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HAC-NPR1-TGA 복합체에 의한 애기장대 면역의 후성유전학적 조절

Epigenetic regulation of Arabidopsis immunity by the HAC-NPR1-TGA Complex

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Abstract

Epigenetic regulation of Arabidopsis immunity by the HAC-NPR1-TGA Complex

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Unlike animals, plants lack specialized immune cells. Instead, plants have developed multiple layers of sophisticated immune responses through massive expression of immune-related genes including the *pathogenesis related* (*PR*) genes to respond to bacterial or viral pathogenic attacks. Upon pathogenic attack, plants turn on the innate immune system as the first line of defense. The immune response is initiated at the site of infection by accumulating salicylic acid (SA). Then, as a major signaling molecule in plant immunity, SA is accumulated at distal tissues to protect the entire plant against successive attacks by various pathogen. This "whole plant" resistance response is referred as systemic acquired resistance (SAR). SA

signal results in the activation of the master immune regulator, NPR1, which is recruited by TGACG SEQUENCE-SPECIFIC BINDING PROTEIN (TGA) transcription factors to numerous downstream *PR* genes. However, despite the critical role of NPR1 in SA-triggered immunity, the biochemical mechanism of NPR1 as a transcriptional co-activator remain largely unknown.

Epigenetics is the study of heritable changes in gene expression which are not based on changes in DNA sequence. Histone acetylation is an epigenetic modification that occurs at the lysine residue of N-terminal histone tail. Histone acetyltransferases (HATs) transfer the acetyl group ($COCH_3$) from acetyl coenzyme A (acetyl-CoA) to the NH_3^+ amino group of histones. HATs are also known as transcription co-activators, leading to transcriptional activation.

In Arabidopsis, recent studies reported that CBP/p300 HAC family proteins possess histone acetyltransferase activities. The two types of zinc finger domains ZnF-TAZ and ZnF-ZZ in CBP/p300 families are known to be important for mediating protein–protein interactions. Epigenetic regulation through several HACs is known to play crucial roles in flowering, various developmental processes, and ethylene signaling pathway.

In this study, I showed that the CBP/p300-family histone acetyltransferases, HAC1 and HAC5 (HAC1/5) are required for SA-triggered immunity and *PR* induction in Arabidopsis. During SA-triggered immune response, HAC1 form a complex with NPR1 and TGAs to activate *PR* genes by histone acetylation. Thus, this study reveals the function of HAC1 as a co-activator of NPR1

and the precise biochemical mechanism of NPR1-mediated transcriptional activation. Furthermore, this study also proposes epigenetic reprogramming acts as an essential part of plant immune system which allows plants to efficiently switch their regular developmental program to a defense program upon pathogenic attack.

Key words: CBP/p300, Histone acetyltransferase, HAC1, NPR1, SA, Immune system, Pathogen.

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Abbreviations

CBP	CREB-binding protein
ChIP	chromatin immunoprecipitation
ChIP-seq	chromatin immunoprecipitation followed by sequencing
Co-IP assay	Co-immunoprecipitation assay
DNA	deoxyribonucleic acid
EDTA	ethylene diaminetetraacetic acid
FDR	false discovery rate
GFP	green fluorescence protein
HA	hemagglutinin
HACs	histone acetyltransferase
HAG1	histone acetyltransferase GCN5
HAT	histone acetyltransferase
INA	2,6-Dichloroisonicotinic acid
KD	kilo dalton
LD	long day

MG132	carbobenzoxy-Leu-Leu-leucinal
mRNA	messenger ribonucleic acid
NPR1	nonexpresser of PR genes 1
OD	optical density
PAGE	polyacrylamide gel
PCR	polymerase chain reaction
PI	propidium iodide
PR	pathogenesis-related gene
Pst DC3000	P. syringae pathovar tomato DC3000
qPCR	quantitative polymerase chain reaction
RNA-seq	RNA sequencing
RT	reverse-transcription
SA	salicylic acid
SD	short day
SDS	sodium dodecyl sulfate
SE	standard error
TAZ domains	transcriptional adapter zinc binding domains

T-DNA	transfer DNA
TGA	TGACG sequence-specific binding protein
UBQ	ubiquitin
WT	wild type

1. General introduction

1.1 Epigenetic control and gene expression

Epigenetics is the study of mitotically and/or meiotically heritable changes in gene expression which are not based on changes in the underlying DNA sequence. The activity and/or function of various genes can be regulated epigenetically through covalent modifications of DNA, histone proteins, DNA-binding proteins, as well as noncoding RNAs. These epigenetic mechanisms are crucial for nearly all biological processes in eukaryotes.

In eukaryotic cells, genetic information is carried in the chromosomal DNA. The DNA is associated with various proteins and RNAs to form a structure called “chromatin". Epigenetic modifications control the structure of chromatin to regulate transcription. The main mechanisms underlying epigenetic regulation include histone modification, DNA methylation, ATP-dependent chromatin remodeling and noncoding RNA-mediated silencing. Epigenetic variations sensitively respond to the environmental and intercellular signals and regulate cue-specific gene expression and thus cause morphological and functional changes in cells. Epigenetic factors are well conserved in eukaryotic organisms and various homologs are identified among plants and animals (Istvan *et al*., 2013).

1.1.1 Histone modification

The structure of the chromatin undergoes various dynamic changes

according to the needs of the cell. The eukaryotic chromatin is comprised of 147 bp of DNA wrapped around histone proteins to form a fundamental structural unit referred as nucleosomes. The core histone protein consists of two H3, H4 histone dimers and two H2A, H2B dimers while histones H1 and H5 act as linker histones (Handy *et al*., 2011). The core histones are packaged into a globular structure whereas the N-terminal tail of each core histone is exposed from the core histone and loosely extended. Various enzymes modify multiple sites within the histone N-terminal tails resulting in an "open" or "closed" chromatin structure. The open chromatin structure also referred as euchromatin, is the loose chromatin state that is accessible to RNA polymerase and transcription factors resulting in gene activation. On the other hand, heterochromatin is the condensed chromatin state consisting transcriptionally inactive genes and repetitive sequences. Thus, gene expression can be regulated at the transcriptional level depending on the chromatin structure itself.

Histones are covalently modified in different residues such as lysine, arginine, serine, and threonine. Among them, lysine residues are important substrates for various modifications such as acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. Acetylation and methylation are major histone modifications which occur in the lysine residues of histones H3, H4, H2A, and H2B tails. Histone H3 can be acetylated at several lysine residues including K9, 14, 18, 23, and 5. Histone H3 can also be methylated on K4, 9, 27, 36, 79, and arginine residue (H3R2). In histone H4, K5, 8, 12, and 16 can be acetylated and K20 and R3 residues can be methylated (zhang *et al*., 2010).

Four types of correlations are established based on analyses of genome-wide profiles of histone modifications and gene expression: suppressed, active, poised, and bivalent (Weng *et al.,* 2012). In the suppressed state, the closed chromatin structure results in suppression of gene transcription. In contrast, gene transcription is activated in open chromatin structure. However, even if the chromatin is at open state with high levels of active marks such as H3K4me3, gene transcription can still be low. This chromatin state is referred as the poised state. When the chromatin is poised, gene transcription can be rapidly activated upon activation signal. The bivalent chromatin state was first identified in developmental gene promoters of embryonic stem cells (Bluma and David, 2014). In bivalent chromatin, both active and repressive marks are present at high levels. The bivalent chromatin state enables the chromatin to change to an open or closed conformation through cell differentiation and upon activation signals (Bluma and David, 2014; Azuara *et al.,* 2006; Bernstein *et al.,* 2006; Mikkelsen *et al.,* 2007). Among various histone modifications, H3K9me2/3 and H3K27me3 are major repressive markers. In contrast, H3Ac, H4Ac, H3K4me3, and H3K36me3 are active markers. The repressive mark H3K27me3 and the active mark H3K4me3 are the most common bivalent marks found in stem cells. H3K27me3 suppresses gene expression while H3K4me3 mark reactivates them when needed. Various histone modifications can make crosstalks. They affect each other negatively or positively and regulate gene expression.

1.1.1.1. Histone acetylation

Histone acetylation was first reported by Allfrey *et al* in 1964. Histone acetylation occurs at the lysine residue of the N-terminal histone tail. The modifying enzymes that catalyze histone acetylation are called histone acetyltransferases (HATs). HATs transfer the acetyl group ($COCH_3$) from acetyl-coenzyme A (acetyl-CoA) to the NH_3^+ amino group of histones, mainly H3 and H4. HATs are also known as transcription co-activators.

Histone acetylation reduces the interaction between nucleosomes and DNA. The decondensed chromatin structure allows access to transcription factors, DNA binding activators, and transcriptional co-activators. Thus, histone acetylation is involved in gene activation. Histone acetylation is involved in the regulation of various cellular processes including chromatin dynamics, differentiation, cell cycle progression, DNA replication, and DNA repair. Furthermore, histone acetylation is also involved transcriptional initiation and elongation (Barski *et al.,* 2007).

HATs are divided into two classes according to their subcellular localization, Type-A and Type-B. Type-A HATs are localized in the nucleus and many of them possess a bromodomain, a specific domain that can recognize acetylated histones. Type-A HATs acts on histone H2A, H2B, H3, and H4. They are further classified into five families based on their sequence homology of their catalytic domains: the CREB-binding protein (CBP/p300) family, Gcn5-related N-acetyltransferases (GNATs), the more recently reported MOZ, Ybf2, Sas2, and Tip60 (MYST) family, TATA-binding protein-associated factor (TAFII250), and the

nuclear receptor co-activators (ACTR)/steroid receptor co-activators (SRC) family (Yasuto *et al*., 2017; Wang *et al.*, 2009). The CBP/p300 family HATs generally act as transcriptional co-activators in the regulation of gene expression (Goodman and Smolik, 2000; Das *et al.*, 2009; Wang *et al.*, 2010). They are involved in a wide range of cellular activities such as DNA repair, cell growth, and cell differentiation (Zhang *et al.,* 1998). GNATs are involved in many cellular processes such as cellular growth and cell differentiation. MYST HATs are involved in cell proliferation and transcriptional regulation (Michael *et al*., 2003). Type-B HATs are localized in the cytoplasm and lack the bromodomain. They acetylate synthesized histone H4K5 and H4K12 as well as the specific site within H3. Type B HATs are also known to acetylate newly synthesized histones H3 and H4 (Mackay *et al.*, 1992).

Four groups HAT family proteins are identified in the Arabidopsis genome (Table 1-1) (Pandey *et al.*, 2002; Liu *et al.*, 2012). Among them, there are five CBP/p300 HAC family members, named HAC1, HAC2, HAC4, HAC5, and HAC12 (Figure 1-1). Arabidopsis HAC1, HAC4, HAC5, and HAC12 possess HAT activities but unlike in animals, HAC2 does not have HAT activity (Li *et al.*, 2014). Previous studies have reported that CBP/p300 HAC proteins can specifically acetylate histones H3 and H4. For instance, HAC1 can specifically acetylate H4K14 and HAC1, HAC5 and HAC12 can acetylate H3K9 (Earley *et al.*, 2007). HAC family proteins also share the CBP-type HAT domain, the partially conserved KIX domain, as well as the partially conserved PHD finger motif. Moreover, two types of zinc finger domains ZnF-TAZ and ZnF-ZZ in HAC family proteins are important for mediating protein-protein interactions (Li *et al.*, 2014).

In Arabidopsis, CBP/p300 HAC proteins are reported to regulate flowering time, ethylene (ET) signaling, and environmental stress-dependent pattern-triggered immunity (PTI) (Han *et al.,* 2006; Li *et al.*, 2014; Singh *et al.*, 2014). HAC1, HAC5, and HAC12 are reported to be functionally redundant in the regulation of flowering. They promote flowering by negatively regulating the expression of *FLOWERING LOCUS C* (*FLC*) which is a major floral repressor (Deng *et al.*, 2007; Han *et al.*, 2007). Accordingly, mutation of HAC1 cause late flowering phenotypes, and the late flowering phenotype is enhanced in the *hac1/hac5* and *hac1/hac12* mutant (Han *et al.*, 2007). HACs are also known to be involved in ET signaling pathway. In *hac1hac5* double mutant the transcription level of several ethylene-reactive genes including *ERF1, ERF4, ERF6*, and *ERF11* are significantly increased (Li *et al.*, 2014). HAC1 also plays an important role in UV-B signaling (Fina *et al.,* 2017). Additionally, HAC1 is known to be responsible for bacterial resistance and PTI priming after exposure to environmental stress such as cold, heat, and salt stress (Singh *et al.*, 2014).

The GNAT family HATs are named HAG1/GCN5, HAG2/HAT1, and HAG3/ELP3. Arabidopsis HAG1 is involved in floral development, cold tolerance as well as root and shoot development (Benhamed *et al.,* 2006; Bertrand *et al.,* 2003; Long *et al.,* 2006; Vlachonasios *et al.,* 2003). Two MYST HAT family proteins are named as HAM1/HAG4 and HAM2/HAG5, respectively. Arabidopsis TAFII250 homologs were identified and named as HAF1 and HAF2. HAF2 plays a role in the regulation of light-induced gene expression. Typically, HAF2 is known to function in the regulation of both red/far-red and blue light signaling pathways (Benhamed *et*

al., 2006; Bertrand *et al.,* 2005).

1.1.1.2. Histone deacetylation

Acetylated histones can be reversibly deacetylated by histone deacetylases (HDACs). HDACs alter the compaction of chromatin by removing the acetyl group of lysine residues from both histone and non-histone proteins. The compacted chromatin structure prevents access of transcription factors and RNA polymerases to the target DNA leading to transcriptional repression. Histone acetylation is mainly catalyzed at histone H3K9, 14, 18, and 23 and histone H4K8, 12, 16, and 20 residues (Fuchs *et al.,* 2006).

There are 18 HDACs divided into four classes in higher eukaryotes: Class I (HDAC1, 2, 3, and 8), Class II (HDAC4, 5, 6, 7, 9, and 10), Class III (SIRT1, 2, 3, 4, 5, 6, and 7), and Class IV (HDAC11) (Table 1-1) (Pandey *et al.*, 2002).

In Arabidopsis, HDACs can be classified into three groups based on the sequence similarity (Yang and Seto, 2007). Among 18 Arabidopsis HDAC proteins, 12 HDACs belong to the yeast reduced potassium deficiency (RPD3/HDA1) superfamily, which is called as HDA. The other 2 sirtuins (SRTs) share similarity to the yeast silent information regulator 2 (SIR2). Additionally, 4 members belong to plant-specific histone deacetylase 2 (HD2) family, known as HD-tuins (HDT). Members of RPD3/HDA1 superfamily are further divided into three classes: Class I (HDA6, 7, 9, 10, 17, 19), Class II (HDA5, 14, 15, 18), and Class III (HDA2) which

possess an incomplete HDAC domain (Pandey *et al.,* 2002).

HDACs play important roles in the regulation of various aspects of Arabidopsis life cycle including plant growth, flowering, circadian regulation, seed development, germination, as well as in ET, jasmonic acid (JA), salicylic acid (SA) signaling pathway and basal defense to pathogens (Wang *et al.*, 2014). Among the Class I proteins, HISTONE DEACETYLASE 6 (HDA6), HISTONE DEACETYLASE 9 (HDA9), and HISTONE DEACETYLASE 19 (HDA19) are the most intensely investigated for their biological function. HDA6 is involved in the regulation of flowering time by directly interacting with lysine-specific demethylase 1 type histone demethylase FLOWERING LOCUS D (FLD), FVE/MSI4, and MSI5 (Jiang *et al.,* 2007; Gu *et al.,* 2011).

Additionally, it has been reported that the crosstalk between histone deacetylation and demethylation is mediated by the physical association of HDA6 and FLD (Yu *et al.,* 2011). HDA6 also acts as a negative regulator of the JA signaling pathway. HDA6 is recruited by JASMONATE ZIM-DOMAIN 1 (JAZ1) to repress the expression of *ETHYLENE INSENSITIVE 3* (*EIN3*), thereby inhibiting JA signaling (Zhu *et al.*, 2011). Additionally, HDA6 and HDA19 redundantly co-repress the expression of embryogenesis-related genes such as *LEAFY COTYLEDON 1* (*LEC1*), *FUSCA 3 (FUS3),* and *ABA INSENSITIVE 3 (ABI3)* by forming multi-functional complexes with other co-factors (Tanaka *et al.,* 2008). Besides its role in embryogenesis, HDA19 plays a crucial role in plant development and the loss of *HDA19* result in various developmental abnormalities (Tanaka *et al.*, 2008). HDA9

is involved in the regulation of flowering time by directly targeting *AGAMOUS-LIKE 19 (AGL19)* and repressing its expression (Kim *et al.,* 2013; Kang *et al.*, 2015). Moreover, HDA19 functions in SA biosynthesis and regulates expression of SA-regulated defense genes through histone deacetylation. It is also known to be involved in the repression of *pathogenesis related 1* (*PR1*) expression (Choi *et al.,* 2012). HDA19 is known to be a positive regulator of basal disease resistance in plants and represses the expression of the transcription factors *WRKY DNA-BINDING PROTEIN 38* (*WRKY38*) and *WRKY DNA-BINDING PROTEIN 62* (*WRKY62*). WRKY38 and WRKY62 are both known to negatively regulate the expression of *pathogenesis related* (*PR*) genes. Accordingly, mutation in *HDA19* abolishes the resistance to *Pseudomonas syringae* pv. tomato DC3000 (*Pst* DC3000) (Kim *et al.*, 2008).

HDA15 is involved in the repression of chlorophyll biosynthesis and photosynthesis. HDA15 interacts with PHYTOCHROME INTERACTING FACTOR 3 (PIF3) which act as a negative regulator in light responses. Together, they repress chlorophyll biosynthesis and chlorophyll biosynthesis-dependent gene expression in dark condition (Liu *et al.,* 2013). HDA18 plays a key role in the cell fate control of Arabidopsis root epidermis (Xu *et al.,* 2005; Liu *et al.,* 2013). HD2C associates with HDA6 and enhances the transcription level of abiotic stress-responsive genes, such as *ABI1*, *ABI2*, and *ERF4*. Moreover, it has been demonstrated that HD2C and HDA6 regulate rRNA gene expression through histone modifications (Luo *et al.,* 2012).

1.1.2 DNA methylation.

DNA methylation is a widely-studied epigenetic mechanism that is typically involved in transcriptional repression. DNA methylation is involved in a variety of biological processes and is conserved in higher eukaryotic organisms including plants, animals, fungi, budding yeast *Saccharomyces cerevisiae*, and the nematode worm *Caenorhabditis elegans* (Colot *et al.*, 1999).

DNA methylation occurs at cytosine residues. The methyl group ($-CH_3$) is added to cytosine bases of the DNA resulting in 5-methylcytosine (5-mC). The methylated status is stable and inherited to the next generation. DNA methylation is usually distributed in CpG islands in animals and plants.

In mammals, DNA methylation mainly occurs in symmetric CG context, although non-CG methylation is ubiquitously distributed in embryonic stem cells. In plants, DNA methylation occurs in the contexts of CG, CHG, and CHH (where H = A, C, or T) (Ramsahoye *et al.*, 2000; Lister *et al.*, 2009). The methyltransferases which catalyze DNA methylation are DNA METHYLTRANSFERASE 1 (DNMT1), DNA (CYTOSINE-5)-METHYLTRANSFERASE 3A (DNMT3A), and DNA (CYTOSINE-5)-METHYLTRANSFERASE 3B (DNMT3B). DNMT3A and DNMT3B are involved in establishing *de novo* DNA methylation patterns during germ cell development (Zhao and Chen, 2014).

The Arabidopsis genome encodes four classes of DNA methyltransferases,

METHYLTRANSFERASE 1 (MET1), CHROMOMETHYLASE 3 (CMT3), DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2), and DNA METHYLTRANSFERASE 2 (DNMT2). MET1 is the main CG methyltransferase in Arabidopsis. MET1 is orthologous to the mammalian DNMT1 which contain the bromo-adjacent homology (BAH) domain and function to maintain CG methylation (Simon *et al.*, 2005). In addition to MET1, DECREASE IN DNA METHYLATION 1 (DDM1), a factor involved in SWI2/SNF2-like chromatin remodeling, is also known to regulate CG methylation. Mutation in *DDM1* resulted in the loss of CG methylation and H3K9 methylation (Gendrel *et al*., 2002; Johnson *et al*., 2002). The histone deacetylase HDA6 is also known to be required to maintain DNA methylation. Mutation in *HDA6* resulted in reduced cytosine methylation at CG as well as CHG sites. Moreover, the expression of the targets of RNA-directed DNA methylation (RdDM) pathway, a main siRNA-mediated epigenetic pathway, were derepressed suggesting that HDA6 also function in RdDM (Aufsatz *et al*., 2002; Probst *et al.*, 2004; Matzeke and Mosher, 2014). *De novo* DNA methylation is established by DRM2, an orthologue of the mammalian DNMT3 which is regulated by the RdDM pathway (Cao *et al.*, 2003; Pontes *et al.*, 2006). CMT3 is a plant-specific DNA methyltransferase and is required for the maintenance of DNA methylation at CHG sites (Simon *et al.*, 2005).

Importantly, H3K9 and DNA methylation are known to be closely related. KRYPTONITE (KYP) and its homologs SU (VAR) 3-9 HOMOLOG 5 (SUVH5) and SU (VAR) 3-9 HOMOLOG 6 (SUVH6) are known as typical H3K9 histone methyltransferases and are required for maintaining CMT3-dependent CHG

methylation (Hume *et al*., 2017).

The removal of the methylated state of DNA, or DNA demethylation, is catalyzed by DNA glycosylases. In Arabidopsis, DEMETER (DME), REPRESSOR OF SILENCING 1 (ROS1), DEMETER-LIKE 2 (DML2), and DEMETER-LIKE 3 (DML3) are known to possess DNA glycosylase activity. They recognize and remove methylated cytosine leading to DNA demethylation (Pilar *et al*., 2008).

1.1.3 ATP-dependent chromatin remodeling

Various types of ATP-dependent chromatin remodeling complexes play different roles in eukaryotic cells. During ATP-dependent remodeling, the position and structure of the nucleosome is altered by sliding, ejecting, or restructuring the nucleosome using the energy obtained from ATP hydrolysis. In this manner, the conformational changes in histone-DNA interaction allow access for transcription factors or recruitment of transcription machinery to the genomic region. In addition, ATP-dependent chromatin remodeling complexes associate with histone chaperones to alter histone H2A-H2B or remove the octameric core from the DNA. In eukaryotes, the ATP-dependent chromatin remodeling complexes are divided into four classes: switching defective/sucrose non-fermenting (SWI/SNF), imitation SWI (ISWI), chromodomain (CHD), and the INO80 groups, respectively (Eisen *et al.*, 1995; Vignali *et al.*, 2000; Weisz *et al.*, 2001; Jerzmanowski *et al.*, 2007).

42 SNF2 family ATPases are annotated and categorized into 24 distinct

subfamilies in Arabidopsis (Flaus *et al.,* 2006). Among them, PHOTOPERIOD INDEPENDENT EARLY FLOWERING 1 (PIE1) is homologous to SWR1 and is known to play a key role in the repression of floral transition (Noh and Amasino, 2003; Kumar and Wigge, 2010). SPLAYED (SYP) and BRAHMA (BRM) are identified as SNF2 subfamily involved in development and immunity in Arabidopsis (Wagner and Meyerowitz, 2002; Bezhani *et al.*, 2007). DECREASED DNA METHYLATION 1 (DDM1) of the LYMPHOID SPECIFIC HELICASE (LSH) subfamily are also known to function as immune regulators. SYD and BRM have functional redundancy in regulating some defense-related genes (Bezhani *et al.*, 2007; Walley *et al.*, 2008). In addition, the expression of the SA-responsive gene *PR1* is increased in *syd-2* mutant upon *Pst* DC3000 infection indicating that SYD negatively regulates SA pathway (Walley *et al.,* 2008).

1.1.4 Noncoding RNA-mediated silencing

Recent studies using genome-wide analysis have shown that 90% of the eukaryotic genome is transcribed. However, only 1-2% of the genome encodes proteins. This implies that a very large number of RNAs do not have the potential to encode protein. This type of RNAs is called noncoding RNAs (ncRNAs). NcRNAs are transcribed from the intergenic region or antisense strand of protein-coding genes. They regulate the expression of their target genes in an epigenetic manner at the transcriptional or post transcriptional level.

NcRNAs can be divided into two groups: the short ncRNAs (snRNAs) and long

ncRNAs (lncRNAs). First, short ncRNAs are less than 200 nucleotides long. Among various short ncRNAs, micro RNAs (miRNAs) and small interfering RNAs (siRNAs) are the most studied. Both animals and plant miRNAs are about 20-22 nt long. They are cleaved by RNase III-like DICER enzymes. In Arabidopsis, DICER-LIKE 1 (DCL1) proteins are known to be involved in the biogenesis of miRNAs (Speth *et al*., 2013). SiRNAs are cleaved by endoribonuclease DICER-LIKE 3 (DCL3) and loaded to ARGONAUTE 4 (AGO4). The siRNA-AGO4 complex associates with DRM2 and regulates target gene transcription (Ramachndran and Chen, 2009). Unlike short ncRNAs, the long ncRNAs (lncRNAs) are greater than 200 nucleotides long. According to a previous report two lncRNAs, COLD INDUCED LONG ANTISENSE INTRAGENIC RNA (COOLAIR) and COLD ASSISTED INTRONIC (COLDAIR) function in the repression of *FLC* expression during vernalization through the association with POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) (Heo *et al.,* 2011).

1.2 The plant immune system

In nature, plants live in a dynamic environment and are constantly threatened by different types of attackers including fungi, bacteria, viruses, and microbial pathogens. However, unlike animals, plants cannot flee from danger. In turn, plants are equipped with a highly sophisticated immune system that can respond to their attackers and withstand challenges. The ability of the plants to defend themselves against various environmental, abiotic stresses is critical for their

survival and reproductive success.

Plant pathogens can be divided into two types based on their lifestyle, the necrotrophs and biotrophs (Corné *et al.,* 2009). Necrotrophs invade and rapidly destroy the host cell to obtain nutrients from dead cells. They are inhibited by JA and ET-related defense system. In contrast, biotroph pathogens absorb nutrients from living cells. They are sensitive to SA-dependent defenses (Corné *et al.,* 2009).

1.2.1 The innate immune system of plants

Unlike animals, plants are in lack of the adaptive immune system. However, plants can compensate this weakness by sensitively recognizing pathogens and responding to their attack by activating specific defense mechanisms through the innate immune system (Chisholm *et al.,* 2006; Dodds *et al.,* 2010). Upon pathogen attack, the primary immune response allows plants recognize common features of various microbial pathogens. These microbial determinants are named as pathogen-associated molecular patterns (PAMPs). Flagellin from gram-negative bacteria represents a typical PAMPs recognized by the immune system of Arabidopsis (Corné *et al.,* 2009).

PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI) are the two main systems of the plant immune response. In PTI, the pattern-recognition receptors (PRR) of the host plant recognizes the PAMPs upon pathogen infection. Then PRRs initiate downstream signaling cascades and activate the immune response to protect the host. However, the co-evolution of pathogens and their host

plants allowed pathogens to acquire effector molecules which can suppress PTI and promote virulence in the host cell leading to effector-triggered susceptibility (ETS). To cope with ETS, plants acquired resistance (R) proteins that recognize these attacker-specific effectors, resulting in a sophisticated immune response referred as effector-triggered immunity (ETI) (Corné *et al.,* 2009).

1.2.2 PAMP-triggered immunity in Arabidopsis

During PTI, the leu-rich repeat transmembrane receptor kinase (LRR-RLK) FLAGELLIN SENSITIVE2 (FLS2) plays a crucial role in flagellin perception. FLS2 contains the extracellular LRR domain and has similar characteristics with the Toll receptors in Drosophila and Toll-like receptors in mammals. Generally, LRR domains are important for protein-protein interactions (Delphin *et al.,* 2006). FLS2 initiate PTI through the association with other proteins such as BRI1-ASSOCIATED KINASE 1 (BAK1) and other LRR-RLKs **(**Delphine *et al.,* 2006).

1.2.3 Systemic acquired resistance (SAR) and salicylic acid (SA) signaling

Following pathogen attack, the plant defense response is induced at the site of infection by accumulating SA. Then a systemic defense response is triggered in the whole plant to protect the undamaged tissues against successive attack by various pathogens. This "whole-plant" resistance response is referred as systemic acquired resistance (SAR). SAR is a sophisticated defense strategy that allows plants to

protect themselves in long-term against a broad range of pathogens. SAR can be activated by PTI and ETI-mediated pathogen recognition and is related to increased levels of SA (Corné *et al.*, 2009).

SA is an important phenolic metabolite that regulates many biological processes in prokaryotic and eukaryotic organisms. In plants, SA plays essential roles in plant development, cell growth, leaf senescence, seed germination, fruit production as well as disease resistance. Plants produce SA as a key defense signal after pathogen attack. SA plays crucial roles in delivering the extracellular PAMP signal into the cell. Cellular accumulation of SA elevates the expression of *PR* genes which contribute to the onset of SAR. The SA-dependent immune response pathway is typically triggered against microbial biotrophic pathogens (Glazebrook *et al.*, 2005).

SA is synthesized from two different biosynthetic pathways: from cinnamate via PHENYLALANINE AMMONIA LYASE (PAL) pathway, and the other from isochorismate via ISOCHORISMATE SYNTHASE (ICS/SID2) pathway (Garcion and M´etraux, 2006). The Arabidopsis genome encodes two ICS genes, *ICS1*/*SID2* and *ICS2*. Previous studies have reported that mutation in *ICS1* impairs SA biosynthesis (Dewdney *et al.*, 2000; Wildermuth *et al.*, 2001). Four PAL genes are encoded in Arabidopsis: *PAL1*, *PAL2*, *PAL3*, and *PAL4*. In *pal1pal2pal3pal4* quadruple mutants, basal and pathogen-induced SA levels are highly reduced (Huang *et al*., 2010).

In Arabidopsis, SA accumulation is promoted by *PHYTOALEXIN*

DEFICIENT 4 (*PAD4*) and *ENHANCED DISEASE SUSCEPTIBILITY 1* (*EDS1*). In addition, the transcription level of *PAD4* and *EDS1* is upregulated by SA treatment (Cui *et al*., 2017). The feedback loop involving SA accumulation and the upregulation of *PAD4* and *EDS1* expression amplifies the defense output.

Both PAD4 and EDS1 encode lipase-like proteins and function in basal and R protein-mediated immune response (Bart *et al*., 2001). The TIR-NBS-LRR-type R proteins in plants are determinants of the immune response which recognize effector molecules and initiate ETI. The interaction of EDS1 and PAD4 proteins in pathogen-challenged or healthy plant cells were demonstrated through *in vivo* co-immunoprecipitation assays (Co-IP) (Bart *et al.*, 2001).

In Arabidopsis, pathogen-activated expression of *ICS1* and *ENHANCED DISEASE SUSCEPTIBILITY 5 (EDS5/SID1)* is blocked in *EDS1* and *PAD4* loss of function mutants. This result indicates that EDS1 and PAD4 play roles upstream of ICS1 and EDS5 in the production of SA (Glazebrook *et al*., 2003). The CALMODULIN BINDING PROTEIN 60g (CBP60g) and its homolog SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1 (SARD1), two CaM-binding transcription factors bind to the *ICS1* promoter and activate its transcription during calcium signaling-dependent SA biosynthesis. Accordingly, *cbp60g sard1* double mutant shows partial deficiency in pathogen-induced SA biosynthesis (Wang *et al.*, 2011).

1.2.4 NON-EXPRESSER OF PR GENES1 (NPR1)

NON-EXPRESSER OF PR GENES 1 (NPR1) is a transcription co-activator and master regulator of SA-induced immune response required for the activation of SA-regulated defense genes (Delaney *et al.,* 1995; Glazebrook *et al.,* 1996; Shah *et al.,* 1997; Beckers and Spoel, 2006; Loake and Grant, 2007; Spoel *et al.,* 2009). NPR1 was first identified through a mutant screening (Cao *et al.,* 1994; Wang *et al.,* 2006). In *npr1*mutant, the SA-mediated *PR* gene expression and SA signaling are blocked in the presence of SA or 2, 6-dichloroisonicotinic acid (INA: synthetic SA analog) leading to defective pathogen resistance. The Cys521/Cys529 transactivation domain of NPR1 is critical for its binding with SA and for its function as a co-activator (Yue *et al.,* 2012).

Following pathogen attack, the accumulation of SA leads to a cellular redox change. This leads to the monomerization of NPR1 oligomers by thioredoxins (TRXh3 and TRXh5) facilitating its translocation into the nucleus for the regulation of SA-response genes including the *PR* genes (Carolin and Kenichi, 2014). Mutation in *NPR1* results in decreased transcription of defense-responsive genes and pathogen resistance (Cao *et al.,* 1994; Shah *et al.,* 1997). A previous study has shown that 193 SA and NPR1-dependent genes are significantly induced through complete Arabidopsis transcriptome microarray (CATMAv2) analysis (Blanco *et al.,* 2009). Furthermore, NPR1 and its paralogues NON-EXPRESSER OF PR GENES 3 (NPR3) and NON-EXPRESSER OF PR GENES 4 (NPR4) are proposed to act as SA receptors (Zheng *et al.,* 2012; Yue *et al.,* 2012).

NPR1 contains the nuclear localization sequence (NLS) yet the endogenous

NPR1 proteins localize both in the cytosol and nucleus (Despre´ s *et al.*, 2000). NPR1 has two protein-protein interaction motifs: Ankyrin repeat domain and broad-complex, tramtrack, and bric-a-brac/pox virus and zinc finger (BTB/POZ) domain (Aravind and Koonin, 1999; Bardwell and Treisman, 1994).

In Arabidopsis, the transcription factor TGACG SEQUENCE-SPECIFIC BINDING PROTEIN (TGA2) functions as a transcriptional repressor for basal repression of *PR* genes. Upon activation of SAR, TGA2 recruits NPR1 and activates *PR* genes. The ankyrin repeat domain is essential for the interaction of TGA2 with NPR1 and mutation of the ankyrin repeat abolishes NPR1-TGA complex formation and *PR* gene expression (Cao *et al.*, 1997; Ryals *et al.*, 1997; Zhang *et al.*, 1999; Despres *et al.*, 2003). A previous study demonstrated that BTB/POZ domain in NPR1 is important for the transactivation function of the TGA2-NPR1 enhanceosome. Stoichiometry analysis demonstrated that TGA2-NPR1 enhanceosome may form a stoichiometry of 2:2 (TGA2 and NPR1) (Patrick *et al*., 2009).

NPR1 is also known to function upstream of the WRKY transcription factor. In Arabidopsis, 74 WRKY transcription factors are involved in the defense response. *WRKY18, WRKY38, WRKY53, WRKY54, WRKY58,* and *WRKY70* are known as direct targets of NPR1 (Wang *et al*., 2006).

Upon SA induction, NPR1 is sumoylated by small SMALL UBIQUITIN-LIKE MODIFIER 3 (SUMO3). NPR1 contains three putative SIM sequence [VIL]-x-[VIL]-[VIL] or [VIL]-[VIL]-x-[VIL], but only SIM3 is involved in the NPR1-

SUMO3 interaction (Kerscher *et al.*, 2007; Wang *et al.*, 2011). In addition, NPR1-SUMO3 interaction is necessary for Ser11/Ser15 phosphorylation to produce active forms of NPR1 through the signal amplification loop. The activated form of NPR1 interacts with TGA transcription factor to activate the transcription of *PR1* (Abdelaty *et al.*, 2015). Moreover, upon SA accumulation, sumoylation of NPR1 causes its dissociation with the transcriptional repressor, WRKY70 (Saleha *et al.*, 2015).

1.2.5 PATHOGENESIS-RELATED (PR) GENES

PR proteins were first identified in tobacco by Gianinazzi and Martin in 1970. They were shown to be hypersensitive to tobacco mosaic virus (TMV) in tobacco leaves (Kauffmann *et al*., 1987). PR proteins are classified into 17 families and they accumulate upon pathogen infections (Ichiro *et al*., 2008). Among various *PR* genes, *PR1* is the most abundantly induced and generally used as a marker gene for SAR in various plant species.

The Arabidopsis genome encodes 22 *PR1*-like genes (Tamara *et al.,* 2017). However, *PR1* (At2g14610) is the only pathogen-inducible gene and other *PR1*-like genes do not respond to pathogens (Loon *et al*., 2006). Moreover, *PR1* is the most abundantly induced gene among other immune genes that are induced by pathogen attack.

Besides *PR1, pathogenesis related 2* (*PR2*) and *pathogenesis related 5* (*PR5*) are also known as major defense genes that are activated by SA-dependent

signaling and pathogen infection in Arabidopsis. In contrast, *PR3* and *PR4* are increased by JA and ET-dependent signaling pathways (Thomma *et al.*, 1998; Seo *et al*., 2008). *PR* genes are also involved in abiotic stress responses and plant development (Seo *et al*., 2008).

The *PR1* promoter contains three cis-regulatory elements LS5, LS7, and LS10. LS5 and LS7 have the TGA binding sequence, TGACG. TGA2 was shown to bind to the LS5 element of the *PR1* promoter (Lebel *et al.*, 1998).

1.2.6 TGA transcription factor

TGACG SEQUENCE-SPECIFIC BINDING PROTEIN (TGA) are bZIP family transcription factors crucial for the regulation of *PR* genes through the physical interaction with the positive regulator, NPR1. In Arabidopsis, 10 genes are identified to encode TGA transcription factors and they are classified into five classes as demonstrated in Figure 1-2. Clade I, II, and III are mainly involved in plant defense whereas clade IV and V are reported to be involved in flower development (Gutsche *et al.*, 2016).

The DNA binding activity of TGA is crucial for the NPR1-TGA complex to activate *PR1* and *PR2* transcription upon pathogen attack as NPR1 lacks the DNA binding domain. Accordingly, *tga2-1tga5-1tga6-1* triple-knockout mutant result in altered *PR* genes expression and defective SAR. TGA2, TGA3, TGA5, TGA6, and TGA7 are known to interact with NPR1 in yeast. Their interaction was also observed

through transient assay in plants (Kesarwani *et al.*, 2007). In Arabidopsis, NPR1 was observed to interact specifically with TGA2 and TGA5 through Co-IP assay. This interaction was enhanced by SA or INA treatment (Weihua and Dong, 2002).

TGA2, TGA5, and TGA6 act as transcriptional repressors of *PR1* in uninfected conditions. However, upon pathogen infection or SA treatment, they act as transcriptional activators (Zhang *et al.*, 2003; Choi *et al.,* 2012). Upon pathogen infection or SA treatment, TGA2 and TGA5 interact with NPR1 and is recruited to the *PR* locus to activate *PR* gene transcription (Zhang *et al.*, 2003; Rochon *et al.*, 2006; Kesarwani *et al.*, 2007; Boyle *et al.*, 2009). In the absence of SA, TGA2 forms an oligomer state and acts as a transcriptional repressor of the target gene. In particular, the N-terminal suppression domain of TGA2 plays an important role in the binding of TGA2 oligomers to DNA. Both TGA1 and TGA4 regulate plant defense in NPR1-independent manners (Shearer *et al.*, 2012). A recent study demonstrated that TGA1 and TGA4 are involved in SA biosynthesis by modulating plant immune transcription factors, such as SARD1 and CBP60g (Sun *et al.*, 2017). TGA3 is known to interact with the cytokinin-activated transcription factor ARABIDOPSIS RESPONSE REGULATOR 2 (ARR2) to induce *PR1* gene activation in a cytokinin-dependent manner. Thus, the resistance of Arabidopsis to *Pst* DC3000 is enhanced by cytokinin (Choi *et al*., 2010).

1.2.7 SA-JA-ET crosstalk in plant immune response

Plant hormones play important roles in plant growth and development.

Among various plant hormones, SA, JA, and ET are known to act as important signal molecules and critical regulators in the plant defense pathway (Corné *et al.,* 2009).

In Arabidopsis, activation of SA pathway is known to suppress JA-mediated defenses. NPR1, a key regulator and a transcriptional co-activator in SA-mediated defense pathway is also required for the SA-JA crosstalk. SA-mediated suppression of JA-responsive gene expression is blocked in the *NPR1* mutant (Spoel *et al*., 2003). By contrast, in wild-type Col-0 plant, the expression of JA-responsive marker gene *PLANT DEFENSIN 1.2* (*PDF1.2*) and *VEGETATIVE STORAGE PROTEIN 2* (*VSP2*) are suppressed by exogenous SA treatment **(**Spoel *et al*., 2003). TGA is also required for SA-mediated suppression of the JA-responsive gene expression. The interaction between TGA2 with the SA-mediated gene, *GLUTAREDOXIN 48 (GRX480)* suppresses JA-responsive marker gene *PDF1.2* expression (Ndamukong *et al*., 2007). Moreover, Clade II TGAs are important activators of JA-ET induced defense response (Zander et al., 2010). WRKY50 and WRKY51, two WRKY transcription factors which are known to be important for SA-dependent defense responses, are also required for SA-mediated suppression of JA signaling (Gao *et al*., 2011). In addition, *WRKY62* is induced by SA and JA signals, but not in the *npr1* mutant indicating that WRKY62 functions downstream of NPR1 and may be involved in the SA-JA crosstalk (Mao *et al*., 2007). *WRKY8, WRKY11, WRKY17, WRKY18, WRKY40, WRKY60,* and *WRKY41* are also reported to be involved in SA-JA crosstalk (Corné *et al.,* 2012).

ETHYLENE STABILIZED TRANSCRIPTION FACTORS 3 (EIN3/EIL1)

is involved in the inhibition of PAMP-responsive gene expression including *ICS/SID2* and reduces the accumulation of SA (Corné *et al.*, 2012; Chen *et al*., 2009).

1.2.8 Epigenetic control of the SA-dependent defense

Recent studies have revealed the importance of epigenetic control of SA-dependent genes in plant defense system. For instance, ARABIDOPSIS TRITHORAX 1 (ATX1), a SET domain protein comprising histone methyltransferase activity, is known to regulate the transcription of *WRKY70*, which is a key regulator of Arabidopsis immunity. *WRKY70* transcription is activated by ATX1-mediated H3K4me3 at the *WRKY70* promoter (Venegas *et al.,* 2007). In addition, another epigenetic regulator PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1 (PIE1) is also involved in regulating several defense-related genes. Moreover, histone variant H2A.Z is also reported to regulate the priming process in SAR through the interaction with PIE1 (March *et al.*, 2007).

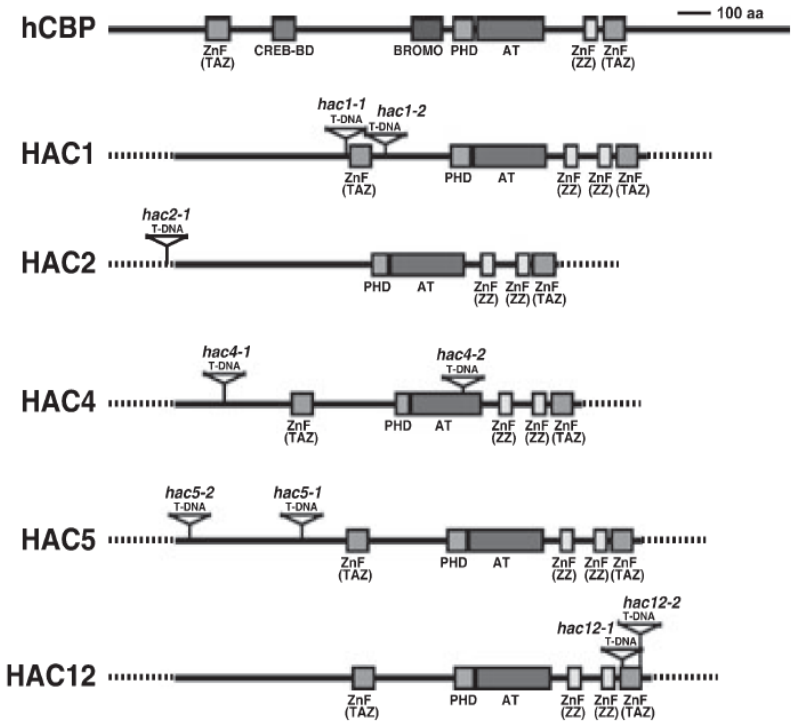
Various HDACs are also known to be involved in the transcriptional repression of defense-related genes in Arabidopsis. First, HDA19 (AtHD1), an RPD3/HDA1 family gene, is known to be involved in plant defense system. The overexpression of *HDA19* activates several JA and ET responsive gene expression and displays increased resistance to necrotrophic pathogen *Alternaria brassicicola* **(**Zhou *et al.,* 2005). Also, the expression of SA biosynthetic genes and SA levels are increased in *hda19* mutants. HDA19 directly deacetylates histones at *PR1* and *PR2* promoters to repress SA biosynthesis and SA acid-mediated defense responses (Choi

et al., 2012).

A member of the SIRT family of HDACs, SRT2, is involved in repressing SA biosynthesis genes, such as *PAD4*, *EDS5*, and *ICS1* (Wang *et al.,* 2010). ELONGATOR COMPLEX SUBUNIT 2 (ELP2) is also known to regulate histone acetylation levels at several defense gene loci and functions in the pathogen-induced dynamic DNA methylation. Accordingly, loss of function mutants of *ELP2* result in reduced histone acetylation levels in the coding region of defense genes (Wang *et al.,* 2013).

Table 1-1. HAT and HDAC family in *Arabidopsis thaliana.*

	HAT and HDAC family		Arabidopsis gene name	MIPS
HAT	CBP		HAC1	At1g79000
			HAC2	At1g67220
			HAC4	At1g55970
			HAC5	At3g12980
			HAC12	At1g16710
	GNAT		HAG1 (atGCN5)	At3g54610
			HAG2	At5g56740
			HAG3	At5g50320
	MYST		HAG4	At5g64610
			HAG5	At5g09740
	TAFII250		HAF1	At1g32750
			HAF2	At3g19040
HDAC	RPD3/HDA1	Class I	HDA6	At5g63110
			HDA7	At5g35600
			HDA9	At3g44680
			HDA10	At3g44660
			HDA17	At3g44490
			HDA19	At4g38130
		Class II	HDA2	At5g26040
		Class III	HDA5	At5g61060
			HDA8	At1g08460
			HDA14	At4g33470
			HDA15	At3g18520
			HDA18	At5g61070
	HD2		HDT1(AtHD2A)	At3g44750
			HDT2(AtHD2B)	At5g22650
			HDT3	At5g03740
			HDT4	At2g27840
	SIR2		SRT1	At5g55760
			SRT2	At5g09230



Han *et al.*, 2007

Figure 1-1. CBP/p300 HAC family in *Arabidopsis thaliana.*

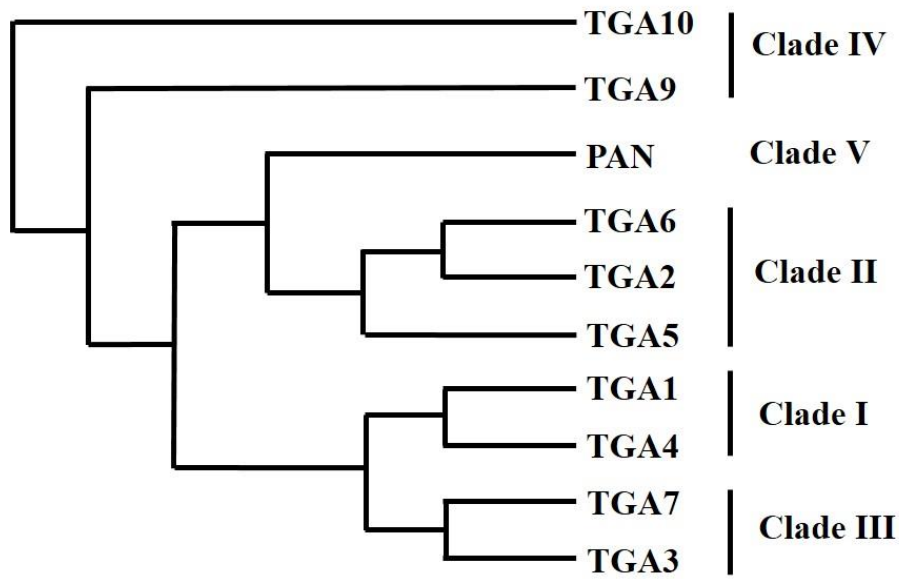


Figure 1-2. The family of TGA transcription factors in *Arabidopsis thaliana.*

Epigenetic reprogramming by the HAC-NPR1-TGA complex confers immunity in Arabidopsis

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This part has been submitted to *Nature Communication* as "Hongshi Jin[1†], Sun-Mee Choi[1†], Min-Jeong Kang[1], Se-Hun Yun[1], Dong-Jin Kwon[1], Yoo-Sun Noh[1,2*], and Bosl Noh[3*] (2017). Epigenetic reprogramming by the HAC-NPR1-TGA complex confers immunity in Arabidopsis" and is currently under revision.

2.1 Materials and methods

2.1.1 Plant materials and growth conditions

All the Arabidopsis mutants (*hac1-1*, *hac1-2 hac5-2, hac1-1 hac12-1*, *haf1-2 haf2-5, hag1-6, ham1-1 ham2-1, npr1-1*, *tga2 tga5 tga6*) and transgenic plants used in this study are in the Columbia-0 (Col) background. Details of the HAT mutants (Kim, J.-Y *et al.,* 2015), *npr1-1*(Cao, H *et al.,* 1994) and *tga2 tga5 tga6* (Table. 2-1) are described elsewhere. List of all the transgenic or multiple-mutant plants used in this study and the ways to generate them are summarized in Table S3. All plants were grown under 100 μE m^{-2} sec^{-1} cool white fluorescence light (8 hr light/16 hr dark photoperiod; short days (SD)) at 22℃. For INA treatment, 4-week-old plants were sprayed with water or INA (Sigma-Aldrich) as previously described (Choi *et al.,* 2012).

2.1.2 Pathogen infection

Pathogen inoculation was performed as described (Choi *et al.,* 2012). Three days after inoculation, three inoculated leaf discs each from different plants were combined and homogenized in sterile H_2O, with at least three times of replication. Leaf extracts were plated on King's B medium and incubated at 28℃ for 2 days, and then bacterial growth was determined by counting the colony-forming units.

2.1.3 Plasmid construction

A *HAC1* genomic DNA including the *HAC1* ORF was generated by PCR with HAC1-gate-F/HAC1-R7 (Table. 2-2), cloned into pENTR/SD/D-TOPO (Invitrogen), and then recombined into pGWB511, resulting in *35S::HAC1:FLAG-DES*. For the construction of *pNPR1::NPR1c:GFP-DES*, an *NPR1* cDNA amplified by PCR with NPR1 ORF-F (NdeI)/NPR1 ORF-R (Table. 2-2) was cloned into pENTR/SD/D-TOPO, and then an *NPR1* promoter covering 1.7 kb upstream of the start codon generated by PCR with NPR1 P-F (NotI) / NPR1 P-R (NdeI) (Extended Data Table. 2) was inserted into pENTR/SD/D-TOPO in front of the *NPR1* ORF. Finally, the resulting *pNPR1::NPR1-ENTR* was integrated into pEarlyGate301-GFP in which the HA tag of pEarleyGate301 (Earley *et al.,* 2006) was replaced by the GFP:6xHis tag from pEarleyGate103 (Earley *et al.,* 2006) For the construction of *pTGA2::TGA2c:FLAG-DES*, a *TGA2* cDNA generated by PCR with TGA2 ORF-F (NdeI)/TGA2-R (w/o stop) (Table. 2-2) was cloned into pENTR/SD/D-TOPO, and then a *TGA2* promoter covering 1.5 kb upstream of the start codon generated by PCR with TGA2 P-F (NotI)/TGA2 P-R (NdeI) (Table. 2-2) was inserted into pENTR/SD/D-TOPO in front of the *TGA2* ORF. Subsequently, *pTGA2::TGA2c:FLAG-ENTR* was integrated into ImpGWB 510 (Nakagawa *et al.,* 2007) resulting in *pTGA2::TGA2c:FLAG-DES*. All the constructs were introduced into plants by floral dip method (Clough *et al.,* 1988) via *Agrobacterium tumefaciens* strain GV301 or C58C1.

2.1.4 Protein purification and immunoblotting

Proteins from nuclear and non-nuclear fractions were prepared as previously

described (Kang *et al.,* 2015; Kinkema *et al.,* 2000) Proteins were quantified using the Protein Assay Kit (Bio-Rad), separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and subsequently transferred onto nitrocellulose membranes (Millipore). For the detection of proteins, the following antibodies were used with indicated dilutions: α-HA (1:3,000; Abcam ab9110), α-GFP (1:4,000; Roche 11814460001), α-TGA2/5 antiserum (1:3,000; gift from C. Gatz), α-FLAG (1:3,000; Sigma-Aldrich A8592), α-H3 (1:10,000; Abcam ab1791), and α-Tubulin (Sigma-Aldrich T9026).

2.1.5 Co-IP assay

Co-IP assay was performed as previously described (Mou, Z *et al.,* 2003) with minor modifications. Briefly, total proteins were extracted from 4-week-old plants by grinding in liquid N2 and homogenizing in extraction buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 Mm $MgCl_2$, 5 mM EDTA, 10% glycerol, 60 μM MG132, 100 mM *β-glycerophosphate***,** 20 mM sodium fluoride, protease inhibitors, and phenylmethylsulfonyl fluoride (PMSF). After preclearing with protein-A agarose beads, proteins were incubated with α-HA agarose beads (Sigma-Aldrich A2095) or α-GFP (Roche) coupled to protein-A agarose (Santa Cruz) at 4°C for 3 hr. For protein elution, the beads were boiled in 2 x SDS sample buffer, and the supernatant obtained after centrifugation was saved and used for protein detection.

2.1.6 Yeast-Two-Hybrid assay

NPR1 cDNA fragments encoding NPR1 BTB/POZ-ANK (1-369 aa), NPR1 Δ370

(370-593 aa), and NPR1 Δ513 (513-593 aa) were amplified by PCR with primers NdeI-NPR-F/SmaI-NPR1-ANK-R, NdeI-NPR1 Δ370-F/ NPR1-Stop-R, and NdeI-NPR1 Δ513-F/ NPR1-Stop-R, respectively (Extended Data Table. 3). *HAC1* cDNA fragments encoding HAC1-N (7-896 aa), HAC1-C1 (875-1,335 aa), HAC1-C2 (991-1,536 aa), HAC1-C3 (1,356-1,697 aa), TAZ^N (624 ~ 716 aa), and TAZ^C (1,575-1,667 aa) were also generated by PCR with primers SmaI-HAC1-N-F/SalI-HAC1-N-R, NcoI-HAC1-C1-F/BamHI-HAC1-C1-R, NcoI-HAC1-C2-F/BamHI-HAC1-C2-R, NdeI- TAZ^N-F/ TAZ^N –R, and NdeI- TAZ^C-F/ TAZ^C -R, respectively (Extended Data Table. 3). *NPR1* and *HAC1* cDNA fragments were cloned into pGADT7 and pGBKT7 vectors (Clontech), respectively, and introduced into yeast strain AH109 by lithium acetate method as described in the Clontech yeast protocol handbook. Interactions were assessed by yeast growth on synthetic drop-out medium lacking leucine, tryptophan, adenine, and histidine in the presence of 1 or 3 mM 3-AT.

2.1.7 Gel filtration assay

Proteins were prepared by homogenizing 4-week-old plant tissues in extraction buffer (20 mM Tris-HCl pH 7.5, 200 mM NaCl, 10% glycerol, 60 μM MG132, 100 mM *β-glycerophosphate***,** 20 mM sodium fluoride, protease inhibitors, and PMSF) followed by 20 min of incubation at 4°C. After centrifugation at 13,000 rpm for 5 min, the supernatant was saved and filtered through a 0.45 μm filter (Millipore). 1.5 mg of total proteins were injected on the Superdex 200 10/300GL column (GE Healthcare Life Sciences) and fractionated by the AKTA fast protein liquid chromatography system (Amersham Biosciences). Proteins in each fraction were

concentrated using acetone (Junsei), separated by SDS-PAGE, and transferred onto nitrocellulose membranes (Millipore) for immunoblot analysis.

2.1.8 RNA extraction and RT-qPCR analysis

Total RNA extraction and reverse transcription were performed as described previously (Choi *et al.,* 2012). The sequences of primers used for reverse transcription followed by quantitative real-time PCR (RT-qPCR) are provided in Table. 2-4.

2.1.9 ChIP assay

ChIP assay was performed as previously described (Choi, S.-M *et al., 2012,* Han, S.K *et al.,* 2007) using 4-week-old plants grown in SD. Antibodies used for ChIP were α-H3Ac (Millipore 06-599), α-FLAG (Sigma-Aldrich F1804), α-HA (Abcam ab9110), and α-GFP (Life technologies A6455). The amount of immunoprecipitated chromatin was measured by qPCR using primers listed in Table. 2-5. The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2015) was used to calculate the relative amount of amplified products in samples.

2.1.10 RNA sequencing analysis

Total RNA was isolated using Tri Reagent (MRC) and further purified with RNeasy MiniKit (Qiagen) to have $OD_{260/280}$ ratio of 1.8 to 2.2. RNA-seq library was constructed and sequenced on the Illumina HiSeq™ 2000 at Beijing Genomics Institute (Hong Kong). Reads were aligned to the Arabidopsis reference genome

using SOAPaligner/soap2 allowing mismatches of no more than 2 bases. Gene-expression level was calculated by using RPKM method (Reads per kb per Million reads). Differentially expressed genes (DEGs) were selected with FDR ≤ 0.01 and $|\log_2 \text{Ratio}| \geq 1$ as thresholds.

2.1.11 ChIP sequencing analysis

ChIP was performed as previously described (Choi *et al., 2012;* Han *et al.,* 2007) with minor modifications. Protein-DNA immune-complex was precipitated using agarose A beads (Santa Cruz 2001) instead of salmon sperm DNA/Protein A agarose beads to avoid the contamination of ChIPed DNA with salmon sperm DNA. 12-20 ng of DNA pooled from 6 independent ChIPs was used for library construction after quality check with 2100 Bioanalyzer (Agilent). Library construction and sequencing on Illumina HiSeq^{TM} 2000 were performed at Beijing Genomics Institute (Hong Kong). Reads were aligned to the TAIR10 Arabidopsis genome by using SOAP2 aligner and BWA, and uniquely mapped reads were used for further analysis. Using MACS2 version 2.1.0 normalized signals respective to Col input were obtained and H3Ac-enriched peaks were identified (P value < 1.00e-02). The wiggle files obtained from peak scanning was visualized and analyzed by using Integrative Genomics Viewer (IGV).

2.1.12 Sequential ChIP assay

Two-week-old seedlings were treated with water or 300 μM INA for 12 hr before

harvest. Sequential ChIP was performed as previously described (Bernstein, B. E *et al.,* 2006) with minor modifications. Chromatin was isolated form cross-linked seedlings by using 450 ml of nuclei lysis buffer (50mM Tris-HCl pH 8.0, 10 mM EDTA, 1% SDS, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and protease inhibitors), sonicated and subjected to immunoprecipitation with α-HA (Abcam ab9110) antibody. Immune complexes were eluted by gentle agitation in 300 μl of elution buffer (30 mM DTT, 500 mM NaCl, and 0.1% SDS) at 37℃ for 30 min and purified using Zeba™ Spin Desalting Column (Thermo Fisher Scientific 89882). Eluted chromatin was diluted with 600 μl of ChIP dilution buffer (50mM Tris-HCl pH 8.0 and 0.1% SDS), subjected to the second immunoprecipitation with α-GFP (Life Technologies A6455) antibody, and then eluted with elution buffer (1% SDS and 0.1 M $NaHCO_3$). DNA was isolated by reverse-crosslinking and proteinase K treatment and purified using QIAquick PCR Purification Kit (Qiagen). Quantification of immunoprecipitated DNA and the evaluation of the relative amount of amplified products in samples were performed as described in the ChIP assay section.

2.1.13 Confocal Microscopy

Images of GFP fluorescence were observed with confocal laser microscope Carl Zeiss LSM700.

Table 2-1. List of all transgenic or multiple-mutant plants used in this study.

Transgenic plant	How to generate
HAC1:HA	Kim et al, 2015 (*28*)
35S::HAC1:FLAG	By introducing *35S::HAC1:FLAG-DES* into Col
35S::NPR1:GFP	Obtained from X. Dong (Duke University, Durham, North Carolina, USA)
NPR1:GFP in npr1-1	*By introducing pNPR1::NPR1c:GFP-DES into npr1-1*
TGA2:FLAG	By introducing *pTGA2::TGA2c:FLAG-DES* into Col
HAC1:HA in npr1-1	*By crossing HAC1:HA with npr1-1*
HAC1:HA in tga2 tga5 tga6	*By crossing HAC1:HA with tga2 tga5 tga6*
HAC1:HA 35S::NPR1:GFP	*By crossing HAC1:HA with 35S::NPR1:GFP in npr1-1*
HAC1:HA 35S::NPR1:GFP in *tga2 tga5 tga6*	*By crossing HAC1:HA 35S::NPR1:GFP with tga2 tga5 tga6*
HAC1:HA NPR1:GFP	*By crossing HAC1:HA with NPR1:GFP in npr1-1*
35S::NPR1:GFP in hac1-2 hac5-2	By crossing *35S::NPR1:GFP* with *hac1-2* (+/-) *hac5-2* (-/-) and PCR-based genotyping in the following generations

35S::NPR1:GFP TGA2g:FLAG in *hac1-2* (+/-) *hac5-2* (-/-)	By crossing *35S::NPR1:GFP* in *hac1-2* (+/-) *hac5-2* (-/-) with *TGA2g:FLAG* and PCR-based genotyping in the following generations
35S::NPR1:GFP TGA2g:FLAG in *hac1-2* (-/-) *hac5-2* (-/-)	By crossing *35S::NPR1:GFP* in *hac1-2* (+/-) *hac5-2* (-/-) with *TGA2g:FLAG* and PCR-based genotyping in the following generations
npr1-1 hac1-2 hac5-2	By crossing *npr1-1* with *hac1-2* (+/-) *hac5-2* (-/-)
tga2 tga5 tga6	*By crossing tga2 tga5 with tga6*

Table 2-2. Primers used for *HAC1:HA*, *NPR1:GFP*, and *TGA2:FLAG* constructs.

Name	Sequence
HAC1-gate-F	5’-CACCGATTTGGGAAAACCTGAATTCATTCGCT-3’
HAC1-R7	5’-ACCTGAGCCCCCAGCGACTTCTGCAGCTC-3’
NPR1 ORF-F (NdeI)	5’-CACCCATATGGACACCACCAT TGATGGATTCG-3’
NPR1 ORF-R (w/o stop)	5’-CCGACGACGATGAGAGAGTTT-3’
NPR1 P-F (NotI)	5’-CAAGGCGGCCGCGTTACTGTATAGAAAATAGTGTCCC-3’
NPR1 P-R (NdeI)	5’-CTTGCATATGCAACAGGTTCCGATGAATTGAAATTC-3’
TGA2 ORF-F (NdeI)	5’-CACCCATATGGCTGATACCAGTCCGAGAAC-3’
TGA2-R (w/o stop)	5’-CTCTCTGGGTCGAGCAAGCCATAAGG-3’
TGA2 P-F (NotI)	5’-CAAGGCGGCCGCTAATGAGTTAAGAATAGAGAATG-3’
TGA2 P-R (NdeI)	5’-CTTGCATATGATTACTTTCTCACCACTTTTCTGTAC-3’

Table 2-3. Primers used for Yeast-Two-Hybrid constructs.

Name	Sequence
Nde Ⅰ-NPR1-F	5’-ACCATATGATGGACACCACCATTGATGGATTCGC-3’
Sma Ⅰ-NPR1-ANK-R	5’-AATCCCGGGTCATGCGATCATGAGTGCGGTTCTACC-3’
Nde Ⅰ-NPR1 Δ370-F	5’-ATTCATATGAAACAAGCCACTATGGCGGTTG-3’
Nde Ⅰ-NPR1 Δ513-F	5’-ATTCATATGGCAGTGCTCGACCAGATTATG-3’
NPR1-Stop-R	5’-TCACCGACGACGATGAGAGAGTTTACGG-3’
Sma Ⅰ-HAC1-N-F	5’-CGCCCGGGTATGTCGGGGCAGGTTTCAAAC-3’
Sal Ⅰ-HAC1-N-R	5’-GCAGTCGACTTTTGATGTATGTTCAGTAG-3’
Nco Ⅰ-HAC1-C1-F	5’-GGGCCATGGAGGTGGAGAAAGAACCTGAATCACTT-3’
BamH Ⅰ-HAC1-C1-R	5’-CCCGGATCCTTTCTTGAGCATTCCTTTCTTATTTCC-3’
Nco Ⅰ-HAC1-C2-F	5’-GGGCCATGGAGTTTTGTATTCCATGTTATAATGAATCCC-3’
BamH Ⅰ-HAC1-C2-R	5’-CCCGGATCCGCCTTGACCAGTTTCAATGTCAAG-3’
Nco Ⅰ-HAC1-C3-F	5’-GGGCCATGGAGACCATTACTAAAAGGGCTCTAAAAG-3’
BamH Ⅰ-HAC1-C3-R	5’-CCCGGATCCTTAACCTGAGCCCCCAGCGA-3’
Nde Ⅰ - TAZ^N-F	5’-ATTCATATGAGAAATGGAAATGGCAACCGGGATCCG-3’
TAZ^N-R	5’-TCACTGTTGCTGTAGGTAGGCCTTCACAGG-3’
Nde Ⅰ - TAZ^C-F	5’-ATTCATATGGCTCAAAACAAAGAAGCGAGGCAATTGC-3’
TAZ^C-R	5’-TCATCTCAGATGCTCCTTTAGGTCCCTGC-3’

Table 2-4. Primers used for RT-qPCR analyses.

Gene	Name	Sequence
UBQ10	qUBQ-F	5′-GGCCTTGTATAATCCCTGATGAATAAG-3′
	qUBQ-R	5′-AAAGAGATAACAGGAACGGAAACATAGT-3′
Tubulin	TUB-F	5′-GTGGTAGTGAAGAATCAAGAGCACC-3′
	TUB-R	5′-GAACCCTAAAGTTCTCAGGCTCCAC-3′
PR1	qPR1-F	5′-GCCGTGAACATGTGGGTTAG-3′
	qPR1-R	5′-GGCACATCCGAGTCTCACTG-3′
PR2	qPR2-F	5′-GATCGTTGGAAATCGTGGTG-3′
	qPR2-R	5′-TAGCTTTCCCTGGCCTTCTC-3′
AT1G13340	F	5′-CTCAAGCCATCTCTGATGTCAC-3′
	R	5′-TCGAGAGTGTTTTGGTCTTTGA-3′
AT1G21310	F	5′-GTCTCCAATGGCCTCTTTAGTG-3′
	R	5′-GGTGGTGGAGGAGAAGAATAGA-3′
AT1G33960	F	5′-TTATCGACTTGGTCAGAAAGCA-3′
	R	5′-TTCTGAATGCCCTTTTGATTCT-3′
AT1G35230	F	5′-TACTGAATCTCCACCAGCTCCT-3′
	R	5′-ACGAGGGAGACTCTGCTAACTG-3′
AT1G68620	F	5′-ATGGACCAGTCGTAGACGAAGT-3′

	R	5′-GGGTAGTGAGGGATCAACACAT-3′
AT1G75040	F	5′-TGCTTAAGGTCATGGATCAGAA-3′
	R	5′-CAAGTTTCCGGCTTATCGTTAG-3′
AT2G13810	F	5′-CTGGTTATGTTGCATCCAGAAA-3′
	R	5′-AACGTGGACTACCATCTTCGAT-3′
AT2G24850	F	5′-GAAAGACCATTTTGTTCCCAAC-3′
	R	5′-TGGGTGCGTAAGAGTTAGCC-3′
AT2G26400	F	5′-ATATGAAGGCAATGCGTCTTTT-3′
	R	5′-TGGACACACCTCACATAAGTCC-3′
AT3G03470	F	5′-AGAAGGAGGGAAGAAGAGGAAA-3′
	R	5′-TCTGGATACTTCACCATGATCG-3′
AT3G22910	F	5′-CCCTCGTTCTCTTTCATACACC-3′
	R	5′-CTAAGCCATTAGGACCACCAAG-3′
AT3G26170	F	5′-GAAAAGATCGAAGAGCTCGTGT-3′
	R	5′-CGTTGAGTCTCTTGTGTTGTCC-3′
AT3G28510	F	5′-TGTGAAGGTGAAGTGGTATTCG-3′
	R	5′-GGTGTCGCCTATGGAAACTAAG-3′
AT3G45860	F	5′-CGCTTCATACTCTACCGGATTC-3′

	R	5′-TTACGGCAAACTTCTTGTGAGA-3′
AT3G51860	F	5′-TTCCATGCAAAACTCTCAAGAA-3′
	R	5′-TTGTAAGAATTGGCAAGAATGG-3′
AT4G04500	F	5′-GTTTGGCAGAGATTCCAAAAAC-3′
	R	5′-TGTGTCTTCAATCACATGTTCG-3′
AT4G10500	F	5′-ACTCCATTGCTTTCCCATAGAA-3′
	R	5′-CTCTGAGATGGCCTCAAGAAGT-3′
AT4G23310	F	5′-GCCTCCTCTAGCTACTCCAGTG-3′
	R	5′-CACAGTTATGGCAAACTTCTGG-3′
AT5G01600	F	5′-CTCCTAAGCCACTACTCCCTCA-3′
	R	5′-ATGTTGTTTGTGTCCACCGTAG-3′
AT5G24200	F	5′-CCCAACTCGATAGTACCTCCAC-3′
	R	5′-GAAGGCGATACGAATGTTAAGC-3′
AT5G64000	F	5′-GGAGCTCCAACCTGATAAACTG-3′
	R	5′-GAGAGCTGGTGTACGATTCCTC-3′
AT5G64510	F	5′-GAAGTCAAGGTTTCTGGGTTTG-3′
	R	5′-GTATTCCCATCGGTTCACATCT-3′
AT1G09560	F	5′-TTCCAGAAGAACAATGGTGATG-3′

	R	5′-CAAGATGTTGTCAGGAACAGGA-3′
AT1G15520	F	5′-TTGATCGTCTCAGGAAAGGAAT-3′
	R	5′-CCATTTGATGAGCCTCTCTAGC-3′
AT1G21240	F	5′-GAAGTCCCCTTGTTGGTCTATG-3′
	R	5′-AGAGTTCCAGCGACTTCTATCG-3′
AT1G51660	F	5′-CCTCTTCCTCTCCCACCTACTT-3′
	R	5′-TCCGATACGGTTACCTCTCACT-3′
AT2G13790	F	5′-TGAAGAAGACCCAGAGGTTCAT-3′
	R	5′-CCAAAACCACCTCTACCCAATA-3′
AT2G47130	F	5′-AGCTTTCTCGACTTGAATCTGG-3′
	R	5′-GCTGGTCGTACATACGATTGAC-3′
AT3G23120	F	5′-GCCAACTTAACCAAGCTTTCTC-3′
	R	5′-GGATTTGAAGTGATTGGAGGAG-3′
AT3G51440	F	5′-CACTCTCTACCAACTCGACACG-3′
	R	5′-ATTGAGAAGACCGACTCCGATA-3′
AT3G60420	F	5′-GGCCTTTAGAACTGGTCAGAGA-3′
	R	5′-GACATAGCAATGGGATCGAAAT-3′
AT4G13510	F	5′-GCCTCTGCTGACTACTCCAACT-3′

	R	5′-AAACCCGGTTAAGAAAGAGGAA-3′
AT4G18250	F	5′-CTATGCTCCATCGACTCAACAG-3′
	R	5′-ACCGTACTCAACACTGATGGTG-3′
AT2G23170	F	5′-GGGATCAACTTGAAACCAATGT-3′
	R	5′-TAGCTCCACAAGTTCGGATTTT-3′
AT4G23810	F	5′-AGATGTTACCAAAGTGGTCAGAAA-3′
	R	5′-TAACTCCTTGGGAATTTGGCGCCT-3′
AT5G01850	F	5′-TACCAAGGAAGGTATGGTCGTC-3′
	R	5′-ATATTGACCTCACGGACGAAAC-3′
AT5G08380	F	5′-GGTCGAGTCTTCGAGATCAGTT-3′
	R	5′-ATGTTGCAGCTAAAATGGTTCC-3′
AT5G08760	F	5′-AGGTACTCGGATTCTCCTCGAT-3′
	R	5′-GCCTTTTCCTTTGGTTGAGTTA-3′
AT5G17060	F	5′-GTGGTGGATTCCTTAGATCGAG-3′
	R	5′-CTCTCATGTCCTGTTTGTTTGC-3′
AT5G22060	F	5′-ATGATCAATATGGGGAAGATGC-3′
	R	5′-CCACCACTACCAAAGAAGGAAG-3′
AT5G44568	F	5′-AACAAAGGAACAAATTGCGTTT-3′

	R	5′-CCTTTATCTTTAGGCGATGCAG-3′
AT5G48540	F	5′-GACGCTTGTCTCAAAGACTCCT-3′
	R	5′-TCCTCTACATTGAGCAAGTCCA-3′
AT5G54610	F	5′-CTCAATCGGGTAGTGTTGATGA-3′
	R	5′-TGGAGAGGTGTGTGGATGATAG-3′

Table 2-5. Primers used for ChIP assays.

Locus	Name	Sequence
UBQ11	ChIP-F	5′-TCAGTATATGTCTCGCAGCAAACTATC-3′
	ChIP-R	5′-GACGACTCGGTCGGTCACG-3′
ACTIN2	ChIP-F	5′-GATCCGTTCGCTTGATTTTGC-3′
	ChIP-F	5′-ACAAGCACGGATCGAATCACA-3′
PR1-P2	P2-F	5′-ATGGGTGATCTATTGACTGTTT-3′
	P6-R	5′-ATCACTCTTGCCTATGGCTG-3′
PR1-P3	P3-F	5′-GCCAAACTGTCCGATACGATT-3′
	P7-R	5′-TGTCATTCAGTTGTTTTGTGTTTTT-3′
PR1-P4	P5-F	5′-CGATTAAAAATCGAGAATAGCCAG -3′
	P8-R	5′-ACGTGAGATCTATAGTTAAC-3′
PR1-P5	P5-F	5′-CAATGGCAAAGCTACCGATACGAAACA-3′
	P5-R	5′-CGATTAAAAATCGAGAATAGCCAG-3′
PR2-P1	P4-F	5′-CCCCAGGCTTGGCTCTATAA-3′
	P1-R	5′-GGCTAAGCTCCTTGATTCAGAC-3′
PR2-P2	P5-F	5′-GACGTACGATTAACGGCCAA-3′
	P5-R	5′-TATGATGAATCGCCCAAACC-3′

PR2-P3	P6-F	5′-ATCATGAAGGGGGAAAACGA-3′
	P6-R	5′-TTGGCTTGTGGGTCTAAGGA-3′
AT1G13340	F	5′-CGTAACGCGTCTTCTCCTCT-3′
	R	5′-TGAGAGAGTCTGGCTTGACG-3′
AT1G21310	F	5′-TGAGAGTCGGAGAGACCAACA-3′
	R	5′-TTGCCACTAAAGAGGCCATT-3′
AT1G75040	F	5′-GCA AAG AAA ATT CAG AGA ACC AA-3′
	R	5′-TTGTGATGAACACGAGGAAGA-3′
AT3G26470	F	5′-TATGGAGATGTCACTTGTCACTTC-3′
	R	5′-TCTCACCGGCGAAATAATCAGTG-3′
AT4G04500	F	5′-TGCTACCACACCTAGTCAAGTCA-3′
	R	5′-AAAAGTCGCCGTAGCAAATG-3′
AT4G13890	F	5′-TGGAATAACTTGCTAAAGGCATCA-3′
	R	5′-GACGAAGTCAAGATGCGTGTTTCCC-3′
AT4G23310	F	5′-TTCCACAGTGCAACAAGACC-3′
	R	5′-TGGGACAGCTGTGGTATCTG-3′
AT5G45090	F	5′-GATTGAGAGATTAAGAGAGAGCTTT-3′
	R	5′-CCTCCGCCTTCTTGTTTCTCCAGT-3′

AT5G64000	F	5′-AGATGGTTTGCACCAACTCC-3′
	R	5′-TTGTTACCTGGCTGAGACGA-3′

2.2 Text

Plant immunity depends on massive expression of *pathogenesis-related* (*PR*) genes whose transcription is de-repressed by pathogen-induced signals. Salicylic acid (SA) acts as a major signaling molecule in plant immunity trigged by bacterial or viral pathogens. SA signal results in the activation of the master immune regulator, NPR1, which is recruited by TGA transcription factors to numerous downstream *PR* genes. Despite its key role in SA-triggered immunity, the biochemical mechanism for the transcriptional coactivator function of NPR1 remains obscure. I show that the CBP/p300-family histone acetyltransferases, HACs, form a complex with NPR1 and TGAs and de-repress *PR* genes by histone acetylation during the SA-triggered immune response. Thus, my study reveals the biochemical mechanism of NPR1-mediated transcriptional activation and the key epigenetic aspects of the central immune system in plants.

Although plants lack specialized immune cells, they have developed sophisticated defense systems against pathogenic attacks. Salicylic acid (SA), a key signaling molecule in plant immunity (Pieterse *et al.,* 2012; Vlot *et al.,* 2009), induces a transcription reprogramming through the master immune-regulator NPR1(Cao *et al.,* 1994; Cao *et al.,* 1997; Delaney *et al.,* 1995; Shah *et al.,* 1997). NPR1 acts as a transcriptional coactivator for nearly two-thousand genes by interacting with TGA-family transcription factors (Rochon *et al.,* 2006; Després *et al.,* 2000; Zhang *et al.,* 2003; Zhang *et al.,* 1999; Zhou *et al.,* 2000). SA regulates NPR1 activity at multiple levels: 1) SA-triggered redox changes result in NPR1 monomerization and nuclear translocation (Mou *et al.,* 2003; Tada *et al.,* 2008); 2)

SA-mediated posttranslational modifications of NPR1 influence its transcriptional activity and turnover (Fu, Z.Q *et al.,* 2012; Saleh, A *et al.,* 2015; Spoel, S.H *et al.,* 2009) ; and 3) SA binding to NPR1 causes a conformational change, enabling its transcriptional coactivator function (Kuai *et al.,* 2015; Manohar *et al.,* 2015; Wu *et al.,* 2012).

Despite the essential role of NPR1 in SA-triggered transcription of *pathogenesis-related* genes (*PRs*) during plant defense, the molecular mechanism of its transcriptional coactivator role remains elusive. Here, I show that the CBP/p300-family histone acetyltransferases (HATs), HAC1 and HAC5 (HAC1/5), and NPR1 are both essential and interdependent on each other to develop SA-triggered immunity and *PR* induction. HAC1/5 and NPR1 form a coactivator complex and are recruited to *PR* chromatin through SA-dependent interaction between NPR1 and TGAs, finally relaxing repressive local chromatin and facilitating transcription. In sum, my study demonstrates the mechanism of NPR1-mediated transcriptional activation and proposes epigenetic reprogramming as central part of plant immune system.

We and others have found that histone H3 acetylation (H3Ac) at the Arabidopsis *PR1* locus is increased by pathogen attack or SA treatment, and this increase is tightly associated with *PR1* transcription(Mosher *et al.,* 2006; Koornneef *et al.,* 2008; Choi *et al.,* 2012). Interestingly, the H3Ac increase at *PR1* is undermined by the loss of either NPR1 or the three related Class II TGAs (TGA2, TGA5, and TGA6; TGA2/5/6) (Figure 2-1a) (Koornneef *et al.,* 2008). These inspired my to identify HATs responsible for the SA-induced H3Ac. As H3Ac acts as an active

epigenetic mark, firstly I searched for Arabidopsis HAT mutants with impaired *PR1* and *PR2* induction upon 2, 6-dichloroisonicotinic acid (INA; synthetic SA analog) treatment. The mutants lacking HAG1 (*hag1-6*) (Long *et al.,* 2006) or HAC1 and HAC5 (*hac1-2 hac5-2*; *hac1/5*) (Han *et al.,* 2007) showed severely impaired INA-induced *PR1* and *PR2* transcriptions (Figure 2-1b and Figure 2-2a). Further, the INA-induced H3Ac increase at the *PR1* promoter was barely detectable in *hac1/5*, whereas its increase in *hag1-6* was comparable to wild type (WT; Figure 2-1c and Figure 2-2b), suggesting that HACs are likely to be the responsible HATs. Consistently, upon infection of *Pseudomonas syringae* pv. tomato DC3000 (*Pst* DC3000), the *PR1/2* induction and H3Ac increase were all severely impaired in *hac1/5* and to lesser extents in *hac1* and *hac1/12* (Figure 2-1d, e and Figure 2-2c, d). Basal resistance to *Pst* DC3000 was also substantially decreased by the *hac1/5* mutations (Figure 2-1f). Moreover, a HAC1:HA fusion protein was targeted to the *PR1* promoter in an INA-dependent manner (Figure 2-3a), corroborating the idea that HACs activate SA-dependent plant immunity by promoting *PR* transcription through histone acetylation.

I then determined whether HACs cooperate with NPR1 and TGAs on *PR* chromatin. NPR1:GFP was also targeted to the same *PR1*-promoter regions with HAC1:HA in an INA-dependent manner (Figure 2-3b and Figure 2-4), whereas TGA2:FLAG bound constantly to the P2 region (Figure 2-3c) consistent with its reported dual roles as repressor and activator depending on SA signal (Rochon *et al.,* 2006; Zhang *et al.,* 2003; Kesarwani *et al.,* 2007; Boyle *et al.,* 2009). Sequential ChIP assays using HAC1:HA- and NPR1:GFP-containing transgenic plants showed

the INA-dependent presence of *PR1* promoter-bound NPR1:GFP within the HAC1:HA immunoprecipitate (Figure 2-3d), indicating the colocalization of HAC1 and NPR1 on *PR1* upon INA treatment. These findings, together with the well-known NPR1-TGA interaction and the lack of INA-induced H3Ac increase in *npr1* and *tga2/5/6* mutants (Figure 2-1a), led my to hypothesize that HACs, NPR1, and TGAs might form a complex on *PR* promoters and modulate transcription through chromatin modification. In support of this view, *hac1/5* and *npr1* mutations showed similar and non-additive effects on *PR1* transcription and susceptibility to *Pst* DC3000 (Figure 2-3e, f).

To study whether HAC1, NPR1, and TGAs interact each other, and, if they do, how the SA signal affects their interactions, I examined the subcellular localization of each protein and their interactions before and after INA treatment using stable transgenic Arabidopsis plants. HAC1 and TGA2/5 were always localized within nucleus, whereas the abundance and localization of NPR1 were affected by INA (Figure 2-5) as previously reported (Boyle *et al.,* 2003; Tada *et al.,* 2008; Spoel *et al.,* 2009). HAC1 and TGA2/5 were detected in the NPR1:GFP immunoprecipitate, and reciprocally NPR1 and TGA2/5 were also detected in the HAC1:HA immunoprecipitate (Figure 2-6a, b and Figure 2-7), revealing the existence of a complex containing HAC1, NPR1, and TGA2/5. TGA2/5 but not HAC1 enrichment within NPR1:GFP immunoprecipitate was increased by INA, suggesting that HAC1 might be limiting in complex formation. In contrast, both NPR1 and TGA2/5 enrichment in the HAC1:HA immunoprecipitate were increased by INA, implying the possibility of one HAC1 molecule engaging multiple NPR1 and TGA2/5

molecules as nuclear NPR1 levels increase in response to SA. This model might be a reminiscent of the interaction of p300 and MEF2 on DNA in which the highly conserved TAZ domain of p300 binds to three MEF2:DNA complexes (He, J. *et al.,* 2011). I could observe interactions between the two TAZ domains of HAC1 and the C-terminal region of NPR1 in yeast (Figure 2-8), suggesting that, similar to p300, the HAC1 TAZ domains might be important for the assembly of the HAC-NPR1-TGA complex.

I then investigated binding dependencies among the components of the HAC-NPR1-TGA complex during the assembly process through a series of Co-IP assays. The HAC1-NPR1 interaction was not affected by TGA2/5/6 deficiency (Figure 2-6c, d), nor was the NPR1-TGA2/5 interaction affected by HAC1/5 deficiency (Figure 2-6e). Remarkably, HAC1-TGA2/5 interaction was evidently disrupted by the lack of NPR1 (Figure 2-6f), revealing that HAC1 and TGA2/5 do not interact directly but indirectly through NPR1.

ChIP assays were then used to study the binding hierarchy of HAC, NPR1, and TGA to *PR1* chromatin. INA-induced targeting of NPR1 and HAC1 to *PR1* chromatin was completely abolished in *tga2/5/6* triple mutants (Figure 2-9a, b), and notably, INA-induced HAC1 targeting to *PR1* was undetectable in *npr1* mutants (Figure 2-6g). These results demonstrate that HAC1 and NPR1 are recruited to *PR1* chromatin via the interaction between NPR1 and the DNA-binding protein TGA as expected from the co-IP results (Figure 2-6a-f). Strikingly, in contrast to the HAC1/5-independent NPR1-TGA2/5 interaction (Figure 2-6e), NPR1 but not TGA2 targeting to *PR1* and *PR2* was largely reduced in the absence of HAC1/5 (Figure 2-

6h and Figure 2-9c, d). Therefore, although HAC1/5 may not be required for the interaction between NPR1 and free TGAs, they are likely required for efficient NPR1 binding to TGAs in the chromatin context. One possibility is that HACs might modify the local chromatin environment to be more permissible for NPR1 targeting to DNA-bound TGAs, or SA might induce a conformational change to the HAC-NPR1 complex rendering more efficient interaction with TGAs. In sum, one role of HACs might be to facilitate or/and stabilize the establishment of the functional HAC-NPR1-TGA complex on *PR* chromatin.

To gain further insight into the HAC-NPR1-TGA complex *in vivo*, I performed gel-filtration chromatography assays. Without the SA signal, HAC1:FLAG, NPR1:GFP, and TGA2/5 were predominantly identified in fractions with molecular weights greater than their respective predicted monomeric sizes (Figure 2.10a-d), suggesting their presence within complexes *in vivo(*Mou *et al.,* 2003; Tada *et al.,* 2008; Spoel *et al*., 2009) Noticeably, INA treatments broadened and shifted the elution profiles of HAC1 and NPR1 toward larger-mass ranges and also substantially affected the elution profile of TGA2/5 to form another peak at much higher molecular-weight range (~ fraction #19 in Figure 2-10c, d), resulting in the co-presence of HAC1:FLAG, NPR1:GFP, and TGA2/5 in fractions > 669 KD range. Thus, by SA signal HAC1, NPR1, and TGA2/5 may form a > 669 KD multi-protein complex.

For deeper understanding of the role of HACs in the assembly of the HAC-NPR1-TGA complex, I then compared the elution profiles of NPR1:GFP and TGA2/5 in WT vs. *hac1/5* mutants (Figure 2-10b, c). Without INA, the elution

profiles of NPR1:GFP and TGA2/5 were similar between WT and *hac1/5*. However, after INA treatment, the NPR1:GFP-shift toward higher molecular-weight fractions in WT was not evident in *hac1/5* (Figure 2-10b). Furthermore, TGA2/5 abundance in fractions > 669 KD was drastically reduced or eliminated in *hac1/5*, and instead TGA2/5 were detected in smaller-weight fractions (Figure 2-10c). Thus, HACs are essential components of the INA-induced high molecular-weight complex containing NPR1 and TGAs. Similarly, after INA treatment, TGA2/5 were not detected in the > 669 KD fractions in *npr1* (Figure 2-10d), consistent with the Co-IP results showing NPR1-dependent HAC1-TGA2/5 interaction (Figure 2-6f).

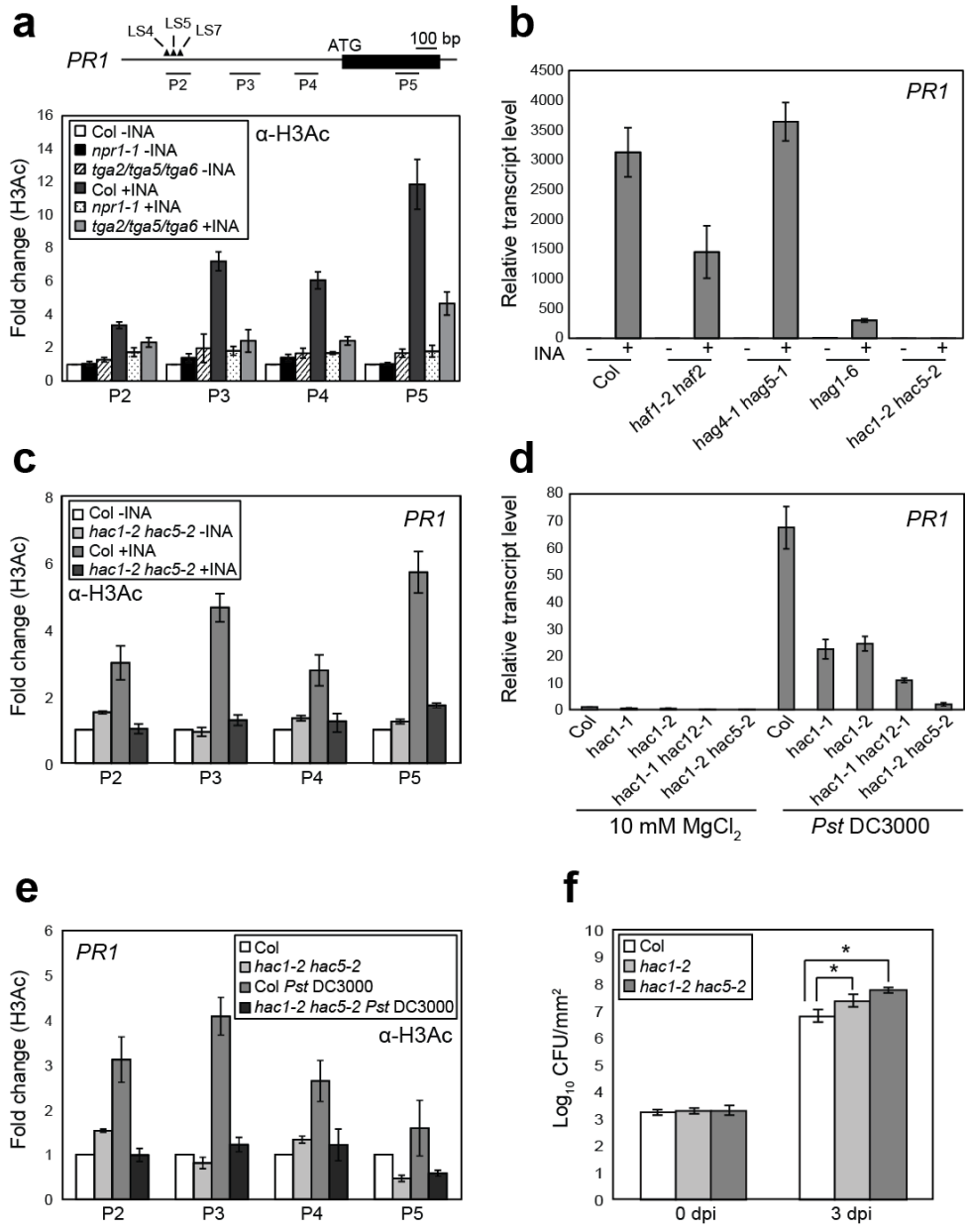
To assess the role of the collaboration between NPR1 and HACs in plant defense at genome-wide level, I performed RNA-seq analyses of transcriptomes of WT, *npr1*, and *hac1/5* either treated with INA or not (Figure 2-10e). ~70% and ~18 % of the genes significantly upregulated by INA in WT were not upregulated in *npr1* and *hac1/5*, respectively. Among the NPR1-dependent genes (2,802), ~21% (582) also showed HAC1/5-dependency (Group 1), whereas the remaining ~79% (2,220) did not (Group 2). The RNA-seq results were confirmed by RT-qPCR analyses of dozens of randomly selected genes from each group (Figure 2-11 and 2-12). Thus, a small but considerable fraction (~15%) of the INA-induced transcriptome in WT is dependent on both NPR1 and HAC1/5 (Group 1), whereas a larger fraction (~56%) requires only NPR1 (Group 2).

Next, by ChIP seq I examined how H3Ac levels at further selected Group 1-gene loci ($\log_2$[(Col+INA)/2, $\log_2$[(*npr1*+INA)/(Col+INA)] £ -2, $\log_2$[(*hac1/5*+INA)/(Col+INA)] £ -2, and FDR ≤ 0.05; Supplementary Table. 2) are

affected by INA and *npr1* or *hac1/5* mutations (Figure 2-9f). At~46%of the loci, H3Ac levels were substantially increased by INA, and 66% of these loci showed compromised H3Ac increases in both *npr1* and *hac1/5* mutants. The ChIP-seq results were reproducible as confirmed by ChIP-qPCR analyses of 11 selected loci (Figure 2-13). These results, together with the RNA-seq results, indicate that the HAC-NPR1-TGA complex constitutes part of the genome-wide transcriptional activator system acting in plant immunity.

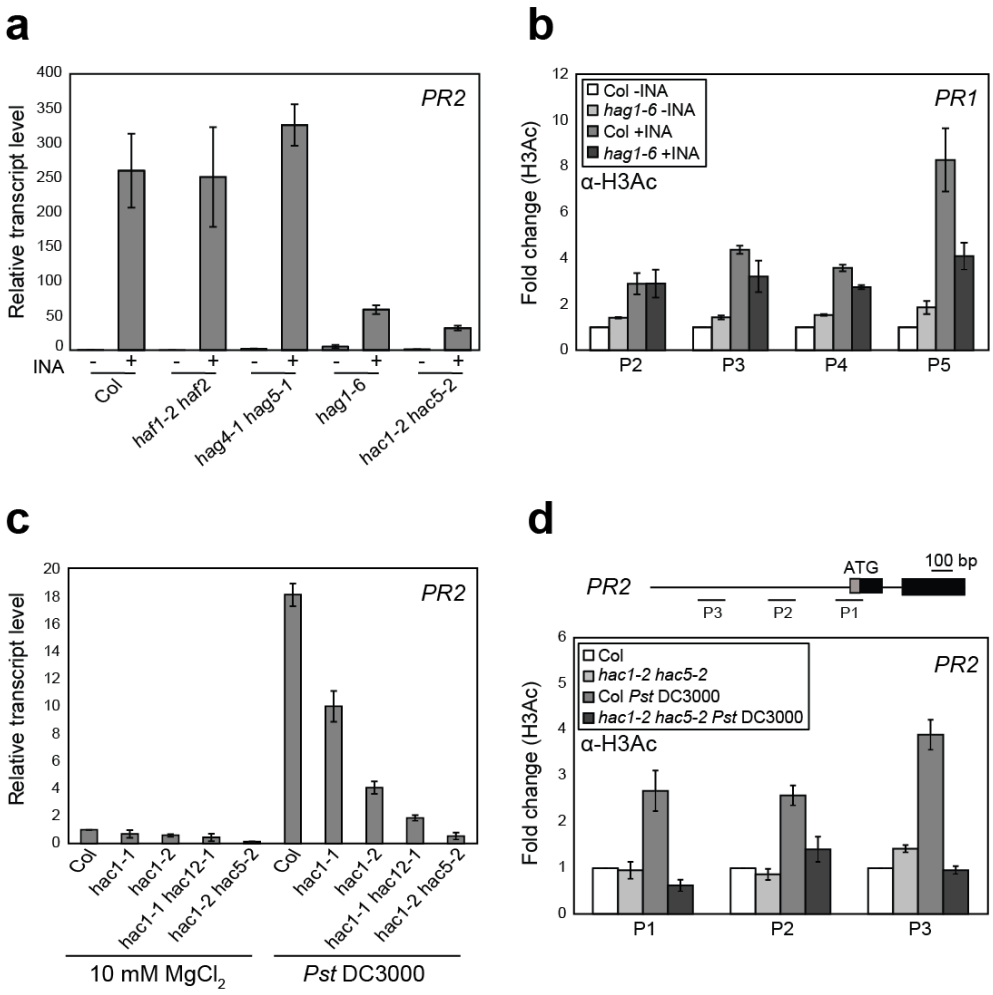
Although NPR1is a well-known master regulator of the SA-dependent immunity and systemic acquired resistance, how it acts as a transcriptional coactivator for over two thousand downstream genes is not fully understood at the molecular level. My study demonstrates that NPR1 acts in concert with HACs as epigenetic partners and that the HAC-NPR1-TGA complex is involved in genome-wide transcriptional reprogramming by a histone acetylation-based mechanism (Figure 2-14). Further, my work indicates that epigenetic reprogramming is a central feature of the immune system in plants which, unlike animals, lack specialized immune cells. My finding of both HAC-dependent and -independent NPR1-regulated genes suggests that NPR1 might act in different modes depending on target chromatin contexts. For example, the degree of chromatin compaction could be a factor in the HAC requirement, or HATs other than HACs might also act in concert with NPR1. Thus, it would be of interest in the future to understand the chromatin features of the Group1 and Group 2 genes or the dependency of the Group 2 transcription on chromatin factors other than HACs.

2.3 Figures



(By Sun-Mee Choi)

Figure 2-1 HAC1 and HAC5 are essential for *PR1* transcription and plant immunity. **a**, H3Ac levels within *PR1* chromatin in Col, *npr1*, and *tga2/5/6*. Schematics of *PR1* showing regions tested for ChIP-qPCR. Black box, exon; solid lines, upstream or downstream regions. **b**, INA-induced *PR1* expression in Col and various HAT mutants. **c**, H3Ac levels within *PR1* chromatin in Col and *hac1/5*. **d**, *PR1* expression in Col and *hac* mutants after *Pst* DC3000 infection. **e**, H3Ac levels within *PR1* chromatin in Col and *hac1/5* after *Pst* DC3000 infection. Values are the means ± SE of three biological experiments performed in triplicates (**a**-**e**). For ChIP-qPCR analyses (**a**, **c**, and **e**), untreated WT levels were set to 1 after normalization by input and the internal control *ACTIN2*. For RT-qPCR analyses (**b**, **d**), Values were normalized to *UBQ10*. **f**, Bacterial cell growth at 0 and 3 days post-infection (dpi) shown as the means ± SE of colony forming units (CFU) from three biological replicates. Asterisks indicate statistically significant differences compared to WT ($P < 0.05$ in a Student's t-test).



(By Sun-Mee Choi)

Figure 2-2 HAC1/5 regulate pathogen-induced *PR2* transcription and histone acetylation. a, Transcript levels of *PR2* in Col and various HAT mutants. **b**, H3Ac levels at *PR1* in Col and *hag1-6* either treated with INA or not. **c** ,**d**, *PR2* transcript levels (**c**) and H3Ac levels at *PR2* (**d**) in Col and *hac* mutants after *Pst* DC3000 infection. Schematics of *PR2* shows the regions tested for ChIP-qPCR (**d**). Means ± SE of three biological experiments performed in triplicates are shown after normalization to *UBQ10* (**a**, **c**) or to input and untagged Col levels (**b**, **d**).

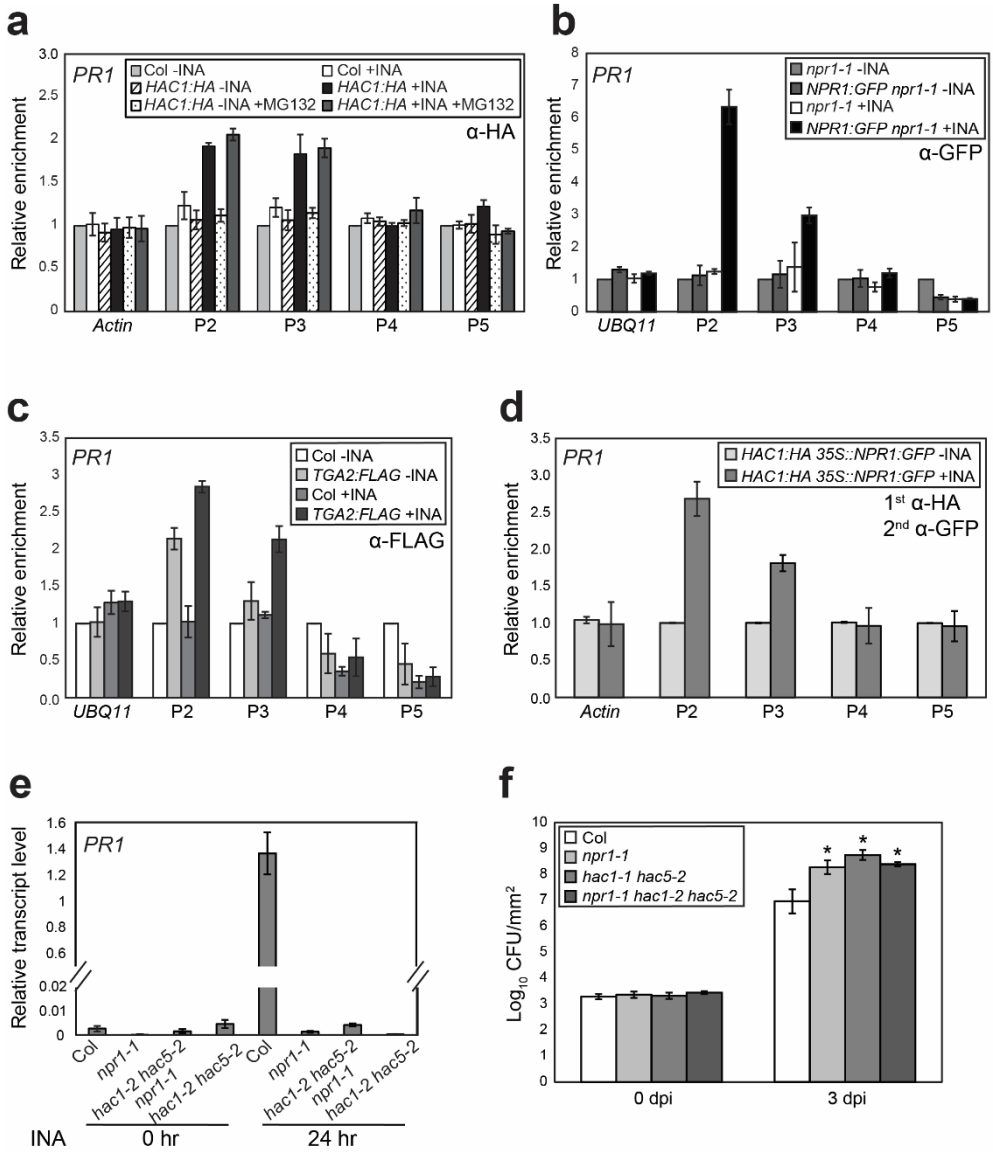
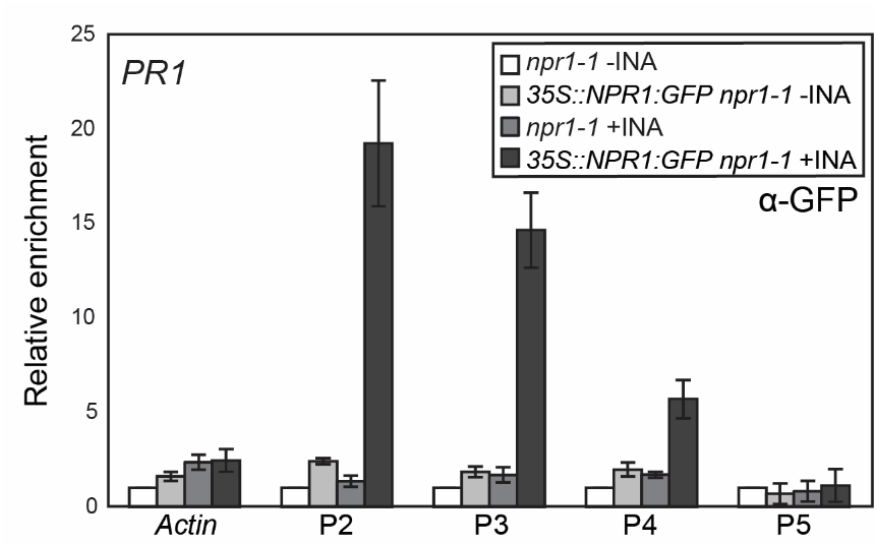

a
PR1
Col -INA
Col +INA
HAC1:HA -INA
HAC1:HA +INA
HAC1:HA -INA +MG132
HAC1:HA +INA +MG132
α-HA
Relative enrichment
Actin
P2
P3
P4
P5
b
PR1
npr1-1 -INA
NPR1:GFP npr1-1 -INA
npr1-1 +INA
NPR1:GFP npr1-1 +INA
α-GFP
Relative enrichment
UBQ11
P2
P3
P4
P5
c
PR1
Col -INA
TGA2:FLAG -INA
Col +INA
TGA2:FLAG +INA
α-FLAG
Relative enrichment
UBQ11
P2
P3
P4
P5
d
PR1
HAC1:HA 35S::NPR1:GFP -INA
HAC1:HA 35S::NPR1:GFP +INA
1st α-HA
2nd α-GFP
Relative enrichment
Actin
P2
P3
P4
P5
e
PR1
Relative transcript level
Col
npr1-1
hac1-2 hac5-2
npr1-1 hac1-2 hac5-2
INA
0 hr
24 hr
f
Col
npr1-1
hac1-1 hac5-2
npr1-1 hac1-2 hac5-2
Log10 CFU/mm2
0 dpi
3 dpi

Figure 2-3 SA signal induces concurrent targeting of HAC1 and NPR1 to *PR1*.

a-c, INA-induced association of HAC1 (a), NPR1 (b), and TGA2 (c) with *PR1* chromatin as determined by ChIP-qPCR. Level of untagged and untreated Col (**a**, **c**) or *npr1* (**b**) was set to 1 after normalization by input. **d**, Co-occupancy of HAC1 and NPR1 at *PR1* loci. Anti-HA immunoprecipitate was re-immunoprecipitated with anti-GFP antibody. The levels of untreated were set to 1 after normalization by input. **e**, RT-qPCR analysis of *PR1* expression in Col, *npr1*, *hac1/5*, and *npr1 hac1/5* upon INA treatment. Values were normalized to *UBQ10*. **f**, Bacterial cell growth in Col, *npr1*, *hac1/5*, and *npr1 hac1/5*. The growth of *Pst* DC3000 at 0 or 3 dpi is shown as the means ± SE of CFU from three biological replicates. Asterisks indicate statistically significant differences from WT ($P < 0.05$ in a Student's t-test). (a, d, e, f: By Sun-Mee Choi)



(By Sun-Mee Choi)

Figure 2-4 NPR1 enrichment within *PR1* chromatin in *35S::NPR1:GFP npr1-1* plants either treated with INA or not. Shown are means ± SE of three independent ChIP experiments performed in triplicates. The untreated *npr1-1* levels were set to 1 after normalization by input.

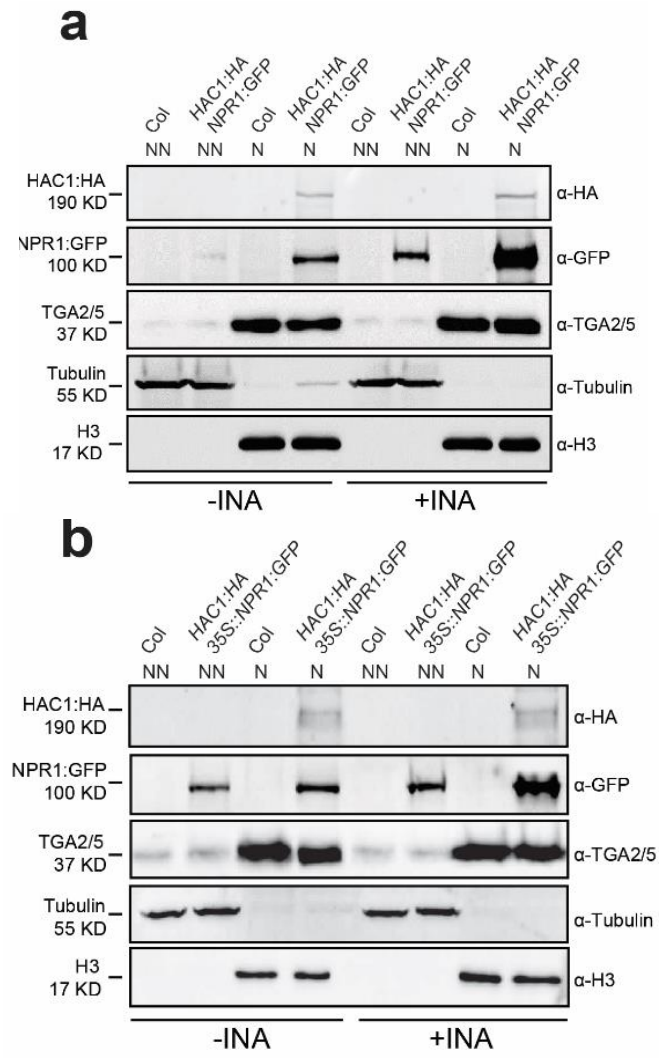
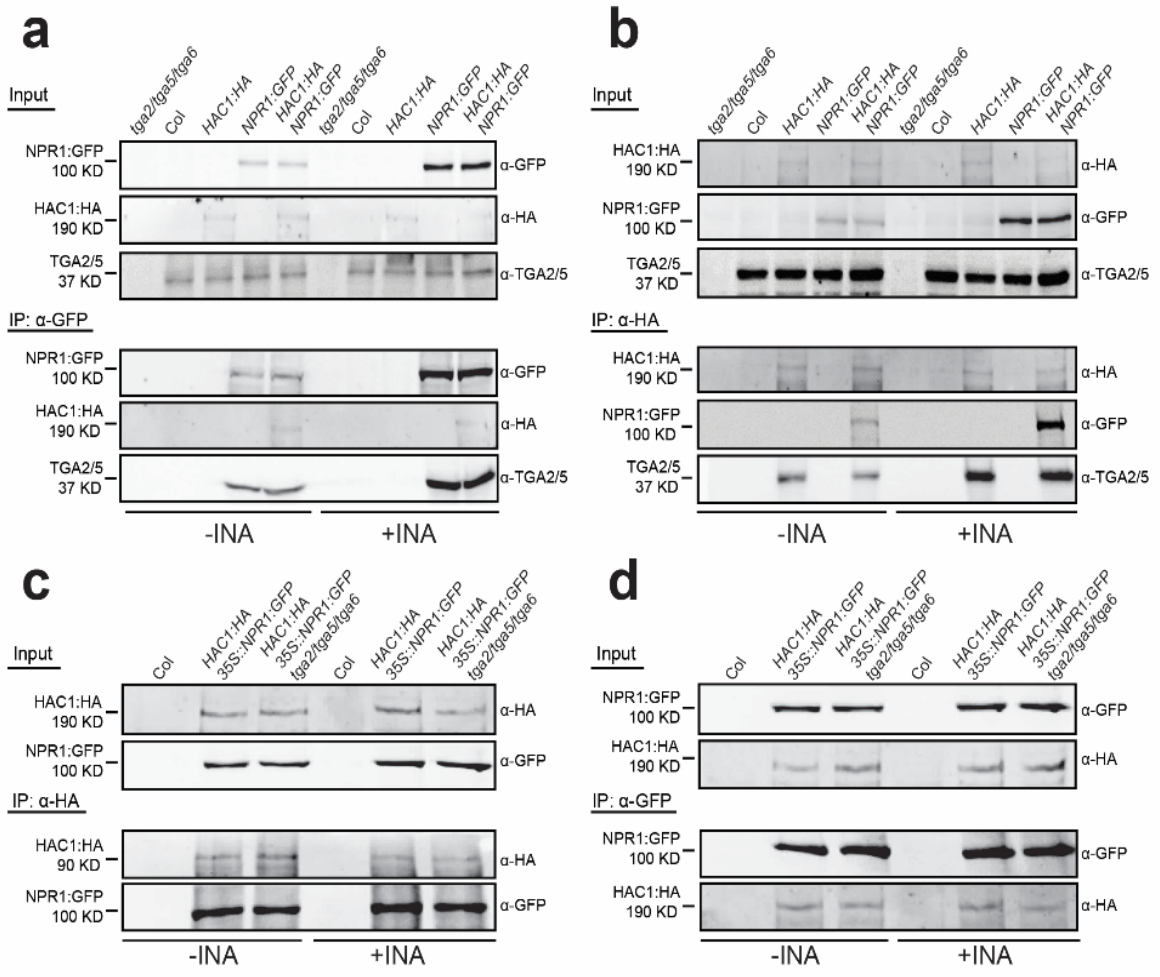

a
Col
HAC1:HA NPR1:GFP
NN
N
HAC1:HA 190 KD
NPR1:GFP 100 KD
TGA2/5 37 KD
Tubulin 55 KD
H3 17 KD
α-HA
α-GFP
α-TGA2/5
α-Tubulin
α-H3
-INA
+INA
b
HAC1:HA 35S::NPR1:GFP
c
-INA
+INA
50 um

Figure 2-5 Subcellular localization of HAC1, NPR1, and TGA2/5. a, b Immunoblot analysis of HAC1:HA, NPR1:GFP, and TGA2/5 proteins in nuclear (N) and non-nuclear (NN) fractions from *HAC1:HA NPR1:GFP* (**a**) or *HAC1:HA 35S::NPR1:GFP* (**b**) double transgenic plants. Histone H3 and tubulin were used as nuclear and non-nuclear protein controls, respectively. **C,** NPR1:GFP localization in leaf cells and primary root cells.


a
Input
tga2/tga5/tga6
Col
HAC1:HA
NPR1:GFP
HAC1:HA
NPR1:GFP
tga2/tga5/tga6
Col
HAC1:HA
NPR1:GFP
HAC1:HA
NPR1:GFP
NPR1:GFP
100 KD
α-GFP
HAC1:HA
190 KD
α-HA
TGA2/5
37 KD
α-TGA2/5
IP: α-GFP
NPR1:GFP
100 KD
α-GFP
HAC1:HA
190 KD
α-HA
TGA2/5
37 KD
α-TGA2/5
-INA
+INA
b
Input
tga2/tga5/tga6
Col
HAC1:HA
NPR1:GFP
HAC1:HA
NPR1:GFP
tga2/tga5/tga6
Col
HAC1:HA
NPR1:GFP
HAC1:HA
NPR1:GFP
HAC1:HA
190 KD
α-HA
NPR1:GFP
100 KD
α-GFP
TGA2/5
37 KD
α-TGA2/5
IP: α-HA
HAC1:HA
190 KD
α-HA
NPR1:GFP
100 KD
α-GFP
TGA2/5
37 KD
α-TGA2/5
-INA
+INA
c
Input
Col
HAC1:HA
35S::NPR1:GFP
HAC1:HA
35S::NPR1:GFP
tga2/tga5/tga6
Col
HAC1:HA
35S::NPR1:GFP
HAC1:HA
35S::NPR1:GFP
tga2/tga5/tga6
HAC1:HA
190 KD
α-HA
NPR1:GFP
100 KD
α-GFP
IP: α-HA
HAC1:HA
90 KD
α-HA
NPR1:GFP
100 KD
α-GFP
-INA
+INA
d
Input
Col
HAC1:HA
35S::NPR1:GFP
HAC1:HA
35S::NPR1:GFP
tga2/tga5/tga6
Col
HAC1:HA
35S::NPR1:GFP
HAC1:HA
35S::NPR1:GFP
tga2/tga5/tga6
NPR1:GFP
100 KD
α-GFP
HAC1:HA
190 KD
α-HA
IP: α-GFP
NPR1:GFP
100 KD
α-GFP
HAC1:HA
190 KD
α-HA
-INA
+INA

e
Input
NPR1:GFP 100 KD
TGA2/5 37KD
IP: α-GFP
Col
35S::NPR1:GFP
hac1-2 hac5-2
α-GFP
α-TGA2/5
-INA
+INA
f
Input
HAC1:HA 190 KD
TGA2/5 37 KD
IP: α-HA
Col
HAC1:HA
npr1-1
α-HA
α-TGA2/5
g
Col +INA
HAC1:HA +INA
HAC1:HA in npr1-1 +INA
α-HA
PR1
UBQ11
Relative enrichment
P2
P3
h
Col -INA
35S::NPR1:GFP -INA
hac1-2/5-2 -INA
Col +INA
35S::NPR1:GFP +INA
hac1-2/5-2 +INA
α-GFP
PR1
UBQ11
Relative enrichment
P2
P3
P4
P5

Figure 2-6 *In vivo* interaction of HAC1 with NPR1 and TGA2/5. a, **b,** Co-IP analyses showing the interaction of HAC1 with NPR1 and TGA2/5, TGA2/5/6-independent HAC1-NPR1 interaction (**c**, **d**), HAC1/5-independent NPR1-TGA2/5 interaction (**e**), and NPR1-dependent HAC1-TGA2/5 interaction (**f**). Proteins prepared from 4-week-old plants treated with water or 300 μM INA for 12 hours before harvest were IPed and immunoblotted with indicated antibodies. Col and *tga2/5/6* were used as negative controls for co-IP assays. **g**, NPR1-dependent targeting of HAC1 to *PR1*. **h**, Reduced NPR1 targeting to *PR1* by *hac1/5* mutations. ChIP-qPCR was performed with indicated antibody and the level of untagged (**g**) or untagged and untreated Col (**h**) was set to 1 after normalization by corresponding input. Means ± SE of three biological experiments performed in triplicates are shown (**g**, **h**).

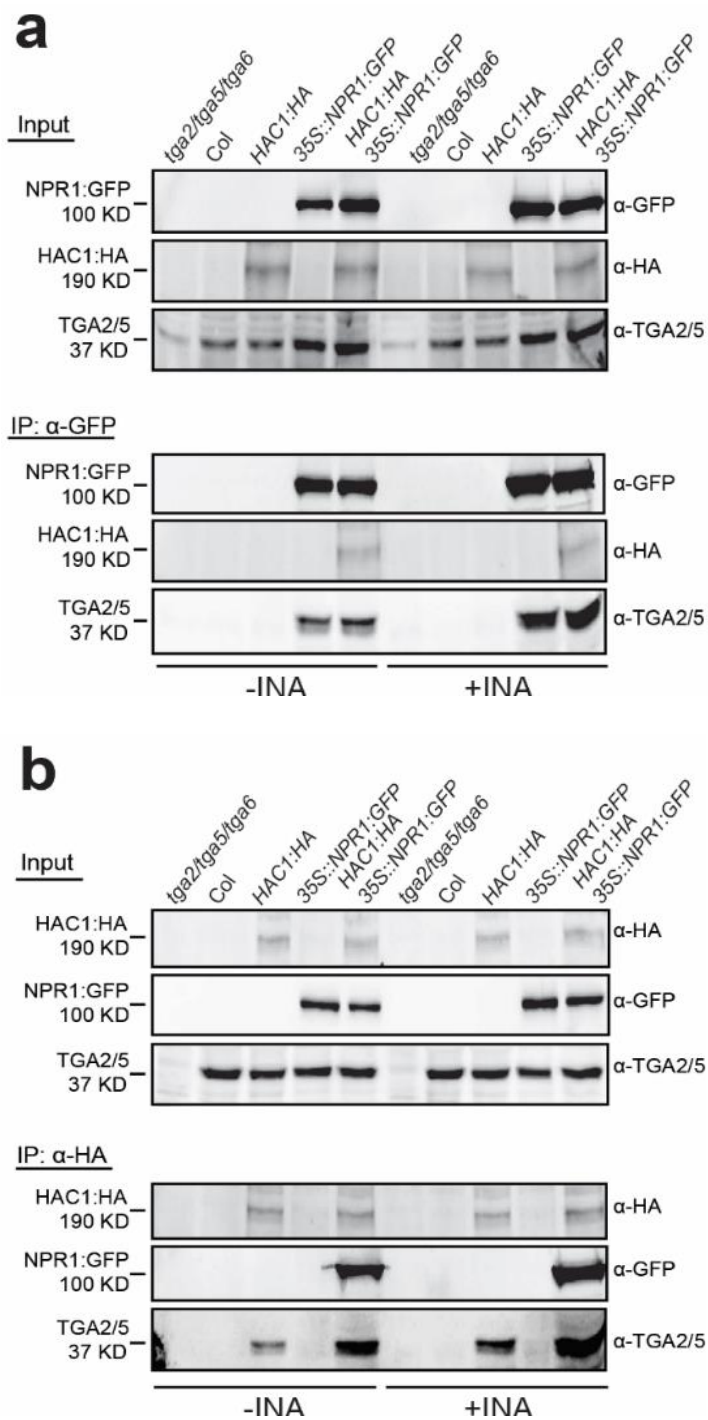
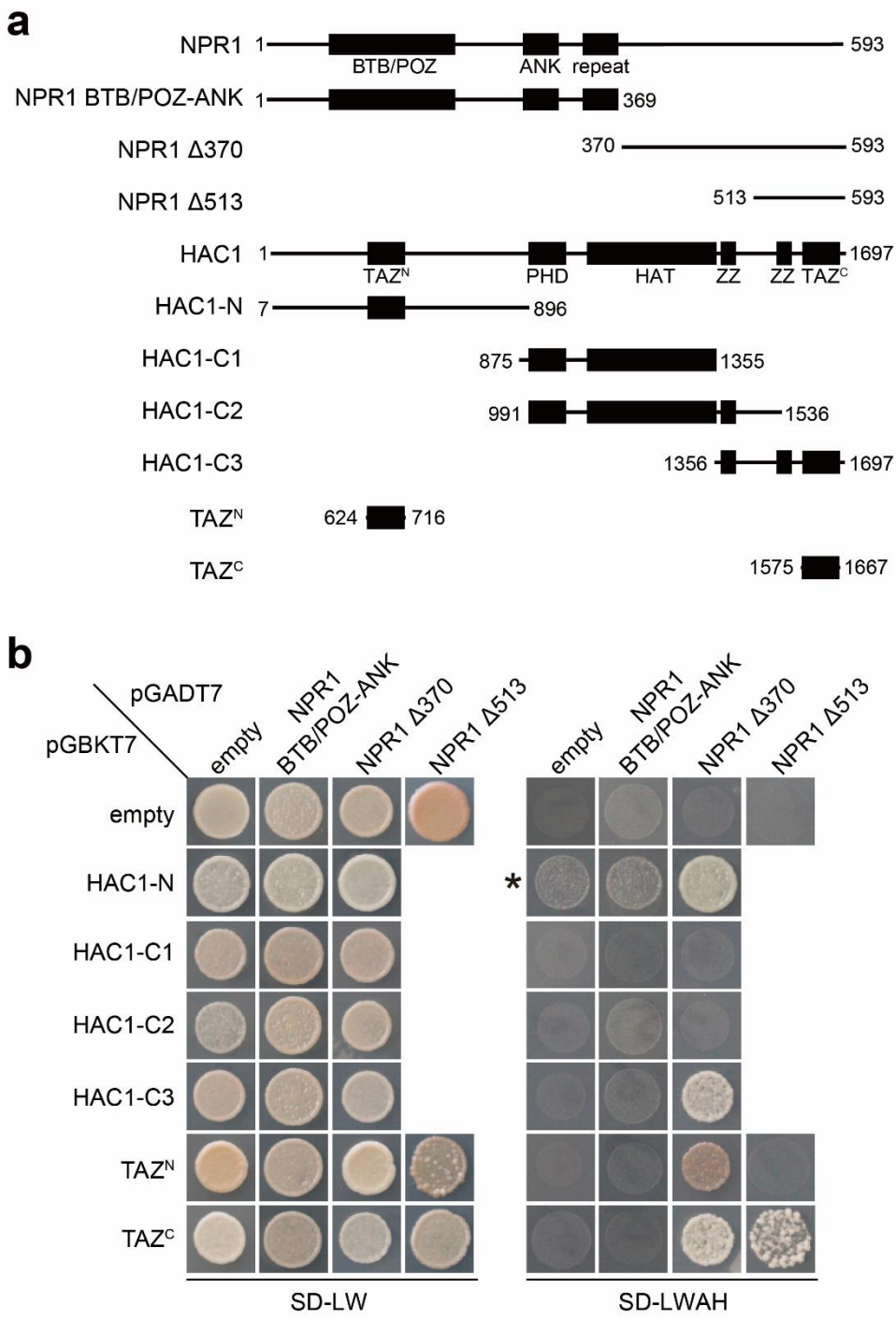

a
Input
tga2/tga5/tga6
Col
HAC1:HA
35S::NPR1:GFP
HAC1:HA
35S::NPR1:GFP
tga2/tga5/tga6
Col
HAC1:HA
35S::NPR1:GFP
HAC1:HA
35S::NPR1:GFP
NPR1:GFP
100 KD
α-GFP
HAC1:HA
190 KD
α-HA
TGA2/5
37 KD
α-TGA2/5
IP: α-GFP
NPR1:GFP
100 KD
α-GFP
HAC1:HA
190 KD
α-HA
TGA2/5
37 KD
α-TGA2/5
-INA
+INA
b
Input
tga2/tga5/tga6
Col
HAC1:HA
35S::NPR1:GFP
HAC1:HA
35S::NPR1:GFP
tga2/tga5/tga6
Col
HAC1:HA
35S::NPR1:GFP
HAC1:HA
35S::NPR1:GFP
HAC1:HA
190 KD
α-HA
NPR1:GFP
100 KD
α-GFP
TGA2/5
37 KD
α-TGA2/5
IP: α-HA
HAC1:HA
190 KD
α-HA
NPR1:GFP
100 KD
α-GFP
TGA2/5
37 KD
α-TGA2/5
-INA
+INA

Figure 2-7 *In vivo* interactions among HAC1, NPR1, and TGA2/5. Proteins prepared from *tga2/5/6*, Col, *HAC1:HA*, *35S::NPR1:GFP*, and *HAC1:HA 35S::NPR1:GFP* plants were immunoprecipitated with anti-GFP (**a**) or anti-HA (**b**) antibody and immunoblotted with anti-GFP, anti-HA, or anti-TGA2/5 antibody.


a
NPR1
1
593
BTB/POZ
ANK repeat
NPR1 BTB/POZ-ANK
1
369
NPR1 Δ370
370
593
NPR1 Δ513
513
593
HAC1
1
1697
TAZN
PHD
HAT
ZZ
ZZ TAZC
HAC1-N
7
896
HAC1-C1
875
1355
HAC1-C2
991
1536
HAC1-C3
1356
1697
TAZN
624
716
TAZC
1575
1667
b
pGADT7
pGBKT7
empty
NPR1 BTB/POZ-ANK
NPR1 Δ370
NPR1 Δ513
empty
NPR1 BTB/POZ-ANK
NPR1 Δ370
NPR1 Δ513
empty
HAC1-N
HAC1-C1
HAC1-C2
HAC1-C3
TAZN
TAZC
*
SD-LW
SD-LWAH

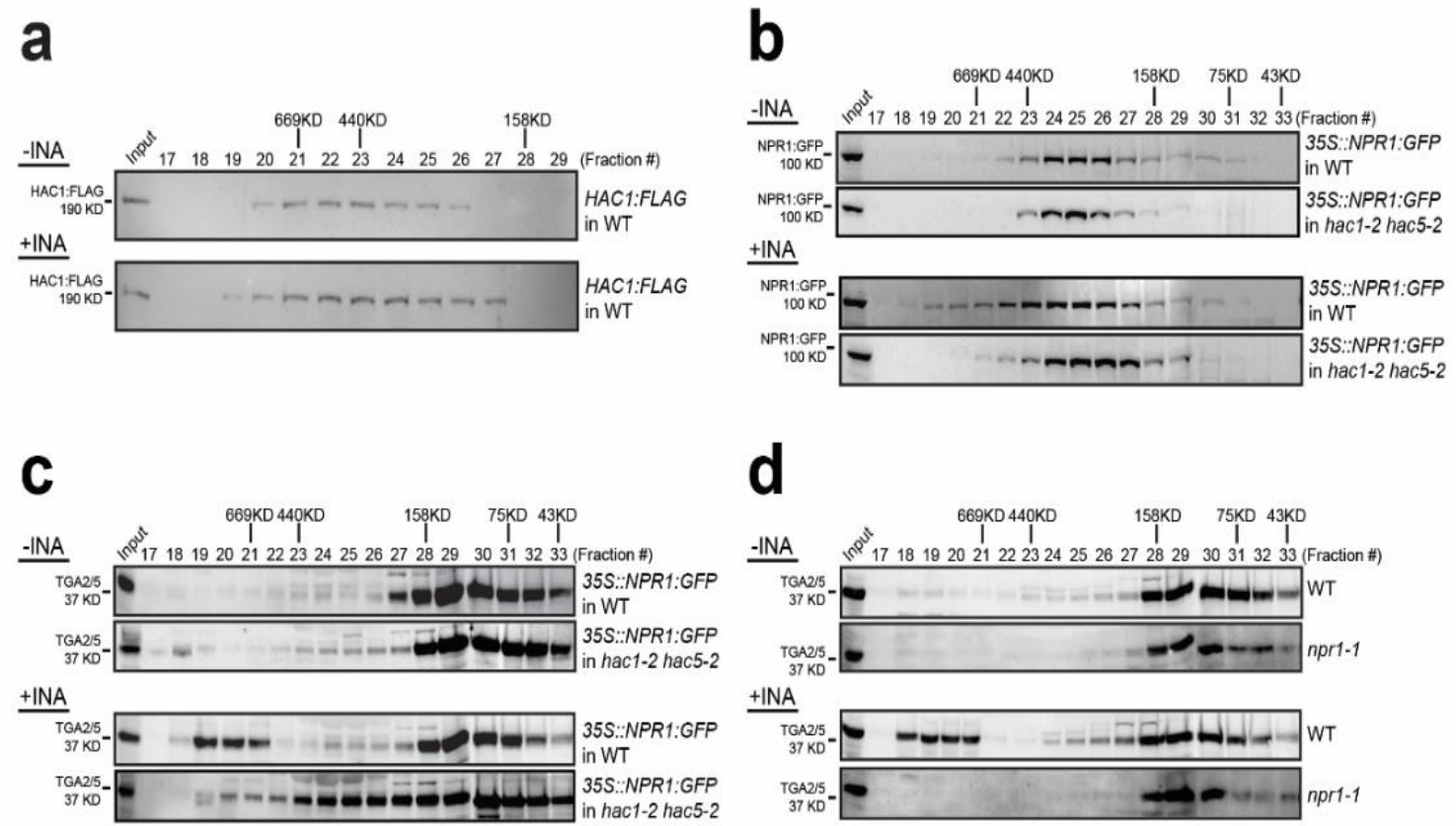
(By Se-Hun Yun)

Figure 2-8 Interaction between HAC1 and NPR1 in yeast. a, Schematics of NPR1 and HAC1 deletions used for Yeast-Two-Hybrid assays. HAC1 deletions were fused to the GAL4 DNA-binding domain and the NPR1 deletions were fused to the GAL4 activation domain. **b**, Yeast transformants were grown on Leu^- Trp^- dropout media (SD-LW) or Leu^- Trp^- $adenine^-$ His^- dropout media (SD-LWAH) containing 1 mM 3-aminotriazole (3-AT) except for the case of HAC1-N as a bait (*) for which 3 mM 3-AT was used

a
PR1
Col +INA
HAC1:HA 35S::NPR1:GFP in WT +INA
HAC1:HA 35S::NPR1:GFP in tga2/5/6 +INA
α-HA
Relative enrichment
UBQ11
P2
P3
b
PR1
Col +INA
HAC1:HA 35S::NPR1:GFP in WT +INA
HAC1:HA 35S::NPR1:GFP in tga2/5/6 +INA
α-GFP
Relative enrichment
UBQ11
P2
P3
c
PR1
Col -INA
TGA2:FLAG 35S::NPR1:GFP hac1-2(+/-) hac5-2(-/-) -INA
TGA2:FLAG 35S::NPR1:GFP hac1-2(-/-) hac5-2(-/-) -INA
Col +INA
TGA2:FLAG 35S::NPR1:GFP hac1-2(+/-) hac5-2(-/-) +INA
TGA2:FLAG 35S::NPR1:GFP hac1-2(-/-) hac5-2(-/-) +INA
α-FLAG
Relative enrichment
UBQ11
P2
d
PR2
Col -INA
35S::NPR1:GFP -INA
35S::NPR1:GFP hac1-2 hac5-2 -INA
Col +INA
35S::NPR1:GFP +INA
35S::NPR1:GFP hac1-2 hac5-2 +INA
α-GFP
Relative enrichment
UBQ11
P1
P2
P3

Figure 2-9 Targeting of HAC1 and NPR1 to *PR1* chromatin requires TGA2/5/6.

a, **b**, ChIP assays showing TGA2/5/6-dependent INA-induced association of HAC1:HA (**a**) and NPR1:GFP (**b**) with *PR1* chromatin. **c**, HAC1/5-independent TGA2/5 targeting to *PR1* chromatin. **d**, Reduced targeting of NPR1:GFP to *PR2* chromatin by *hac1/5* mutations. An asterisk indicates statistically significant difference ($P < 0.05$ in a Student's t-test) between *35S::NPR1:GFP* + INA and *35S::NPR1:GFP hac1-2 hac5-2* + INA. Shown are means ± SE of three independent ChIP experiments performed in triplicates. Either treated (**a**, **b**) or untreated (**c**, **d**) Col levels were set to 1 after normalization by input.


a
669KD 440KD 158KD
-INA
Input 17 18 19 20 21 22 23 24 25 26 27 28 29 (Fraction #)
HAC1:FLAG 190 KD
HAC1:FLAG in WT
+INA
HAC1:FLAG 190 KD
HAC1:FLAG in WT
b
669KD 440KD 158KD 75KD 43KD
-INA
Input 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 (Fraction #)
NPR1:GFP 100 KD
35S::NPR1:GFP in WT
NPR1:GFP 100 KD
35S::NPR1:GFP in hac1-2 hac5-2
+INA
NPR1:GFP 100 KD
35S::NPR1:GFP in WT
NPR1:GFP 100 KD
35S::NPR1:GFP in hac1-2 hac5-2
c
669KD 440KD 158KD 75KD 43KD
-INA
Input 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 (Fraction #)
TGA2/5 37 KD
35S::NPR1:GFP in WT
TGA2/5 37 KD
35S::NPR1:GFP in hac1-2 hac5-2
+INA
TGA2/5 37 KD
35S::NPR1:GFP in WT
TGA2/5 37 KD
35S::NPR1:GFP in hac1-2 hac5-2
d
669KD 440KD 158KD 75KD 43KD
-INA
Input 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 (Fraction #)
TGA2/5 37 KD
WT
TGA2/5 37 KD
npr1-1
+INA
TGA2/5 37 KD
WT
TGA2/5 37 KD
npr1-1

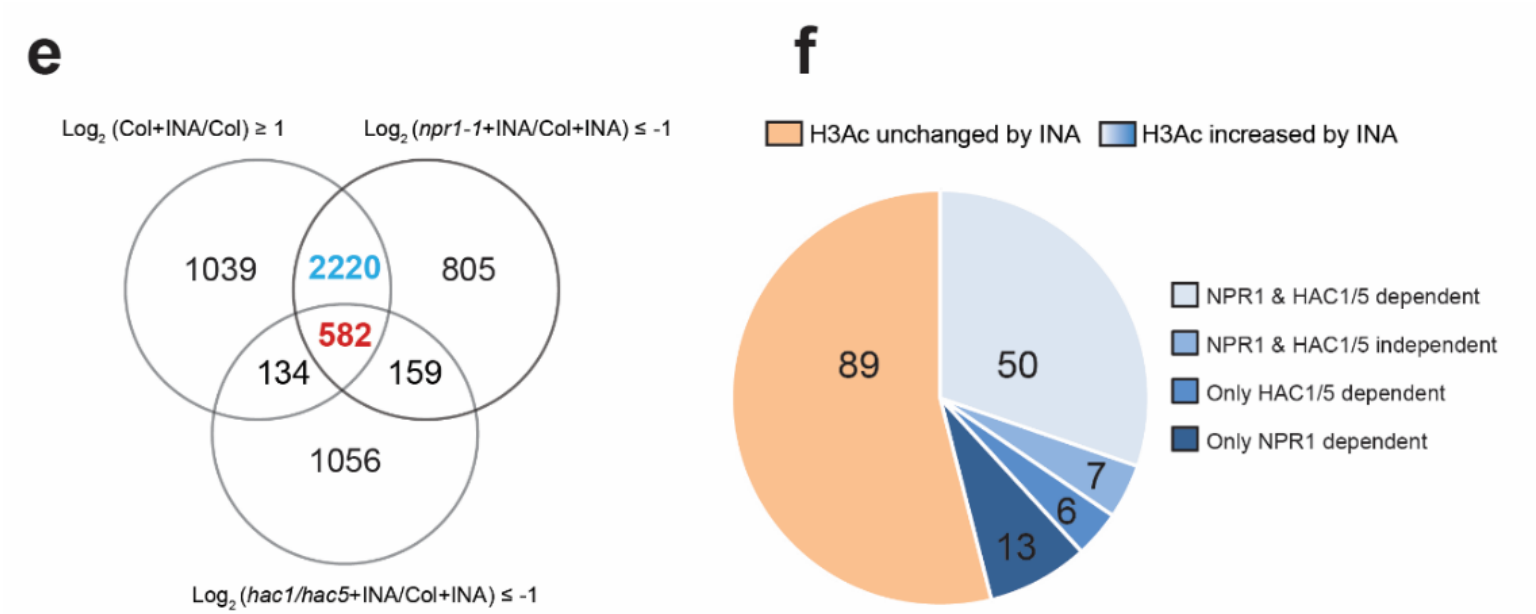
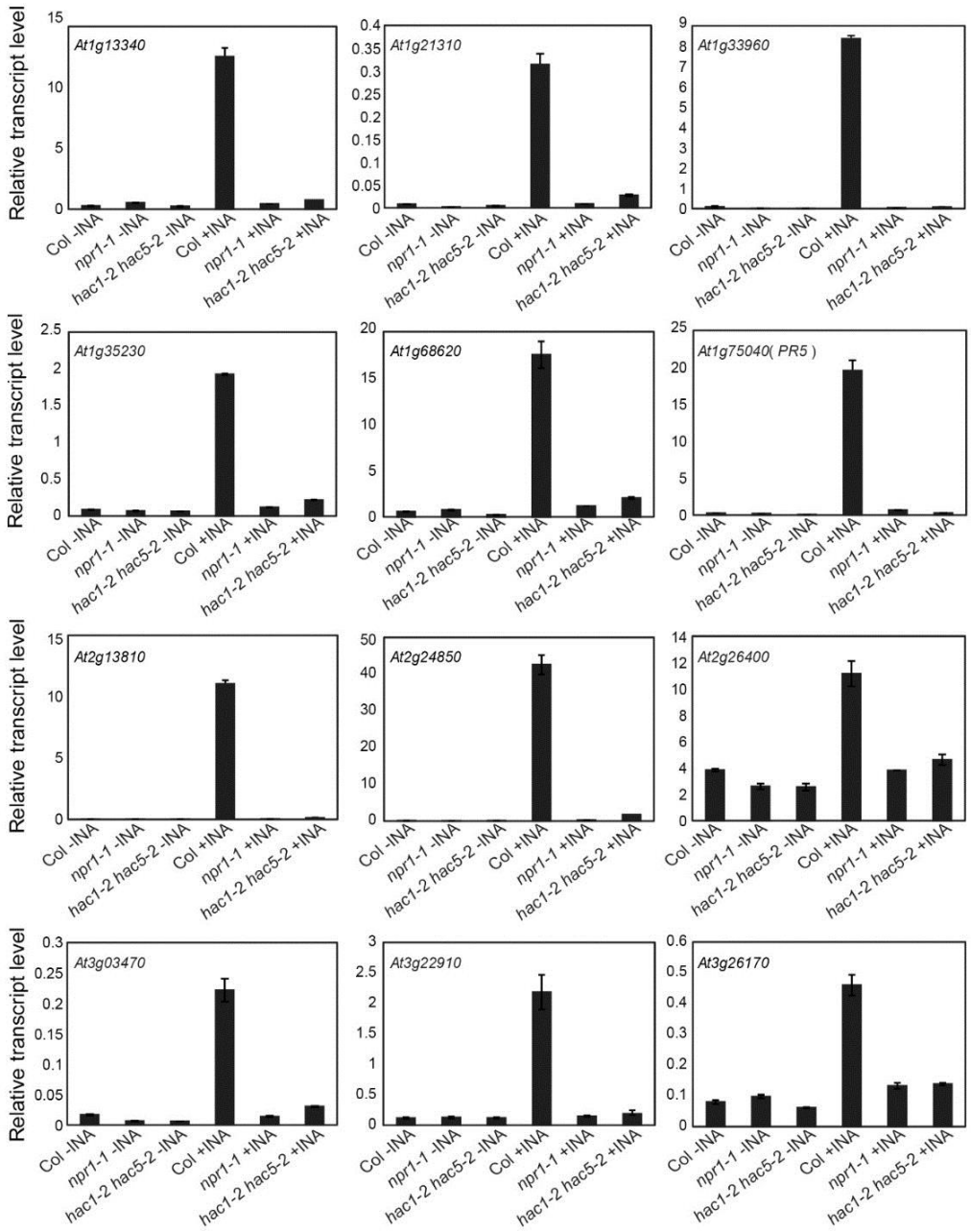
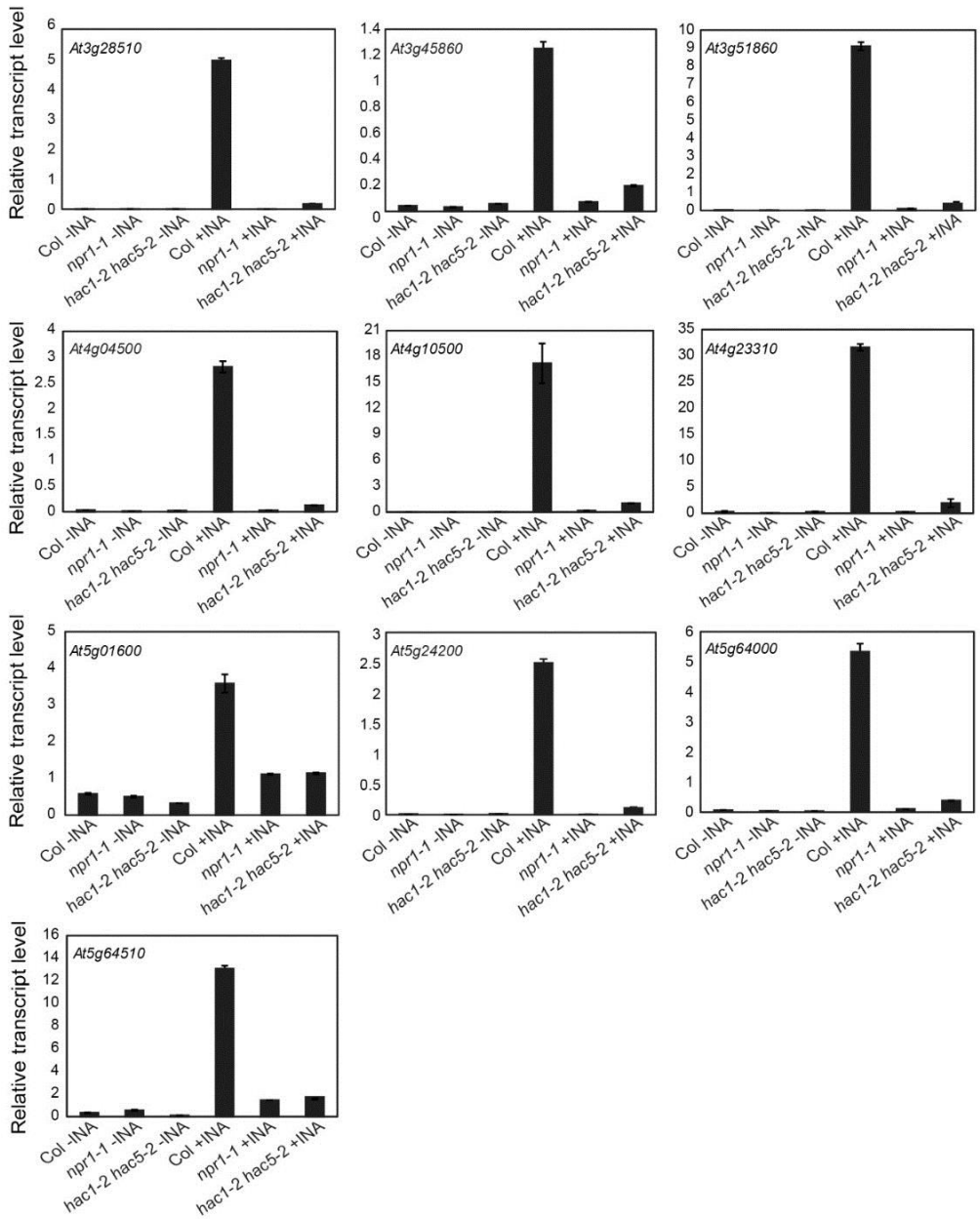

e
Log2 (Col+INA/Col) ≥ 1
Log2 (npr1-1+INA/Col+INA) ≤ -1
1039
2220
805
582
134
159
1056
Log2 (hac1/hac5+INA/Col+INA) ≤ -1
f
H3Ac unchanged by INA
H3Ac increased by INA
89
50
7
6
13
NPR1 & HAC1/5 dependent
NPR1 & HAC1/5 independent
Only HAC1/5 dependent
Only NPR1 dependent

Figure 2-10 Fractionation of the HAC-NPR1-TGA complex and its role in the regulation of SA-induced transcriptome and epigenome dynamics. a-d, Immunoblot analysis of FPLC fractions. Proteins from *HAC1:FLAG* (**a**), *35S::NPR1:GFP* or *35S::NPR1:GFP* in *hac1/5* (**b**, **c**), and WT or *npr1* (**d**) plants were fractionated by FPLC and subjected to immunoblot analyses with indicated antibodies. Molecular-weight standards used (thyroglobulin (660 KD), ferritin (440 KD), and aldolase (158 KD)) were co-fractionated with proteins. **e**, Venn diagram illustrating number of genes induced by INA (FDR $\leq$ 0.2). The number of genes co-regulated by NPR1 and HAC1/5 (Group 1) or regulated by NPR1 only (Group 2) is indicated by red or blue, respectively. **f**, Pie-chart showing the proportion of further selected Group 1-gene loci (see text) with or without increased H3Ac after INA treatment.

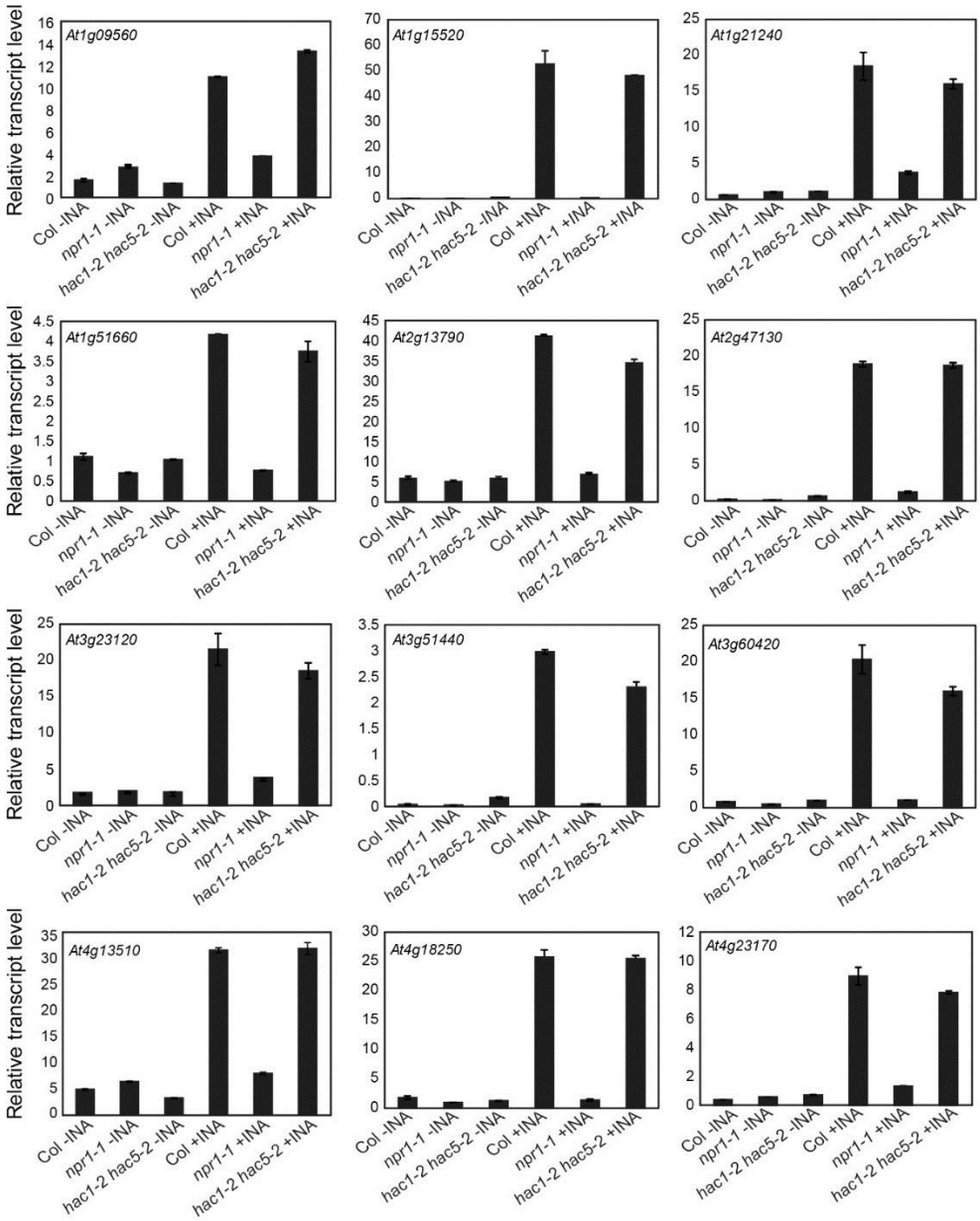


(By Sun-Mee Choi)

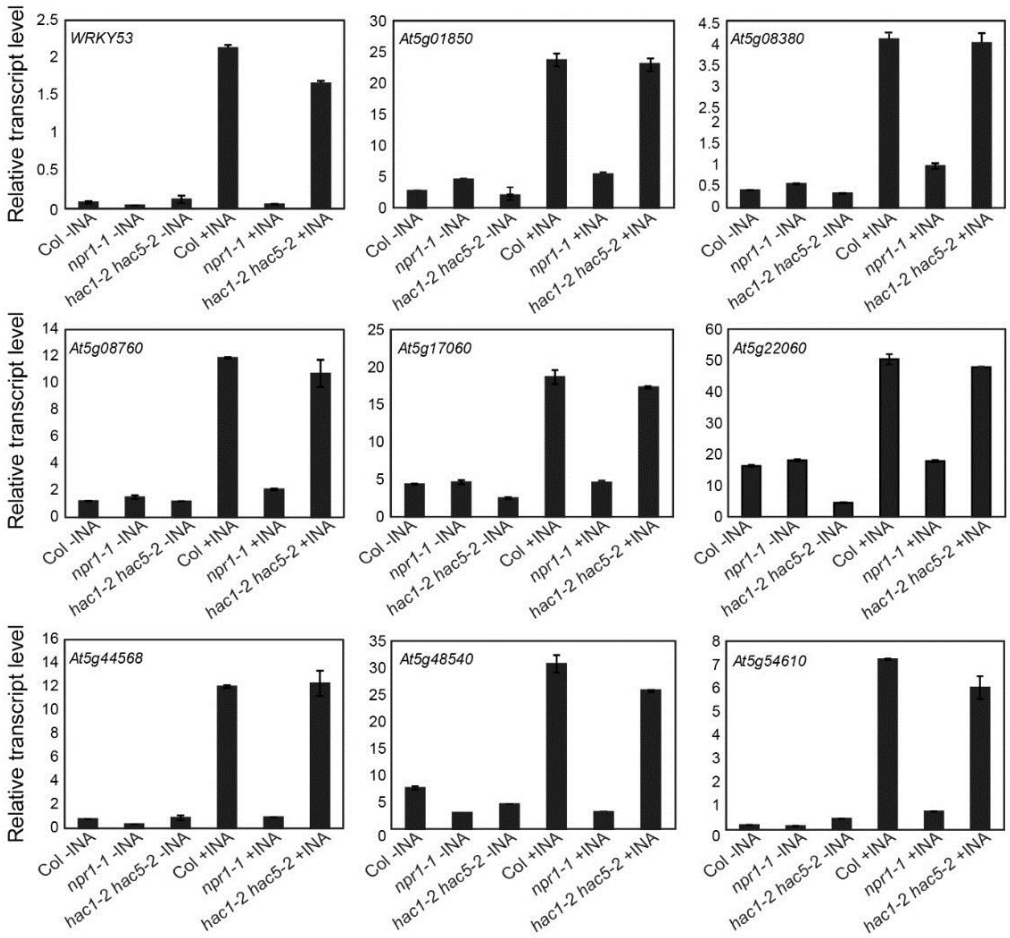


(By Sun-Mee Choi)

Figure 2-11 RT-qPCR analysis of randomly selected 22 Group 1-gene expression in Col, *npr1-1*, and *hac1-2 hac5-2* treated with INA or not. Means ± SD of duplicates are shown after normalization to *Tubulin*.



(By Sun-Mee Choi)



(By Sun-Mee Choi)

Figure. 2-12 RT-qPCR analysis of randomly selected 21 Group 2-gene expression in Col, *npr1-1*, and *hac1-2 hac5-2* treated with INA or not. Means ± SD of duplicates are shown after normalization to *Tubulin*.

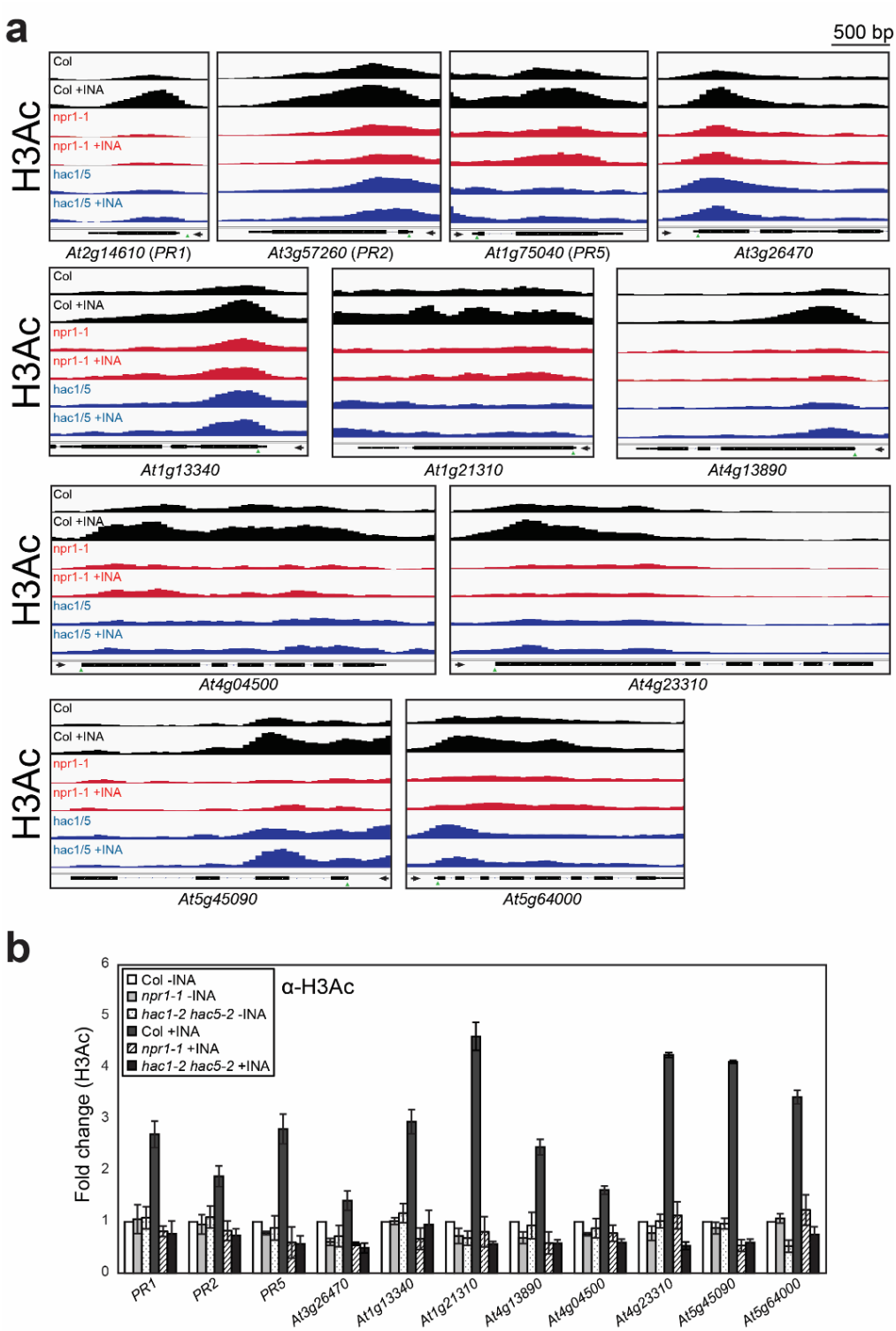

a
500 bp
H3Ac
Col
Col +INA
npr1-1
npr1-1 +INA
hac1/5
hac1/5 +INA
At2g14610 (PR1)
At3g57260 (PR2)
At1g75040 (PR5)
At3g26470
At1g13340
At1g21310
At4g13890
At4g04500
At4g23310
At5g45090
At5g64000
b
α-H3Ac
Col -INA
npr1-1 -INA
hac1-2 hac5-2 -INA
Col +INA
npr1-1 +INA
hac1-2 hac5-2 +INA
Fold change (H3Ac)
0
1
2
3
4
5
6
PR1
PR2
PR5
At3g26470
At1g13340
At1g21310
At4g13890
At4g04500
At4g23310
At5g45090
At5g64000

Figure 2-13 Visualization and confirmation of H3Ac ChIP-seq data. a, Integrative Genomics Viewer (IGV) snapshot images of H3Ac ChIP-seq data for 11 selected Group 1-gene loci in Col (Black), *npr1-1* (red), and *hac1-2 hac5-2* (blue) plants either treated with INA or not. **b**, ChIP-qPCR analysis of H3Ac levels for the 11 loci shown in (**a**). The region in each locus tested for ChIP-qPCR is indicated with green triangle within the schematics in (**a**). Means ± SE of triplicates are shown after normalization to input and Col-INA level.

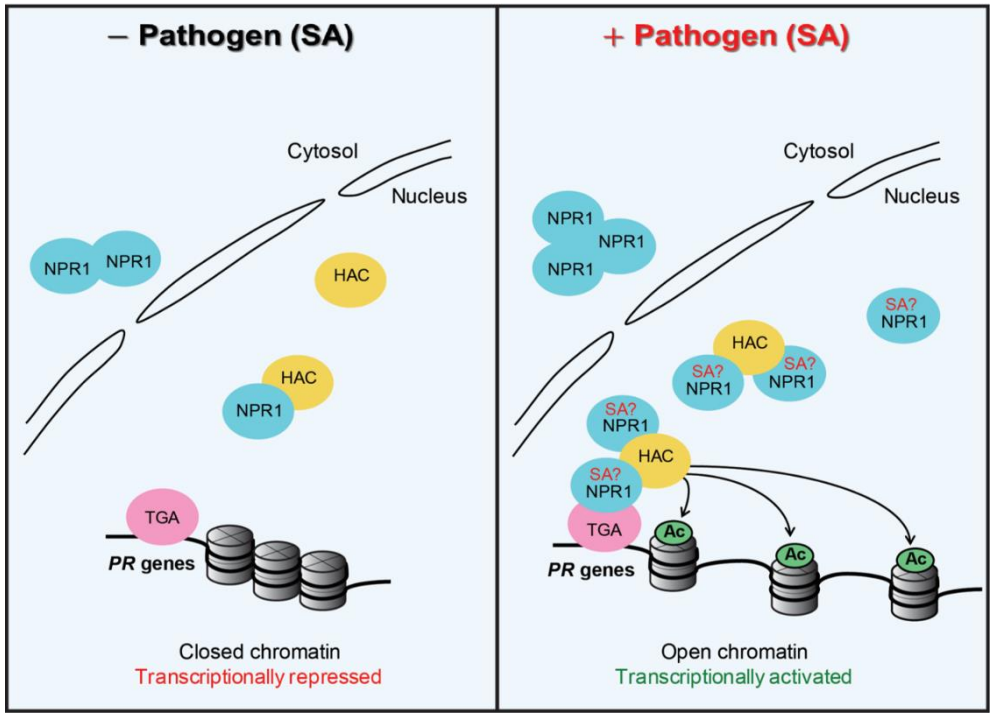

– Pathogen (SA)
Cytosol
Nucleus
NPR1
NPR1
HAC
HAC
NPR1
TGA
PR genes
Closed chromatin
Transcriptionally repressed
+ Pathogen (SA)
Cytosol
Nucleus
NPR1
NPR1
NPR1
SA?
NPR1
HAC
SA?
NPR1
SA?
NPR1
SA?
NPR1
HAC
SA?
NPR1
TGA
Ac
Ac
Ac
PR genes
Open chromatin
Transcriptionally activated

Figure 2-14 Model for the epigenetic reprogramming of *PR* genes by the HAC-NPR1-TGA complex. Under normal condition (left), NPR1 (blue oval) preferentially presents within the cytoplasm as oligomers while its minor fraction is within the nucleus and interacts with HAC (yellow oval). Class II TGA transcription factors (pink oval) bind to *PR* promoters and repress *PR* transcription. Upon pathogen challenge and following SA surge (right), cytoplasmic NPR1 is monomerized, translocated into the nucleus, and interacts with HAC possibly in a multiple:one fashion. The HAC-NPR1 complex is recruited to *PR* promoters through the interaction between NPR1 and TGA, and the resulting HAC-NPR1-TGA complex induces transcriptional activation through histone acetylation (Ac)-dependent chromatin reprogramming. SA may or may not directly induce the formation of the HAC-NPR1-TGA complex during this process.

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국문 초록

식물은 세균성 또는 바이러스성 병원성 공격에 따라 수많은 면역 관련 유전자를 대량 발현시켜 면역 반응을 일으키고, 따라서 면역시스템이 시작된다. 식물이 병원균에 감염되었을 때 방어를 위한 방어메커니즘이 작동되며 면역반응은 감염부위에서 (살리실산) SA 축적을 일으킴으로써 시작된다. SA는 다양한 병원균에 의한 연속적인 공격으로부터 "식물전신"을 보호하기 위해 식물 원위조직 전체에서 축적된다. 이러한 "식물전신"반응 저항성을 전신취득저항성 (SAR)이라고 한다. SA는 핵심 면역 조절자인 NPR1을 활성화시키고, TGA라는 전사 조절 인자에 의해 NPR1은 면역방어유전자 *PR* 유전자 좌로 유도된다. NPR1은 SA에 의해 유발되는 면역시스템에서 중요한 전사 보조활성제로 작용함에도 불구하고, 생화학적 기작의 다양성은 현재까지 많은 연구가 되지 않았다.

후성유전학은 DNA 서열이 변화되지 않으면서 유전자 발현이 유전적으로 변화되는 학문분야이다. 히스톤 아세틸화는 N-말단 히스톤 꼬리의 라이신 잔기에서 co-activator 인 아세틸화효소(HATs)에 의해 아세틸기 ($COCH_3$)를 아세틸-CoA 로부터 NH_3^+ 아미노 그룹으로 전달하므로 일어나는 후성학적 변형이다.

최근 연구에 의하면 애기장대에서 CBP/p300 HAC 단백질은 히스톤 아세틸화효소 활성능력을 보유하고 있다. 또한 ZnF-TAZ 와

ZnF-ZZ 의 두가지 유형의 도메인을 보유하고 있으며 단백질-단백질 간 상호 작용을 매개하는 데 중요한 역할을 한다. HACs 에 의한 후성학적 조절은 애기장대 개화시기, 식물생장 및 에틸렌 신호전달 경로에 영향을 준다고 알려져 있다.

본 연구는 애기장대에서 SA 의존적 면역 반응이 일어나는 동안, HAC1 이 NPR1 과 TGA 전사인자와 그룹을 이루어 *PR* 유전자 좌에 유도되고 HAC1 와 HAC5 은 히스톤 아세틸화 효소로서, 히스톤을 아세틸화 하여 *PR* 유전자 발현을 촉진시킨다는 것을 규명하였다. 따라서 NPR1 의 co-activator 로서의 HAC1 기능과 NPR1 이 매개하는 전사 활성의 정확한 생화학적 메커니즘을 규명하였다. 또한, 후성학적 리프로그래밍은 식물 면역계의 필수적인 부분으로 작용하여 식물이 병원성 공격에 대해 효율적으로 방어할 수 있다는 것을 알 수 있다.

주요어: CBP/p300, 히스톤 아세틸화, HAC1, NPR1, SA, 면역시스템, 병원균.

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