*., 2014; Yang *et al*., 2020). Even one extra copy of the *SNC1* gene can cause severe dwarfism and induce strong defense response (Li *et al*., 2007b; Stokes *et al*., 2002; Yi & Richards, 2009). In addition, expression of *SNC1* is constrained, and the use of the strong 35S promoter can only increase its expression by about four folds (Stokes *et al*., 2002) as higher expression of *SNC1* likely causes gene silencing (Yi & Richards, 2007). Several chromatin-associated proteins were identified as positive regulators of *SNC1* transcription based on their mutation suppression of the autoimmune mutants *bonzai 1* (*bon 1*) or *snc1-1* where *SNC1* expression is increased compared to the wild type (Yang and Hua 2004). ARABIDOPSIS TRITHORAX-RELATED 7 (ATXR7), a H3K4 methyltransferase, is required for the *SNC1* upregulation in the *bon1* mutant (Gou *et al*., 2017), probably through depositing H3K4me3 (Xia *et al*., 2013). HISTONE MONOUBIQUITINATION 1 (HUB1), a E3 ubiquitin ligase, promotes *SNC1* transcription by mono-ubiquitinating histone 2B (H2Bub1) at the *SNC1* locus in the *bon1* mutant (Zou *et al*., 2014). CHROMATIN REMODELING 5 (CHR5) belonging to the Chd subfamily of chromatin remodelers positively regulates *SNC1* expression in the *bon1* mutant and is required for *SNC1*-mediated defense response in *bon1* (Zou *et al*., 2017). MODIFER OF *snc1* (MOS1), a large protein without distinct functional protein domains, is also required for *SNC1* induction in the *bon1* and autoimmunity of *snc1-1* (Li *et al*., 2010; Bao *et al*., 2014). Besides, MOS1 physically interacts with TCP15 related transcription factors that are involved in *SNC1* expression regulation (Zhang *et al*., 2018). In addition to these positive regulators, HIGH EXPRESSION OF OSMOTICALLY

RESPONSIVE GENES 15 (HOS15) functions together with HISTONE DEACETYLASE 9 (HDA9) to repress *SNC1* transcription by removing acetylation of histone 3 on lysine 9 (H3K9ac) at the *SNC1* locus (Yang *et al*., 2020). Despite increasing evidence showing that *SNC1* is regulated at the chromatin level, it is unknown whether and how these chromatin-associated proteins coordinate to fine-tune NLR gene transcription.

Here we presented a systematic investigation of interaction of multiple regulators of *SNC1* expression to gain a better understanding of the transcriptional regulation of NLR genes at the chromatin level. We analyzed single mutants of *atrx7*, *hub1*, *chr5* and *mos1* and their mutants combined with *bon1* or *hos15* (both with increased *SNC1* expression), to determine histone modification states and expression at the *SNC1* gene. We focused on these genes because they have a strong effect on *SNC1* expression and a significant effect on autoimmunity of the *bon1* mutant (for the positive regulators). This will build a framework for the study of other chromatin related regulators such as *SPLAYED* which might have a milder effect on *SNC1* expression regulation (Johnson *et al.*, 2015). For *SNC1* induction either by SA or by the *bon1* mutation, ATXR7 and HUB1 deposit H3K4me3 and H2Bub1 inter-dependently at the *SNC1* locus and their function requires MOS1. CHR5 works independently with ATXR7 and HUB1 to induce *SNC1* transcription. *SNC1* upregulation from the loss of the repressor HOS15 requires functional MOS1 and CHR5. Together, this study revealed how *SNC1* is fine-tuned by different modules of chromatin-associated proteins during SA induction and in autoimmune mutants and furthered our understanding of general gene expression regulation.

Materials and Methods

Plant materials and growth condition

Arabidopsis thaliana Col-0 was used as the wild type control for all analyses in this study. The *atxr7-1* mutant (SALK_149692), *hub1-4* mutant (SALK_122512) and *chr5-1* mutant (SALK_020296) were obtained from Arabidopsis Biological Resource Center. The *mos1-6* and *hos15-4* mutants were as described previously (Bao *et al*., 2014; Yang *et al*., 2020). Plants were grown in soil at 22°C under constant light (100 μmol m^{-2} s^{-1}) and 50% humidity conditions. Two-week-old (13 to 15 days) plants were used for all experiments in this study except for the data in Fig. S7 where 11-day-old plants were used. Primers for genotyping are listed in Table S1.

Salicylic acid (SA) treatment

SA (Sigma, 247588) was dissolved in ethanol to constitute a stock of 0.5 M, and the stock was diluted into 1 mM in water. Plants were evenly sprayed with 1 mM SA or 0.2% ethanol in water (mock treatment). After four hours, two individual plants were pooled as one biological replicate and three biological replicates were collected for analysis.

Gene expression analysis

Gene expression analysis was performed as previous described (Yang *et al*., 2022). In brief, total RNAs were extracted from leave tissues by Trizol reagent (Invitrogen, 15596026). About 0.5 μg of total RNAs per sample was used for cDNA synthesis (TAKARA, RR047A). Each sample was diluted 10 folds before subject to qPCR using iQ SYBR Green supermix (Bio-Rad, 1708880) on the CFX96TMReal-Time System (Bio-Rad). At least three biological replicates were performed for each experiment. Primers for qPCR are listed in Table S1.

ChIP (Chromatin Immunoprecipitation)-qPCR analysis

ChIP experiments were performed as described (Saleh *et al*., 2008) with the following modifications. Isolated nuclei were resuspended in 0.5 mL of cold nuclei lysis buffer and transferred into TPX microtubes (Diagenode, C30010010-300) for sonication using a Bioruptor device (high intensity mode; 40-50 cycles with 30 s ON and 30 s OFF). Sonication efficiency was checked by de-crosslinking and purification of DNA from the 20 μL chromatin preparations followed by DNA separation on 1.5% agarose gel. Samples were sonicated till DNA was fragmented to 200 bp-1000 bp. Sonicated chromatin preparations were diluted to 10 times, and 1 mL diluted samples were used for immunoprecipitation (IP). For each IP, 1.5 μg anti-H3K4me3 (abcam, ab8580), 2 μg anti-H3 (abcam, ab1791), 2 μg anti-H3K9ac (Millipore, 07-352), or 4 μg anti-H2Bub1 (Medimabs, MM-0029-P) were added to the chromatin samples. After incubation at 4°C overnight, 50 μL Dynabeads Protein G (Invitrogen, 10004D) was used to pull down the antibody and its associated DNA fragments. Dynabeads were washed with low salt buffer, high salt buffer, LiCl buffer and TE buffer each twice. DNA was eluted twice using 100 μL elution buffer (1% SDS, 0.1 M $NaHCO_3$) at 65°C. Eluted DNA was de-crosslinked overnight and then purified using QIAGEN MiniElute PCR purification kit

(QIAGEN, 28004). Purified DNA was subject to qPCR analyses. Primers for ChIP-qPCR are listed in Table S1.

FAIRE experiment

The FAIRE experiment was performed as previously described (Omidbakhshfard *et al*., 2014) with minor modifications. About 1.5 g leaf tissue was used for the analyses. After nuclei isolation, 300 μL ice-cold nuclei lysis buffer was added to resuspend the nuclei. The suspension was transferred into TPX microtubes (Diagenode, C30010010-300) and sonicated on a Bioruptor device for 40-45 cycles (high intensity mode; 30 s ON and 30 s OFF) until DNA were fragmented to 200 bp-700 bp. Samples were then centrifuged at 4°C (16,000g) to pellet debris and supernatant was transferred to a new tube. The sonicated chromatin was subject to phenol/chloroform/isoamyl alcohol extraction for twice. The upper, aqueous phase was purified using QIAGEN MiniElute PCR purification kit (QIAGEN, 28004). Purified DNA was subject to qPCR analyses. Primers for FAIRE-qPCR are listed in Table S1.

Measurement of free SA

Free SA was measured by high performance liquid chromatography (HPLC)-mass spectrometry (MS) analysis as described previously (Yang et al., 2022).

Results

SNC1 induction by salicylic acid (SA) requires ATXR7, HUB1, CHR5 and MOS1

To better understand the contribution of ATXR7, HUB1, CHR5 and MOS1 to *SNC1* expression, we determined whether they played a role in regulating *SNC1* expression under normal growth condition. Each single mutant had a close to wild type growth phenotype and showed comparable *SNC1* transcript level compared to wild type Col-0 (hereafter, WT) (Fig. 1a,b). This indicates that these proteins do not play a significant role in *SNC1* basal expression regulation. *SNC1* is induced by *Pst* DC3000 and SA (Yang & Hua, 2004; Zou *et al*., 2014; Yang *et al*., 2020). Because the induction of *SNC1* by SA is less variable from experiment to experiment compared to that by *Pst* DC3000 treatment, we utilized SA treatment to examine

whether these positive regulators identified from autoimmune mutants could contribute to the *SNC1* induction during natural defense responses. *SNC1* expression was analyzed at 1 hour (h), 4 h, 12 h, 24 h and 48 h post-SA treatment in the wild type plants by qRT-PCR. *SNC1* was found to be significantly induced by SA at 1 h, 4 h and 12 h, but not at 24 h and 48 h post-treatment, with the strongest induction at 4 h post-treatment (Fig. S1). It is noted that the extent of *SNC1* induction by SA varied from 1.3 to 5 folds among different sets of experiments but the induction by SA was always significant. We then used 4 h as the time point to assay *SNC1* expression after SA treatment in the mutants of *SNC1* regulators. Compared to prior treatment, *SNC1* expression was induced by 130% in the WT at 4 h after SA treatment while it was not altered by mock treatment (Fig. 1c). The induction of *SNC1* by SA observed in the wild type was reduced to 80%, 90%, 60% in the *atxr7*, *hub1* and *chr5* mutant, respectively, and to only 30% in the *mos1* mutant while no change was observed by mock treatment (Fig. 1c). The relative contribution of each gene to *SNC1* induction by SA was similar to that in the *bon1* mutant, with *MOS1* having the largest effect (Fig. 1c). These results indicate that *ATXR7*, *HUB1*, *CHR5* and *MOS1* are required for *SNC1* induction by SA similarly to that in the *bon1* mutant. In addition, *MOS1* and *CHR5* have a larger effect than *ATXR7* and *HUB1* on *SNC1* induction by SA.

SNC1 induction by SA is companied by an increase of H3K4me3 and H2Bub1 modifications at the _SNC1_ locus

We next tested whether *SNC1* induction by SA was associated with the increased abundance of H3K4me3 and H2Bub1 at the *SNC1* locus by ChIP (chromatin-immunoprecipitation)-qPCR. We chose the P2 region of *SNC1* for analysis because histone modifications at this region are highly correlated with *SNC1* expression (Zou *et al*., 2014; Yang *et al*., 2020). A second region F8 close to the translation start site was also included in the analysis. Both regions have a higher deposition of various active histone modifications including H3K4me3 compared to other regions (Fig. 1d; the Plant Chromatin State Database by Liu *et al*., 2018). We found both H3K4me3 and H2Bub1 were more accumulated on the F8 and P2 region after 1 mM SA treatment compared to that in mock treatment (Fig. 1e). This result indicates that *SNC1* induction by SA is associated with a higher abundance of H3K4me3 and H2Bub1 at the *SNC1* locus, as similarly observed in the *bon1* mutant (Xia *et al*., 2013; Zou *et al*., 2014; Gou *et al*., 2017).

H3K4me3 and H2Bub1 modifications are interdependent in inducing *SNC1* transcription in the *bon1* mutant and by SA treatment

The *bon1* mutant accumulated more free SA compared to the wild type (Fig. S2), and SA-mediated defense response makes a major contribution to the autoimmunity of *bon1* (Yang and Hua, 2004). This suggests that the *bon1* mutant grown under non-pathogenic conditions has a state that mimics the wild type after SA treatment or pathogen infection. As the above mentioned three genes are each required for the upregulation of *SNC1* transcript by SA and the *bon1* mutation, , we utilized the *bon1* mutant to further investigate the molecular details of *SNC1* regulation in immunity. The two E3 ubiquitin ligases HUB1 and HUB2 are each required for *SNC1* expression and the *hub1 hub2* double mutant is the same as the two single mutants (Zou et al., 2014), which is consistent with these two proteins working together in depositing H2Bub1 (Cao et al., 2008). For simplicity, we used HUB1 for further analyses since they have equivalent and non-overlapping function in regulating *SNC1* expression (Zou et al., 2014). We first analyzed the interaction between the deposition of two positive histone modifications, H3K4me3 mediated by ATXR7 and H2Bub1 mediated by HUB1. The *bon1 atxr7 hub1* triple mutant was generated and characterized for its growth and immunity phenotypes. As reported earlier, the *atxr7* and *hub1* mutation reduced the *SNC1* expression in the *bon1* mutant, accompanied by the alleviation of the growth defects of the *bon1* mutant (Fig. 2a,b; Fig. S3a; Zou *et al*., 2014; Gou *et al*., 2017). The *bon1 atxr7 hub1* triple mutant had slightly more biomass as compared to either the *bon1 atxr7* mutant or the *bon1 hub1* mutant (Fig. 2a; Fig. S3a). Of note, *SNC1* expression in the triple mutant was the same as in the *bon1 atxr7* mutant which had a lower *SNC1* expression compared to the *bon1 hub1* mutant (Fig. 2b). These results suggest that ATXR7 and HUB1 do not promote *SNC1* gene expression in an additive manner. We further examined the interaction of *ATXR7* and *HUB1* in *SNC1* induction by SA. SA-induced *SNC1* expression was significantly reduced in the *atrx7* and *hub1* mutants compared to that in the wild type, and it was not further reduced in the double mutant compared to either the single mutant (Fig. 2c). These results indicate that ATXR7 and HUB1 are required for *SNC1* upregulation both in the *bon1* mutant and by SA treatment, and they do not have additive effects on *SNC1* induction.

Because ATXR7 and HUB1 are responsible for depositing H3K4me3 and H2Bub1 respectively at the *SNC1* region in the *bon1* mutant, we determined whether or not these two modifications, H3K4me3 and H2Bub1, are inter-dependent at the *SNC1* locus in the *bon1 atxr7*

and *bon1 hub1* double mutants by ChIP-qPCR. At both the F8 and P2 regions of the *SNC1* locus, the *bon1* mutant had a higher abundance of H3K4me3 compared to WT (Fig. 2d). This higher accumulation of H3K4me3 abundance was reduced to the wild type level by the *atxr7* mutation in *bon1 atrx7* (Fig. 2d), indicating that ATXR7 was the major methyltransferase catalyzing H3K4me3 modification for *SNC1* induction in the *bon1* mutant. As previously reported (Zou *et al*., 2014), H2Bub1 was significantly more enriched in the *bon1* mutant compared to that in WT and the abundance of H2Bub1 was totally abolished in the *bon1 hub1* mutant (Fig. 2d), suggesting that the H2Bub1 enrichment in the *bon1* mutant is dependent on HUB1. Of note, the *hub1* mutation also significantly reduced H3K4me3 abundance and the *atxr7* mutation reduced H2Bub1 abundance in the *bon1* mutant (Fig. 2d). These results suggest that both ATXR7 and HUB1 are required for proper H3K4me3 and H2Bub1 deposition at the *SNC1* locus in the *bon1* mutant.

Similar inter-dependence of the two modifications was also observed for SA induction. Both H3K4me3 and H2Bub1 modifications are increased at the F8 and P2 region of *SNC1* in the wild type after SA treatment compared to the mock treatment (Fig. 2e). This higher abundance of H3K4me3 and H2Bub1 after SA treatment was abolished by either the *atxr7* or the *hub1* mutation (Fig.2e), indicating that ATXR7 and HUB1 are both required for the deposition of H3K4me3 and H2Bub1 modifications at the *SNC1* locus after SA treatment (Fig. 2e). Taken together, these results demonstrate that ATXR7 mediated H3K4me3 and HUB1 mediated H2Bub1 modifications at the *SNC1* region are dependent on each other.

Since ATXR7 and HUB1 are two general positive regulators of gene expression, it is possible that ATXR7 might promote RNA expression of *HUB1* to influence the deposition of H2Bub1 modification and *SNC1* expression, and vice versa. To test this, we first examined the transcript level of *ATXR7* and *HUB1* and found that they were not altered by the *bon1* mutation or by SA treatment (Fig. S4). In addition, *ATXR7* expression was not altered by the *hub1* mutation under normal condition or under SA treatment. Likewise, the *HUB1* expression was not altered by the *atrx7* mutation under normal condition or SA treatment (Fig. S4b). These results strongly suggest that the co-modifications of H3K4me3 and H2Bub1 at the *SNC1* might result from the interdependence of the direct regulation by ATXR7 and HUB1 at the *SNC1* locus.

MOS1 is required for the deposition of H3K4me3 and H2Bub1 at the *SNC1* locus in the *bon1* mutant and after SA treatment

Because *SNC1* induction in the *bon1* mutant and by SA treatment was abolished by the loss of MOS1 function (Bao *et al*., 2014; Yang *et al*., 2020; Fig. 1c), we hypothesized that MOS1 is a key transcriptional regulator of *SNC1*. We asked whether or not MOS1 regulates *SNC1* transcription by influencing histone modifications at the *SNC1* locus. To this end, we determined the abundance of H3K4me3 and H2Bub1 at the F8 and P2 region of *SNC1* locus in the *bon1 mos1* mutant and in the *mos1* mutant after SA treatment by ChIP-qPCR. Both H3K4me3 and H2Bub1 were significantly reduced in the *bon1 mos1* mutant compared to that in the *bon1* mutant (Fig. 2f) and the increased H3K4me3 and H2Bub1 modifications at the *SNC1* locus after SA treatment were abolished in the *mos1* mutant (Fig. 2g), indicating that MOS1 is required for the deposition of H3K4me3 and H2Bub1 at the *SNC1* locus.

Since MOS1 is a postulated transcriptional regulator, we determined whether or not MOS1 promotes the expression of *ATXR7* and *HUB1* and therefore is required for their function. The expression of *ATXR7* and *HUB1* transcripts was not reduced in the *mos1* mutant under mock or SA treatment (Fig. S4b), indicating that *MOS1* is not a positive regulator of transcript levels of *ATXR7* and *HUB1*. These results suggest that MOS1 is required for the ATXR7 and HUB1 proteins to function at the *SNC1* locus to induce *SNC1* expression. Interestingly, the *HUB1* transcript level was slightly increased in the *mos1* mutant compared to the wild type under mock treatment (Fig. S4b), suggesting a feedback regulation on *HUB1* expression from a reduced HUB1 activity.

We subsequently tested whether or not MOS1 might interact with ATXR7 and HUB1 directly to influence H3K4me3 and H2Bub1. A yeast two-hybrid (Y2H) assay and a Bimolecular Fluorescence Complementation (BiFC) assay were performed, and no positive interactions were observed between MOS1 and ATXR7 or MOS1 and HUB1 (Fig. S5). This suggests that MOS1 may not have a direct physical interaction with ATXR7 and HUB1 in these heterologous systems.

CHR5 is not required for H3K4me3 modification in *SNC1* induction in the *bon1* mutant and after SA treatment

HUB1 and CHR5 were shown to work independently to promote *SNC1* expression in *bon1* (Zou *et al*., 2014). To investigate the interaction between ATXR7 and CHR5, we characterized

342 the growth and immunity phenotypes of the *bon1 atxr7 chr5* triple mutant. The triple mutant
343 displayed larger rosette and had further reduced *SNC1* expression than *bon1 atxr7* or *bon1 chr5*
344 double mutant, and the effects of *atxr7* and *chr5* appeared to be additive (not enhancing each
345 other) in the *bon1* mutant (Fig. 3a,b; Fig. S3b). In addition, SA-induced *SNC1* upregulation
346 was reduced in the *atxr7* and *chr5* single mutant, and it was further reduced in the *atxr7 chr5*
347 double mutant (Fig. 3c). These results suggest that ATXR7 and CHR5 might work
348 independently in inducing *SNC1* expression. To investigate this further, we determined the
349 H3K4me3 abundance at the F8 and P2 region of *SNC1* in the *bon1 chr5* mutant. ChIP-qPCR
350 analysis revealed that the *chr5* mutation did not affect H3K4me3 level in the *bon1* mutant and
351 the *bon1* and the *bon1 chr5* had the same level of H3K4me3 (Fig. 3d). Additionally, unlike
352 *atxr7* mutation which reduced the SA-induced H3K4me3 modification, the *chr5* mutation did
353 not affect the H3K4me3 abundance after SA treatment and the *chr5* mutant and the wild type
354 had the same level of increase of H3K4me3 after SA treatment (Fig. 3e). Taken together, these
355 results indicate that CHR5 is not required for ATXR7 mediated H3K4me3 modification at the
356 *SNC1* locus.
357
358 **CHR5 does not significantly alter DNA accessibility at the *SNC1* locus**
359
360 CHR5 was reported to promote the expression of a seed maturation gene *FUSCA3* (*FUS3*) by
361 reducing nucleosome occupancy near its transcription start site (Shen *et al*., 2015), and the
362 global nucleosome occupancy is altered in the *chr5* mutant compared to that in the WT (Zhou
363 *et al*., 2017). We tested the hypothesis that CHR5 regulates *SNC1* expression by altering
364 nucleosome occupancy at the *SNC1* locus. A FAIRE (Formaldehyde-Assisted Isolation of
365 Regulatory Elements; Omidbakhshfard *et al*., 2014) experiment was performed in WT, *bon1*
366 and *bon1 chr5* to examine whether the DNA accessibility in the *SNC1* promoter region was
367 altered by the CHR5 protein. We tested seven regions (F1 to F7) residing along the 1200 bp 5'
368 to the *SNC1* translation initiation site (TIS), F8 region within the first exon and the P2 region
369 400 bp 3' to the TIS (Fig. 1d). Chromatin accessibility was higher in F4, F5 and F6 (620 bp to
370 240 bp 5' to TIS) compared to other regions in WT (Fig. 3f). However, no significant difference
371 was observed between WT and the *bon1* mutant and between the *bon1* mutant and the *bon1*
372 *chr5* mutant in any of the regions (Fig. 3f). In addition, the H3 level at the *SNC1* locus was not
373 altered by mutations of *bon1* or *hos15* and it was not altered by SA treatment (Fig. S6). Taken
374 together, these results suggest that *SNC1* expression change may not involve a drastic

chromatin structure reconfiguration and the *chr5* mutation does not drastically alter the chromatin structure in the *SNC1* promoter region.

We subsequently tested whether or not CHR5 is associated with the *SNC1* region and thus directly regulates *SNC1* expression. To this end, a CHR5-GFP fusion protein was transiently expressed in protoplasts prepared from the *chr5* mutant seedlings, and a ChIP-qPCR experiment was performed. As expected, the CHR5-GFP fusion protein was localized in the nucleus when expressed in protoplast (Fig. 3g). This fusion protein was only partially functional as it was able to complement the *PR1* gene expression defect but not the growth defects in the *bon1 chr5* mutant (Fig. S7). A small but significant enrichment of CHR5-GFP fusion protein was observed on the *SNC1* F5 region ($p = 0.0285$) and P2 region ($p = 0.0383$), and not on the two control genes *ACTIN2* and *TA3* (Fig. 3h). This result suggests that CHR5 may directly bind to the *SNC1* locus to promote *SNC1* transcription.

SNC1 induction is accompanied by an increase of H3K9ac after SA treatment but not in the _bon1_ mutant

SNC1 transcript induction is accompanied by H3K9ac modification in the *hos15* mutant and MOS1-dependent H3K4me3 and H2Bub1 modifications in the *bon1* mutant and by SA treatment. To determine whether these inductions are associated with the same histone modifications at the *SNC1* locus, we investigated H3K9ac modification in the *bon1* mutant and H3K4me3 and H2Bub1 in the *hos15* mutant. Consistent with earlier findings (Yang & Hua, 2004; Yang *et al*., 2020), both the *bon1* and *hos15* single mutants displayed dwarfism and had elevated *SNC1* expression compared to WT (Fig. 4a,b), and H3K9ac at the *SNC1* locus was increased in the *hos15* mutant compared to WT as analyzed by ChIP-qPCR (Fig. 4c). The H3K9ac level was not altered in the *bon1* mutant compared to WT (Fig. 4c). In addition, the *bon1 hos15* double mutant had a similar H3K9ac level compared to the *hos15* mutant, although its *SNC1* expression was further increased (Fig. 4b,c) and its fresh weight was further reduced (Fig. S3c). This suggests that *SNC1* induction in the *bon1* mutant does not involve an enhancement of H3K9ac modification at the *SNC1* locus. By contrast, H3K9ac modification is slightly increased at the *SNC1* locus by SA treatment (Fig. 4d). Since HOS15 had the same repression on *SNC1* expression under normal and pathogenic conditions (Yang *et al.*, 2020), the higher H3K9ac at the *SNC1* locus triggered by SA treatment may not involve the regulation of the constitutive HOS15 activity. Taken together, these results indicate that H3K9ac may

contribute to SA-triggered *SNC1* upregulation, and this histone modification associated with *SNC1* induction may differ between the *bon1* mutant and SA treatment.

SNC1 **upregulation in the** ***hos15*** **mutant is accompanied by an increase of H3K4me3**

We examined the H3K4me3 and H2Bub1 abundance at the *SNC1* locus in the *hos15* mutant to determine if the *SNC1* upregulation in this mutant is associated with higher levels of H3K4me3 and/or H2Bub1 as observed in the *bon1* mutant. ChIP-qPCR revealed that the level of H2Bub1 at F8 and P2 region of *SNC1* was the same in the *hos15* mutant as in the WT (Fig. 5a). In contrast, more H3K4me3 was found at the *SNC1* locus in the *hos15* mutant compared to the WT (Fig. 5a). This suggests that *SNC1* upregulation in the *hos15* mutant is associated with H3K4me3 increase but not H2Bub1 increase.

We next tested whether ATXR7 is required for *SNC1* upregulation in the *hos15* mutant by analyzing the *SNC1* expression in the *hos15 atxr7* double mutant. The *atxr7* mutation did not alleviate the growth defects of the *hos15* mutant (Fig. 5b), neither did it reduce the *SNC1* expression defect in the *hos15* mutant (Fig. 5c). These results indicate that H3K9ac-mediated *SNC1* activation in the *hos15* mutant does not require ATXR7. It is yet to be determined whether ATRX7 or other histone methyltransferase are required for depositing H3K4me3 at the *SNC1* locus in the *hos15* mutant and whether or not H3K4me3 increase is not needed for the increased expression of *SNC1* in the *hos15* mutant.

SNC1 **upregulation in the** ***hos15*** **mutant requires MOS1 and is associated with deposition of H3K4me3 at the** ***SNC1*** **locus**

We next asked whether MOS1 was required for *SNC1* upregulation in the *hos15* mutant. To do this, we generated *hos15 mos1* double mutant (Fig. 6a) and analyzed the *SNC1* expression in the *hos15 mos1* double mutant. The *mos1* mutation did not alleviate the growth defects of the *hos15* mutant (Fig. S3d). However, it significantly reduced the *SNC1* expression in the *hos15* mutant, although not to the WT level (Fig. 6b). This is accompanied by a significant reduction of H3K4me3, but not H3K9ac, at the *SNC1* locus by the *mos1* mutation (Fig. 6c). These results indicate that MOS1 is required for the increase of H3K4me3 but not H3K9ac at the *SNC1* locus and H3K4me3 accumulation is associated with full activation of *SNC1* in the *hos15* mutant.

SNC1 upregulation in the *hos15* mutant requires CHR5 function

We then asked whether CHR5 is required for *SNC1* activation in the *hos15* mutant. To do this, we generated *hos15 chr5* double mutant and characterized its growth and *SNC1* expression. The *chr5* mutation did not alleviated the growth defects of the *hos15* mutant (Fig. 7a, Fig. S3e), but it significantly reduced the *SNC1* expression in the *hos15* mutant (Fig. 7b). Because the abundance of H3K9ac and H3K4me3 at the *SNC1* locus is associated with the *SNC1* expression in the *hos15* mutant (Fig. 6; Yang *et al*., 2020) and CHR5 does not affect H3K4me3 abundance at the *SNC1* locus (Fig. 3d,e), we tested whether CHR5 might modulate *SNC1* expression by affecting H3K9ac. The *hos15* single mutant and the *hos15 chr5* double mutant were found to both have more H3K9ac as compared to WT and there was no significant difference of H3K9ac abundance between them (Fig. 7c). This indicates that CHR5 is required for *SNC1* activation in the *hos15* mutant, but it does not affect H3K9ac at the *SNC1* locus.

Discussion

The transcript level of the NLR gene *SNC1* is regulated by several chromatin-associated proteins, but whether and how they coordinate to fine-tune *SNC1* gene transcription was not clear. We show that these regulators function in three modules: two activation modules MOS1 and CHR5, and one repression module HOS15. These modules are differentially responsible for *SNC1* expression under non-pathogenic and defense induction conditions (Fig. 8). Under nonpathogenic condition, HOS15 constitute a repression module to keep *SNC1* expression low by removing the H3K9ac modification. Transcriptional regulation of *SNC1* expression in the *bon1* mutant and by SA treatment are largely similar. Both induce *SNC1* expression through the two activation modules: MOS1 and CHR5. The MOS1 protein mediates ATXR7 and HUB1 for the deposition of H3K4me3 and H2Bub1 at the *SNC1* locus to promote *SNC1* expression. The CHR5 protein functions in parallel or after the MOS1 module. Besides these similarities, However, *SNC1* induction by SA differs from that by *bon1* in the association of an increase of H3K9ac modification. HOS15 also plays a repression role when *SNC1* is activated by SA and the *bon1* mutation. Upregulation of *SNC1* by removing *HOS15* function is partially dependent on the CHR5 module and the MOS1 module. These data reveal that *SNC1* transcription is orchestrated by different sets of chromatin-associated proteins tuning to nonpathogenic and pathogenic conditions.

This study reveals that MOS1 regulates gene expression by promoting active histone modifications. MOS1 is required for increased H3K4me3 conferred by a histone methyltransferase ATXR7 and increased H2Bub1 conferred by a E3 ubiquitin ligase HUB1 at the *SNC1* locus in the *bon1* mutant and after SA treatment (Fig. 2f,g). It is also required for the increased H3K4me3 conferred by additional histone methyltransferase(s) at the *SNC1* locus in the *hos15* mutant (Fig. 6c). This suggests that MOS1 has a larger effect than ATRX7 and HUB1 on *SNC1* activation. This is unlikely resulting from a transcriptional regulation on *ATXR7* and *HUB1* by MOS1 (Fig. S4b), suggesting a regulation at the protein level. Indeed, MOS1 was shown to interact with different transcription factors, including SUF4 for flowering time control (Bao *et al*., 2014) and TCP15-like proteins for immune responses (Zhang *et al*., 2018). Together, these findings suggest that MOS1 is a transcriptional regulator that connects with both transcription factors and histone modification enzymes. No direct interaction between MOS1 and ATRX7 or HUB1 was observed in an Y2H assay or a BiFC assay in *N. benthamiana* (Fig. S5). Therefore, MOS1 might not directly interact with these histone modification enzymes. Further study should investigate whether or not MOS1 interacts with these modification enzymes at specific locus under specific cellular conditions.

This study also reveals an inter-dependence between H3K4me3 and H2Bub1 modifications in *SNC1* activation (Fig. 8). Previous studies in non-plant organisms revealed that H2Bub stimulates the deposition of H3K4me2/3 (Dover *et al*., 2002; Lee *et al*., 2007; Kim *et al*., 2009; Ma *et al*., 2021) and a defect in H2Bub1 reduces the genome-wide H3K4 methylation level (Hwang *et al*., 2003). In Arabidopsis, the global H3K4me3 is not altered in the H2Bub1-defective mutants *hub1* and *ubc1 ubc2* (Cao *et al*., 2008; Gu *et al*., 2009), but H3K4me3 at specific flowering regulator genes including *FLOWERING LOCUS C* (*FLC*) is reduced in the *hub1* mutant (Cao *et al*., 2008). Both H3K4me3 and H2Bub1 modifications activate *FLC* (Cao *et al*., 2008; Pien *et al*., 2008; Gu *et al*., 2009) and they are interdependent in activating *SNC1* expression (Fig. 2d,e). The effects of *atxr7* and *hub1* are not identical for *SNC1* upregulation in the *bon1* mutant (Fig. 2b). In addition to their direct regulation of *SNC1* in depositing H3K4me3 and H2Bub1 into the *SNC1* locus, they may indirectly affect *SNC1* expression through regulating other regulators of *SNC1*, although they do not regulate each other's transcription (Fig. S4b). Therefore, there is a similarity in the regulation by H3K4me3 and H2Bub1 on the expression of *FLC* and *SNC1*, and these two modifications could be inter-dependent at these two loci. Expression of both genes are also regulated by MOS1, but one

negatively and one positively. MOS1 is required for H3K4me3 and H2Bub1 modifications at the *SNC1* locus to promote its expression (Fig. 2f,g). In contrast, MOS1 was reported to repress *FLC* expression (Bao *et al*., 2014) while H3K4me3 and H2Bub1 promote *FLC* expression (Cao *et al*., 2008; Pien *et al*., 2008; Gu *et al*., 2009). Whether or not MOS1 affects these two modifications and other histone modifications at the *FLC* locus is yet to be determined. These findings point to the complex mechanisms of transcriptional regulators and histone modifications on gene expression.

The increase of H3K9ac abundance at the *SNC1* locus is likely the primary effect of the loss of HOS15 as HOS15 is shown to interact with HDA9 for H3K9ac removal. The *hos15* mutant also has an increase of H3K4me3, but not H2Bub1 at the *SNC1* locus (Fig. 5a), suggesting that the increase of H3K9ac induces H3K4me3. On the other side, H3K4me3 increase in *bon1* was not accompanied by an increase of H3K9ac (Fig. 4c), suggesting that these two active histone modifications are not mutually activating each other. The coincidence of H3K9ac and H3K4me3 has been reported in other loci in Arabidopsis (Guo *et al*., 2008; Kim *et al*., 2008; Brusslan *et al*., 2015). For instance, H3K9ac and H3K4me3 share very similar distribution pattern on the coding regions of stress-responsive genes including *RESPONSIVE TO DEHYDRATION* (*RD*) *29A*, *RD29B*, *RD20* and *RELATED TO AP2.4*, and are responsible for the activation of these genes in response to drought stress (Kim *et al*., 2008). It is hypothesized that H3K9ac might serve as a transcriptional initiation signal during the start of transcription, and H3K4me3 is subsequently recruited for maintaining transcriptional activity during transcriptional elongation (Li *et al*., 2007a). This sequential model might apply to the transcriptional activation of the *SNC1* gene expression.

This study finds that the CHR5 protein functions in parallel or after the MOS1 module. How CHR5 regulates *SNC1* expression is not yet known. CHR5 has been shown to affect relative abundance of nucleosome occupancy in the promoter region versus the gene body in Arabidopsis (Zhou *et al*., 2017), and it reduces nucleosome occupancy near the transcriptional start site of the *FUS3* gene and promotes its expression (Shen *et al*., 2015). Here we detected a subtle but significant enrichment of CHR5 protein at the *SNC1* locus (Fig. 3h), suggesting a direct association of CHR5 at the *SNC1* locus. However, no drastic change in DNA accessibility at the *SNC1* promoter region was observed by the FAIRE assay in the *bon1 chr5* mutant and the *bon1* mutant where *SNC1* expression was different (Fig. 3f), suggesting that CHR5 does not drastically alter DNA accessibility at the *SNC1* region. In addition, no change

of DNA accessibility at the *SNC1* locus was observed between *bon1* and the WT either (Fig. 3f), and H3 abundance was not altered when *SNC1* is induced (Fig. S6). This suggests that DNA accessibility or chromatin configuration might not drastically change when *SNC1* expression is induced. CHR5 may directly regulates *SNC1* expression through altering nucleosome occupancy (but too subtle to be detected by FAIRE method) or through affecting other chromatin-based processes such as transcription elongation which was reported for its yeast homolog Chd1 (Simic *et al*., 2003). Future efforts are needed to explore how CHR5 modulates *SNC1* activation.

This study reveals the interaction of three chromatin-associated modules that regulate *SNC1* expression under non-pathogenic condition and upon defense activation in the *bon1* mutant and by SA treatment. *SNC1* is recently shown to enhance disease resistance to a few avirulent *Pst* DC3000 strains (Wang et al, 2022 accepted). Future study could investigate the role of chromatin remodeling in *SNC1* expression in natural plant pathogen interaction. Our findings not only provide molecular details of immune regulation especially NLR control but also furthers our understanding of general gene expression regulation. Transfer of NLR genes has proven to be effective to generate crops with enhanced disease resistance, and therefore a knowledge about NLR expression regulation is key to a successful gene transfer in breeding more resilient plants.

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Author contribution

JH conceived and supervised this study; LY and JH designed the experiments; LY and ZW performed the experiments and data analyses. LY and JH wrote the manuscript with input from ZW.

References

Bannister, A. J., and Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell research*, *21*(3), 381-395.

Bao, Z., Zhang, N., and Hua, J. (2014). Endopolyploidization and flowering time are antagonistically regulated by checkpoint component MAD1 and immunity modulator MOS1. *Nature communications*, *5*(1), 1-10.

Brusslan, J. A., Bonora, G., Rus-Canterbury, A. M., Tariq, F., Jaroszewicz, A., and Pellegrini, M. (2015). A genome-wide chronological study of gene expression and two histone modifications, H3K4me3 and H3K9ac, during developmental leaf senescence. *Plant physiology*, *168*(4), 1246-1261.

Cao, Y., Dai, Y., Cui, S., and Ma, L. (2008). Histone H2B monoubiquitination in the chromatin of *FLOWERING LOCUS C* regulates flowering time in Arabidopsis. *The Plant Cell*, *20*(10), 2586-2602.

Clapier, C. R., Iwasa, J., Cairns, B. R., and Peterson, C. L. (2017). Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. *Nature reviews Molecular cell biology*, *18*(7), 407-422.

Chakraborty, S., Britton, M., Martínez-García, P. J., and Dandekar, A. M. (2016). Deep RNA-Seq profile reveals biodiversity, plant–microbe interactions and a large family of NBS-LRR resistance genes in walnut (Juglans regia) tissues. *Amb Express*, *6*(1), 1-13.

Chen, J., Pang, W., Chen, B., Zhang, C., and Piao, Z. (2016). Transcriptome analysis of Brassica rapa near-isogenic lines carrying clubroot-resistant and–susceptible alleles in response to Plasmodiophora brassicae during early infection. *Frontiers in plant science*, *6*, 1183.

Dover, J., Schneider, J., Tawiah-Boateng, M. A., Wood, A., Dean, K., Johnston, M., and Shilatifard, A. (2002). Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. *Journal of Biological Chemistry*, *277*(32), 28368-28371.

Gou, M., and Hua, J. (2012). Complex regulation of an R gene *SNC1* revealed by autoimmune mutants. *Plant signaling and behavior*, *7*(2), 213-216.

Gou, M., Huang, Q., Qian, W., Zhang, Z., Jia, Z., & Hua, J. (2017). Sumoylation E3 ligase SIZ1 modulates plant immunity partly through the immune receptor gene *SNC1* in Arabidopsis. *Molecular plant-microbe interactions*, *30*(4), 334-342.

Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., … and Yin, Z. (2005). R gene expression induced by a type-III effector triggers disease resistance in rice. *Nature*, *435*(7045), 1122-1125.

Gu, X., Jiang, D., Wang, Y., Bachmair, A., and He, Y. (2009). Repression of the floral transition via histone H2B monoubiquitination. *The Plant Journal*, *57*(3), 522-533.

Guo, L., Zhou, J., Elling, A. A., Charron, J. B. F., and Deng, X. W. (2008). Histone modifications and expression of light-regulated genes in Arabidopsis are cooperatively influenced by changing light conditions. *Plant Physiology*, *147*(4), 2070-2083.

Huang, C. Y., Rangel, D. S., Qin, X., Bui, C., Li, R., Jia, Z., … and Jin, H. (2021). The chromatin-remodeling protein BAF60/SWP73A regulates the plant immune receptor NLRs. *Cell Host and Microbe*, *29*(3), 425-434.

Hwang, W. W., Venkatasubrahmanyam, S., Ianculescu, A. G., Tong, A., Boone, C., and Madhani, H. D. (2003). A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. *Molecular cell*, *11*(1), 261-266.

Jones, J. D., and Dangl, J. L. (2006). The plant immune system. *Nature*, *444*(7117), 323-329.

Johnson, K.C.M., Xia, S., Feng, X., Li, X. (2015). The chromatin remodeler SPLAYED negatively regulates SNC1-mediated immunity. *Plant & Cell Physiology*, *56*(8), 1616-1623.

Kim, J., Guermah, M., McGinty, R. K., Lee, J. S., Tang, Z., Milne, T. A., … and Roeder, R. G. (2009). RAD6-Mediated transcription-coupled H2B ubiquitylation directly stimulates H3K4 methylation in human cells. *Cell*, *137*(3), 459-471.

Kim, J. M., To, T. K., Ishida, J., Morosawa, T., Kawashima, M., Matsui, A., ... and Seki, M. (2008). Alterations of lysine modifications on the histone H3 N-tail under drought stress conditions in Arabidopsis thaliana. *Plant and Cell Physiology*, 49(10), 1580-1588.

Lai, Y., Cuzick, A., Lu, X. M., Wang, J., Katiyar, N., Tsuchiya, T., ... & Eulgem, T. (2019). The Arabidopsis RRM domain protein EDM 3 mediates race‐specific disease resistance by controlling H3K9me2‐dependent alternative polyadenylation of *RPP7* immune receptor transcripts. *The Plant Journal*, *97*(4), 646-660.

Lai, Y., and Eulgem, T. (2018). Transcript-level expression control of plant NLR genes. *Molecular plant pathology*, *19*(5), 1267-1281.

Lee, J. S., Shukla, A., Schneider, J., Swanson, S. K., Washburn, M. P., Florens, L., … and Shilatifard, A. (2007). Histone crosstalk between H2B monoubiquitination and H3 methylation mediated by COMPASS. *Cell*, *131*(6), 1084-1096.

Lee, Y., Park, D., and Iyer, V. R. (2017). The ATP-dependent chromatin remodeler Chd1 is recruited by transcription elongation factors and maintains H3K4me3/H3K36me3 domains at actively transcribed and spliced genes. *Nucleic acids research*, *45*(12), 7180-7190.

Li, B., Carey, M., and Workman, J. L. (2007a). The role of chromatin during transcription. *Cell*, *128*(4), 707-719.

Li, Y., Tessaro, M. J., Li, X., and Zhang, Y. (2010). Regulation of the expression of plant resistance gene *SNC1* by a protein with a conserved BAT2 domain. *Plant physiology*, *153*(3), 1425-1434.

Li, Y., Yang, S., Yang, H., and Hua, J. (2007b). The TIR-NB-LRR gene *SNC1* is regulated at the transcript level by multiple factors. *Molecular plant-microbe interactions*, *20*(11), 1449-1456.

Liu, Y., Tian, T., Zhang, K., You, Q., Yan, H., Zhao, N., … and Su, Z. (2018). PCSD: a plant chromatin state database. *Nucleic acids research*, *46*(D1), D1157-D1167.

Ma, T., Zhang, L., Wang, M., Li, Y., Jian, Y., Wu, L., … and Yin, Y. (2021). Plant defense compound triggers mycotoxin synthesis by regulating H2B ub1 and H3K4 me2/3 deposition. *New Phytologist*, *232*(5), 2106-2123.

Mohr, P. G., and Cahill, D. M. (2007). Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in Arabidopsis infected with Pseudomonas syringae pv. tomato. *Functional and integrative genomics*, *7*(3), 181-191.

Munch, D., Gupta, V., Bachmann, A., Busch, W., Kelly, S., Mun, T., and Andersen, S. U. (2017). Organ-specific NLR resistance gene expression varies with plant symbiotic status. *bioRxiv*, doi: 10.1101/135764

Ngou, B. P. M., Ding, P., and Jones, J. D. (2022). Thirty years of resistance: Zig-zag through the plant immune system. *The Plant Cell*. *34*(5), 1447-1478

Omidbakhshfard, M. A., Winck, F. V., Arvidsson, S., Riaño-Pachón, D. M., and Mueller-Roeber, B. (2014). A step-by-step protocol for formaldehyde-assisted isolation of regulatory elements from Arabidopsis thaliana. *Journal of integrative plant biology*, *56*(6), 527-538.

Palma, K., Thorgrimsen, S., Malinovsky, F. G., Fiil, B. K., Nielsen, H. B., Brodersen, P., … and Mundy, J. (2010). Autoimmunity in Arabidopsis acd11 is mediated by epigenetic regulation of an immune receptor. *PloS pathogens*, *6*(10), e1001137.

Pien, S., Fleury, D., Mylne, J. S., Crevillen, P., Inzé, D., Avramova, Z., ... and Grossniklaus, U. (2008). ARABIDOPSIS TRITHORAX1 dynamically regulates *FLOWERING LOCUS C* activation via histone 3 lysine 4 trimethylation. *The plant cell*, *20*(3), 580-588.

Pfluger, J., and Wagner, D. (2007). Histone modifications and dynamic regulation of genome accessibility in plants. *Current opinion in plant biology*, *10*(6), 645-652.

Pruitt, R. N., Locci, F., Wanke, F., Zhang, L., Saile, S. C., Joe, A., … and Nürnberger, T. (2021). The EDS1–PAD4–ADR1 node mediates Arabidopsis pattern-triggered immunity. *Nature*, 598(7881), 495-499.

Shen, Y., Devic, M., Lepiniec, L., and Zhou, D. X. (2015). Chromodomain, helicase and DNA-binding CHD1 protein, CHR5, are involved in establishing active chromatin state of seed maturation genes. *Plant Biotechnology Journal*, *13*(6), 811-820.

Simic, R., Lindstrom, D. L., Tran, H. G., Roinick, K. L., Costa, P. J., Johnson, A. D., ... and Arndt, K. M. (2003). Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. *The EMBO journal*, 22(8), 1846-1856.

Sims, R. J., Chen, C. F., Santos-Rosa, H., Kouzarides, T., Patel, S. S., and Reinberg, D. (2005). Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. *Journal of Biological Chemistry*, *280*(51), 41789-41792.

Stokes, T. L., Kunkel, B. N., and Richards, E. J. (2002). Epigenetic variation in Arabidopsis disease resistance. *Genes and development*, *16*(2), 171-182.

Tan, X., Meyers, B. C., Kozik, A., West, M. A., Morgante, M., St Clair, D. A., … and Michelmore, R. W. (2007). Global expression analysis of nucleotide binding site-leucine rich repeat-encoding and related genes in Arabidopsis. *BMC plant biology*, *7*(1), 1-20.

Tian, H., Wu, Z., Chen, S., Ao, K., Huang, W., Yaghmaiean, H., ... and Zhang, Y. (2021). Activation of TIR signalling boosts pattern-triggered immunity. *Nature*, 598(7881), 500-503.

Van Wersch, R., Li, X., and Zhang, Y. (2016). Mighty dwarfs: Arabidopsis autoimmune mutants and their usages in genetic dissection of plant immunity. *Frontiers in plant science*, *7*, 1717.

Xia, S., Cheng, Y. T., Huang, S., Win, J., Soards, A., Jinn, T. L., ... and Li, X. (2013). Regulation of transcription of nucleotide-binding leucine-rich repeat-encoding genes *SNC1* and *RPP4* via H3K4 trimethylation. *Plant physiology*, *162*(3), 1694-1705.

Yang, L., Chen, X., Wang, Z., Sun, Q., Hong, A., Zhang, A., ... and Hua, J. (2020). HOS15 and HDA9 negatively regulate immunity through histone deacetylation of intracellular immune receptor NLR genes in Arabidopsis. *New Phytologist*, *226*(2), 507-522.

Yang, L., Wang, Z., and Hua, J. (2021). A meta-analysis reveals opposite effects of biotic and abiotic stresses on transcript levels of Arabidopsis intracellular immune receptor genes. *Frontiers in Plant Science*, *12*, 241.

Yang, L., Wang, Z., Zhang, A., Bhawal, R., Li, C., Zhang, S., ... & Hua, J. (2022). Reduction of the canonical function of a glycolytic enzyme enolase triggers immune responses that further affect metabolism and growth in Arabidopsis. *The Plant Cell*, koab283, https://doi.org/10.1093/plcell/koab283

Yang, S., and Hua, J. (2004). A haplotype-specific Resistance gene regulated by BONZAI1 mediates temperature-dependent growth control in Arabidopsis. *The Plant Cell*, *16*(4), 1060-1071.

Yi, H., & Richards, E. J. (2007). A cluster of disease resistance genes in Arabidopsis is coordinately regulated by transcriptional activation and RNA silencing. *The Plant Cell*, *19*(9), 2929-2939.

Yi, H., and Richards, E. J. (2009). Gene duplication and hypermutation of the pathogen Resistance gene *SNC1* in the Arabidopsis *bal* variant. *Genetics*, *183*(4), 1227-1234.

Zhang, N., Wang, Z., Bao, Z., Yang, L., Wu, D., Shu, X., and Hua, J. (2018). MOS 1 functions closely with TCP transcription factors to modulate immunity and cell cycle in Arabidopsis. *The Plant Journal*, *93*(1), 66-78.

Zou, B., Sun, Q., Zhang, W., Ding, Y., Yang, D. L., Shi, Z., and Hua, J. (2017). The Arabidopsis chromatin-remodeling factor CHR5 regulates plant immune responses and nucleosome occupancy. *Plant and Cell Physiology*, *58*(12), 2202-2216.

Zou, B., Yang, D. L., Shi, Z., Dong, H., and Hua, J. (2014). Monoubiquitination of histone 2B at the disease resistance gene locus regulates its expression and impacts immune responses in Arabidopsis. *Plant physiology*, *165*(1), 309-318.

Supplemental information

Fig. S1 *SNC1* expression is induced by SA.

Fig. S2 Salicylic acid (SA) is increased in the *bon1* mutant.

Fig. S3. Quantification of fresh weight of higher order mutants in this study.

Fig. S4 Transcripts of *ATXR7*, *HUB1* and *MOS1* do not change in multiple mutants or after SA treatment.

Fig. S5 Assays of interactions between MOS1 and ATXR7 or HUB1 by yeast two-hybrid (Y2H) and Bimolecular Fluorescence Complementation (BiFC).

Fig. S6 H3 level does not change when *SNC1* is induced.

Fig. S7 *35S::CHR5:GFP* complements *PR1* gene expression but not growth defects in the *bon1 chr5* mutant.

744 Table S1. Primers used in this study.