The Plant Cell, Vol. 23: 2809–2820, August 2011, www.plantcell.org

REVIEW

Transcription Dynamics in Plant Immunity

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Plant cells maintain sophisticated gene transcription programs to regulate their development, communication, and response to the environment. Environmental stress cues, such as pathogen encounter, lead to dramatic reprogramming of transcription to favor stress responses over normal cellular functions. Transcription reprogramming is conferred by the concerted action of myriad transcription (co)factors that function directly or indirectly to recruit or release RNA Polymerase II. To establish an effective defense response, cells require transcription (co)factors to deploy their activity rapidly, transiently, spatially, and hierarchically. Recent findings suggest that in plant immunity these requirements are met by posttranslational modifications that accurately regulate transcription (co)factor activity as well as by sequential pulse activation of specific gene transcription programs that provide feedback and feedforward properties to the defense gene network. Here, we integrate these recent findings from plant defense studies into the emerging field of transcription dynamics in eukaryotes.

INTRODUCTION

Plants are continuously exposed to numerous assaults by different plant pathogens. To counteract the actions of invading pathogens, plants must rapidly recognize the presence of these pathogens and initiate an immune response that is timely, accurate, and effective. Activation of immunity is accomplished by the action of a multitude of transcriptional regulators that reprogram the transcriptome to favor defense responses over routine cellular requirements. The activity of these transcriptional regulators is orchestrated by a blend of signaling hormones of which salicylic acid (SA), jasmonic acid (JA), and ethylene are particularly important (Pieterse et al., 2009). These signaling hormones regulate specific and overlapping sets of genes that are induced and/or repressed by the action of transcriptional regulators.

The plant's arsenal of transcriptional regulators consists not only of DNA binding transcription factors that function as activators and repressors, but also of cofactors that do not physically associate with the DNA but coactivate or corepress transcription through interaction with DNA binding transcription factors. To efficiently fend off pathogen attack, activation of plant immunity must be rapid and involve massive transcriptional reprogramming of thousands of genes. Thus, as the prime executioners of gene expression, transcription (co)factors must meet several key requirements. First, transcription (co)factors must be able to rapidly perceive the signal relayed by signaling hormones and translate it into a functional response. Therefore, the mechanisms by which transcription (co)factors perceive a signal are likely to be the same or tightly linked to the mechanisms that translate it into a functional response. Second, transcription factors must be able to rapidly locate their cognate DNA binding motif, whereas cofactors that do not directly bind to DNA must swiftly recognize the correct chromatin site at which they are required. Third, while transcription (co)repressors must efficiently suppress the recruitment of RNA Polymerase II (RNAPII), transcription (co)activators must be capable of recruiting RNAPII to the target promoter in a controlled fashion. RNAPII recruitment should be timely and executed numerous times to produce abundant mRNAs according to the intensity or frequency of the upstream signal. Finally, transcription (co)factors must be able to function within larger networks, in that they should function cooperatively or antagonistically to regulate the expression of gene networks that contain feedforward and feedback loops.

Do transcription (co)factors of the plant immune system conform to the above mechanistic requirements? During recent years a number of innovative works have revealed the composition and complex integrated nature of the transcriptional mechanisms that form the regulatory networks comprising plant immunity. In this review, we will discuss these findings in the context of the above transcription (co)factor requirements. Consequently, a discussion of all transcriptional events in plant immunity is beyond the scope of this review. Instead, we will focus on particular transcription (co)factors that have been studied in greater detail, allowing an assessment of their mechanistic requirements. Moreover, we will place particular emphasis on well-studied transcriptional responses orchestrated by SA. As it is becoming increasingly clear that SA communicates with other hormones to fine-tune its action in plant immunity, selected components from other signaling pathways will also be highlighted. Where possible we strive to complement these with reference to excellent, comprehensive reviews detailing the latest knowledge within the respective fields.

SIGNAL PERCEPTION BY TRANSCRIPTION REGULATORS

Many transcription (co)activators of the plant immune response are already abundantly expressed in the absence of pathogen

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www.plantcell.org/cgi/doi/10.1105/tpc.111.087346

threat. To prevent autoimmunity, these (co)activators must by default be either inactive or kept away from chromatin. Several mechanisms have now emerged by which plant cells signal transcription (co)activators to switch from inactive to active states.

Perception of Ca^{2+} Signals

Recent work identifies Ca^{2+} signaling as a central modulator of SA-dependent transcription dynamics. Numerous studies clearly observed rapid transient fluxes in Ca^{2+} accumulation upon activation of plant immune responses (Lecourieux et al., 2006; Ma and Berkowitz, 2007). The regulatory function of Ca^{2+} is performed by an array of Ca^{2+} binding proteins that are thought to orchestrate protein activity. In *Arabidopsis thaliana*, several immune-activated transcription factors that bind Ca^{2+} and are subsequently regulated by this metal have been identified.

Signal Responsive1 (SR1) is a Ca^{2+}/calmodulin binding transcription factor that was shown to be involved in modulating pathogen-induced accumulation of SA. In comparison to wild-type plants, an *sr1-1* loss-of-function mutant displayed increased accumulation of transcripts for the SA-synthesis related genes *Enhanced Disease Susceptible1* (*EDS1*), *EDS5*, *Phytoalexin Deficient4* (*PAD4*), and *Isochorismate Synthase1* (*ICS1*) and accordingly exhibited elevated levels of SA (Du et al., 2009). Of these genes only the *EDS1* gene promoter contained a typical conserved CGCG box to which SR1 was shown to bind and repress *EDS1* gene transcription (Figure 1A). Importantly, in comparison to wild-type plants, *sr1-1* plants show nonsynchronous and accelerated accumulation of SA in response to virulent pathogen challenge, suggesting that SR1 transcription repressor activity may function as a modulator of the kinetics of SA synthesis (Du et al., 2009).

Calmodulin Binding Protein 60-like.g (CBP60g) and Systemic Acquired Resistance Deficient1 (SARD1) function as positive regulators of plant immunity and appear to perform overlapping regulatory roles in pathogen-induced SA accumulation (Wang et al., 2009; Zhang et al., 2010). CBP60g and SARD1 are members of the same protein family, share 39% sequence identity, and possess a highly conserved DNA binding domain. Despite this homology, the regulatory properties of these proteins appear distinct. Activation of CBP60g requires binding of Ca^{2+}/calmodulin to its N-terminal domain, a feature not shared with SARD1. Rather, responses attributed to SARD1 correlate with an increase in mRNA transcript abundance for this gene. Overexpression of SARD1, but not CBP60g, dramatically increases total SA levels and leads to constitutive expression of the pathogenesis-related (PR) marker genes *PR-1* and *PR-2* genes. Chromatin immunoprecipitation established that following pathogen challenge both CBP60g and SARD1 are highly enriched and readily detectable at the promoter region of *ICS1*, encoding a key rate-limiting SA biosynthetic enzyme. Further genetic analysis showed that CBP60g and SARD1 are potent transcription activators of *ICS1* and thus play key regulatory roles in SA biosynthesis (Wang et al., 2009; Zhang et al., 2010). Taken together, these findings hint toward an important role for Ca^{2+} fluxes in the modulation of transcriptional regulator activity at two tiers (*EDS1* and *ICS1*) in the transcription cascade required to produce SA (Figure 1A). How transcriptional regulators decode the information in pathogen-induced Ca^{2+} signatures remains unknown, but binding of Ca^{2+} to their associated calmodulins predicts large conformational changes may underlie this process (Chou et al., 2001).

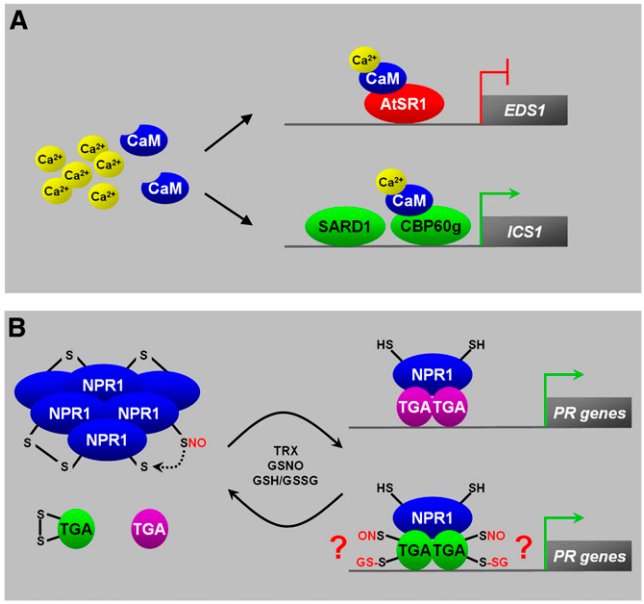

Figure 1. Calcium and Redox Transients Are Perceived by Immune Transcription (Co)factors in *Arabidopsis*.

(A) Ca^{2+}/calmodulin (CaM) binding to the repressor SR1 and the activator CBP60g regulate the SA biosynthetic genes *EDS1* and *ICS1*, respectively. ICS1 is also activated by the Ca^{2+}-independent SARD1 activator.
(B) Redox changes regulate the activity of the coactivator NPR1, which in the resting state forms intermolecular disulfide bonds to generate a high molecular weight, cytosolic oligomer. Oligomer formation is facilitated by *S*-nitrosoglutathione (GSNO)–induced *S*-nitrosylation (SNO). Upon activation of defense, part of the NPR1 oligomer is reduced to monomers by Thioredoxins (TRX) and translocates to the nucleus where it interacts with TGA transcription factors, resulting in activation of *PR* genes. Whereas some TGA factors do not require redox modifications for their activities (see purple TGA factors), others have been proposed to be regulated by reduction of an intramolecular disulfide bond (see green TGA factors), permitting interaction with NPR1. Moreover, in vitro experiments with the latter TGA factors suggest that *S*-nitrosylation and glutathione (GSH/GSSG)-induced *S*-glutathionylation (S-GS) may enhance their DNA binding activities.

Perception of Cellular Redox Changes

Pathogen attack has long been associated with changes in host cell redox. SA and JA, two phytohormones that accumulate after pathogen attack, as well as during infection with avirulent pathogens, previously were shown to impact the cellular redox buffer glutathione (Mateo et al., 2006; Koornneef et al., 2008; Spoel and Loake, 2011). SA not only elevated the cellular amount of glutathione, it also increased the ratio of reduced versus oxidized glutathione. JA, on the other hand, strongly decreased the glutathione pool in favor of the oxidized form (Spoel and Loake,

2011). Such cellular redox changes may be sensed by intrinsically reactive Cys residues in regulatory proteins. Depending on their local environment, Cys residues are highly nucleophilic and capable of accepting different levels of reversible, oxidative modifications that regulate protein function. Interestingly, proteome-wide studies in human cells suggest that surface-exposed, highly reactive Cys residues are relatively rare (Weerapana et al., 2010), adding to the specificity of redox-based posttranslational modifications in signaling processes. Several Cys-containing transcriptional regulators have recently been shown to translate pathogen-induced cellular redox changes into transcription programs in plant immunity.

Much of the transcription reprogramming associated with SA-dependent immune responses is attributable to the transcription coactivator Nonexpresser of *PR* genes1 (NPR1). NPR1 controls the expression of over 2200 immune-related genes in *Arabidopsis* and contains several Cys residues that are conserved between NPR1-like molecules in *Arabidopsis* and other plant species (Mou et al., 2003; Wang et al., 2006). Disulfide bonding between these Cys residues results in the formation of a stable, high molecular weight oligomer that is confined to the cytoplasm. In the relatively reduced cytoplasm, disulfide-mediated oligomerization of NPR1 is catalyzed by nitric oxide donors. Particularly the nitric oxide donor *S*-nitrosoglutathione was shown to trigger *S*-nitrosylation (i.e., the covalent attachment of nitric oxide to Cys thiols) of Cys-156 of NPR1, which facilitated disulfide bond formation and increased oligomer assembly (Tada et al., 2008). Upon pathogen challenge, accumulation of SA triggers transient cellular reduction that is sensed by Cys residues of the NPR1 oligomer. This transient redox change reduces NPR1 disulfide bonds with the help of the redox enzymes Thioredoxin 3 and 5, resulting in the release of NPR1 monomer that translocates to the nucleus to activate gene transcription (Kinkema et al., 2000; Mou et al., 2003; Tada et al., 2008). Thus, NPR1 functions as a sensor of cellular redox by linking redox changes to its molecular conformation, which directly impacts its nucleocytoplasmic localization and, thus, transcriptional activity (Figure 1B).

NPR1 is not the only redox sensor in plant immunity. Remarkably, TGA transcription factors that physically interact with NPR1 to form a transcription transactivating complex (Zhang et al., 1999; Zhou et al., 2000; Rochon et al., 2006; Boyle et al., 2009) are also regulated by cellular redox. Two redox-active Cys residues in TGA1 and TGA4 were shown to form a disulfide bond in resting cells, thereby prohibiting interaction with any available NPR1. Upon SA induction, this disulfide bond is reduced, which permits interaction with NPR1, an association that is hypothesized to induce defense gene transcription (Després et al., 2003). Adding to the complexity of redox sensing by TGA transcription factors, it was recently also shown that the above-mentioned Cys residues in recombinant protein are also subject to in vitro *S*-nitrosylation and *S*-glutathionylation (i.e., the covalent attachment of glutathione to Cys thiols). It was speculated that these posttranslational modifications may promote the transcriptional active state of TGA1 and TGA4 by protecting the two redox-active Cys residues from further oxidation and by enhancing DNA binding activity (Lindermayr et al., 2010) (Figure 1B). However, it remains to be determined if these modifications are functionally relevant in planta. Moreover, how cellular redox synchronizes the transcriptional activities of NPR1 and TGA1/4 remains unknown, but the local action of small redox molecules and redox enzymes may be essential in this process.

REGULATION OF CHROMATIN TARGETING OF TRANSCRIPTION (CO)ACTIVATORS

Eukaryotic transcription (co)factors are thought to locate their specific DNA targets by rapid diffusion through the nucleus and by local scanning or hopping of chromatin. Consequently, most of the cell's nuclear transcription (co)factors are probably, for the most part, associated with nontarget sites on chromatin (Hager et al., 2009). Emerging evidence in plants, however, indicates that transcription (co)activators of plant immunity are actively kept at bay from chromatin by two different molecular mechanisms.

Sequestration of (Co)activators Away from Chromatin

In plants, immune (co)activators have been shown to be sequestered in both the cytoplasm and nucleus to prevent them from untimely activating defense gene transcription. The basic leucine zipper (bZIP) transcription factor bZIP10 is an activator of defense genes involved in pathogen-induced cell death, a potent physiological response that isolates biotrophic pathogens, which require live tissues for proliferation. In resting cells, the zinc-finger protein Lesion Simulating Disease resistance1 (LSD1), a negative regulator of cell death that protects plants from reactive oxygen–triggered stress, interacts with bZIP10 and partially sequesters it in the cytoplasm, thereby curbing bZIP10 accumulation in the nucleus and preventing it from activating cell death responses. It has been hypothesized that the pathogen-induced reactive oxygen burst promotes dissociation of bZIP10 from LSD1, allowing it to translocate to the nucleus and activate immune-related gene expression (Kaminaka et al., 2006). This model is reminiscent of the mechanism by which the transcription activator Nuclear Factor κB (NF-κB) is controlled in animal innate immunity. In resting cells, NF-κB is sequestered in the cytoplasm by the inhibitory protein Inhibitor of κB (IκB). Immune activation leads to the phosphorylation and subsequent degradation of IκB, releasing NF-κB to activate target genes in the nucleus (Hayden and Ghosh, 2004). Unlike NF-κB in animals, however, plant bZIP10 does not exhibit exclusive cytoplasmic localization in resting plant cells but was also found in the nucleus. This suggests that a relative increase in nuclear bZIP10 abundance, rather than mutually exclusive nuclear or cytosolic localization, is necessary for transcription activation. A similar scenario was recently found for the immune regulator EDS1, a critical positive regulator of plant immunity that is localized to both the cytoplasm and nucleus where it forms complexes with other defense coregulators. Upon pathogen attack, part of the cytoplasmic EDS1 pool redistributes to the nucleus, an event that precedes EDS1-dependent gene regulation. In the nucleus, EDS1 may function as a transcription coactivator, as it was reportedly found to interact with transcription factors in yeast two-hybrid assays (García et al., 2010). Thus, similar to bZIP10, the nuclear presence of EDS1 in itself does

not activate gene transcription; rather, a relative increase in the amount of nuclear EDS1 is associated with transcription reprogramming. These findings suggest that nuclear import of immune (co)activators is a key process in establishing successful defense responses (García and Parker, 2009). Accordingly, mutation of the nucleoporin modifier of *snc1*,7, a subunit of the nuclear pore complex, compromises plant immunity due to decreased nuclear accumulation of immune regulators, including EDS1 and NPR1 (Cheng et al., 2009).

Transcription activators are not only sequestered in the cytosol, it appears they may also be kept away from chromatin within the nucleus. The transcription activator WRKY33 shows pathogen-inducible association with defense genes, including *PAD3*, an enzyme required for synthesis of the potent antimicrobial compound camalexin. In unchallenged cells, WRKY33 was shown to form a nuclear complex with the negative regulator Mitogen-Activated Protein Kinase 4 (MPK4) that is dependent on the MPK4 substrate MKS1, presumably titrating WRKY33 away from the chromatin. Upon pathogen infection, however, MPK4 dissociates from the MKS1-WRKY33 complex, which allows WRKY33 to activate defense gene transcription (Andreasson et al., 2005; Qiu et al., 2008). Likewise, the ethylene-responsive transcription factor ERF104 was shown to be sequestered in the nucleus by MPK6 (Bethke et al., 2009). In this case, ERF104 appeared to be a MPK6 substrate and its phosphorylation stabilized the protein. Pathogen perception and associated ethylene accumulation disrupt the ERF104-MPK6 interaction, presumably allowing ERF104 to bind to ethylene-responsive defense genes. Similarly, nuclear sequestration may also control the JA-inducible activator MYC2. The activity of MYC2 is curbed by members of the JAZ (Jasmonate ZIM-domain) family of repressor proteins. Accumulation of JA-Ile activates the ubiquitin ligase Skp1-Cullin1-Fbox^{COI1} (SCF^{COI1}), which targets JAZ proteins for proteasome-mediated degradation, liberating the MYC2 activation domain (Chini et al., 2007; Thines et al., 2007). Further research is required to determine if JAZ proteins sequester MYC2 away from its target sites or if the JAZ-MYC2 complex is constitutively associated with JA-inducible promoters (Hou et al., 2010; Pauwels et al., 2010). Notably, several different plant hormone signaling pathways are regulated in a comparable fashion to JA signaling (Santner and Estelle, 2009), suggesting that this type of gene activation switch is of central importance to plant biology.

Clearance of Transcription (Co)activators by Means of Destruction

Recently, another mechanism by which transcription (co)activators are kept at bay from the chromatin has emerged. Although the SA-responsive coactivator NPR1 forms a cytoplasmic oligomer, it was shown that in the absence of immune inducers small amounts of NPR1 monomer can escape oligomerization and translocate to the nucleus. These monomeric NPR1 escapees are rapidly ubiquitinylated by a Cullin 3–based ubiquitin-ligase and targeted to the proteasome. Mutation of Cullin 3 or pharmacological inhibition of the proteasome resulted in constitutive, low-level activation of defense genes and immunity (Spoel et al., 2009). Thus, nuclear clearance by means of destruction of transcriptional active NPR1 prevents untimely activation of immunity.

Ethylene-responsive genes were previously also shown to be controlled by nuclear activator clearance. In the presence of ethylene, the transcription activator Ethylene Insensitive3 (EIN3) accumulates to high levels in the nucleus where it associates with and activates ethylene-responsive genes. In the absence of ethylene, however, the levels of EIN3 are negligible, suggesting that the activity of EIN3 is at least in part controlled by its abundance. EIN3 is constitutively targeted to the proteasome by the $SCF^{EBF1/2}$ ubiquitin ligase. Mutation of EIN3-Binding Fbox1 (EBF1) and EBF2, the substrate adaptors that specifically recruit EIN3 to the SCF ubiquitin ligase, results in accumulation of EIN3, leading to constitutive activation of ethylene responses in the absence of ethylene (Guo and Ecker, 2003; Potuschak et al., 2003). Hence, clearing nuclear (co)activators like NPR1 and EIN3 is of major importance to silencing specific transcription programs.

The current cumulative data perhaps provide an important clue to the regulatory mechanisms governing plant transcription reprogramming. The data suggest that many transcription (co)activators of primary immune response genes are already abundantly present or synthesized in plant cells. Rather, it is their clearance from the chromatin by means of inhibitory proteins or destruction that keeps these factors from activating gene transcription. This implies that upon receiving particular signals, transcription programs are ready to go in plant cells, provided (co)activators rapidly find their chromatin targets. Likely, transcription regulators in plants will behave similar to ones in animals where studies have shown that transcription factors diffuse throughout the nucleus with great speed and accuracy. In fact, transcription factors are thought to visit the entire volume of a mammalian nucleus and scan the chromatin for specific binding sites in merely a few minutes (Hager et al., 2009).

TRANSCRIPTION ACTIVATION BY IMMUNE REGULATORS

Once transcription (co)activators have found their appropriate location on the chromatin, they are required to initiate gene transcription. For successful transcription initiation, (co)activators may have to overcome (co)repressor activities, restructure the chromatin to create access for general transcription factors of the preinitiation complex, and finally execute multiple rounds of recruiting and releasing RNAPII. In a few select cases, we are beginning to understand some of these initiation steps in the plant immune system.

Activation of the SA-Responsive Model Promoter PR-1

Advances in understanding the mechanisms by which immune regulators activate gene expression come largely from studies on the SA-responsive *PR-1* gene promoter. Using deletion analysis and linker-scanning mutagenesis, the SA-responsive region of the *PR-1* promoter was mapped to two specific locations designated *Linker-Scan 5* (*LS5*) and *LS7* (Lebel et al., 1998). These *cis*-acting elements contain the TGA transcription

factor binding core motif TGACG. Demonstration of direct interaction between the coactivator NPR1 and different TGA factors provided a simple model for SA-responsive NPR1/TGA-mediated induction of *PR-1* (Zhang et al., 1999; Després et al., 2000; Zhou et al., 2000; Subramaniam et al., 2001; Fan and Dong, 2002). However, more recent studies show that regulation of the *PR-1* promoter is far more complex.

Genetic studies have indicated that individual TGA factors have both positive and negative regulatory properties in relation to basal resistance and *PR-1* induction (Kesarwani et al., 2007). While simultaneous knockout of *TGA2*, *TGA5*, and *TGA6* abolished SA-responsive *PR* gene expression and pathogen resistance, basal *PR-1* expression was elevated in these mutants (Zhang et al., 2003), suggesting redundant positive and negative roles for TGA factors. Dissection of the individual contributions of TGA factors to *PR* gene expression or disease resistance showed that TGA2 may function as a repressor, while TGA3/6 factors are activators. While appearing to repress *PR* gene expression, TGA1/4 function as positive regulars of disease resistance (Kesarwani et al., 2007; Lindermayr et al., 2010). More recently, it was proposed that the repressor activity of TGA2 is transformed into an activator activity by its incorporation into a transactivation complex with NPR1 (Rochon et al., 2006; Boyle et al., 2009) (Figure 2). Although this model argues that TGA factors constitutively bind *PR* promoters, it has also been reported that SA induced the recruitment of TGA factors to the *PR-1* promoters in *Arabidopsis* and tobacco (*Nicotiana tabacum*; Johnson et al., 2003; Butterbrodt et al., 2006).

NPR1 and TGA factors function in a wider chromatin context at the *PR-1* promoter. In screens for suppressor mutants of *npr1*, the negative regulator suppressor of *npr1*, inducible1 (SNI1) was identified (Li et al., 1999). Transcription profiling of mutant *sni1* plants showed that SNI1 functions as a specific corepressor of NPR1-dependent genes, including *PR-1*. Its suppressive activity is likely exerted through an ancient conserved mechanism, as SNI1 also functions as a corepressor in yeast (Mosher et al., 2006). Accordingly, suppressor screens on *sni1* mutants have identified the positive regulators *Ras Associated with Diabetes51D* (*RAD51D*), *Breast Cancer2A* (*BRCA2A*), and *suppressor of sni1,2* (*SSN2*), all of which are conserved genes involved in homologous recombination or DNA repair (Durrant et al., 2007; Wang et al., 2010; Song et al., 2011). SA induces the recruitment of RAD51D and SSN2 to the *PR-1* promoter, which leads to the expulsion of SNI1, probably through direct interaction of RAD51D and SSN2 with SNI1 (Figure 2). These events are probably synchronized with the arrival of NPR1 at the *PR-1* promoter, as SSN2 recruitment is dependent on NPR1 and occurs through physical interaction with TGA7 (Song et al., 2011). Importantly, these advances demonstrate that proteins from homologous recombination and DNA repair pathways play surprisingly direct roles in transcription activation of defense genes. What mechanistic role they play exactly remains unknown, but it may well be related to cotranscriptional invasion of the transcribed DNA template by the nascent RNA. The resulting RNA:DNA hybrid renders nontemplate DNA as a single-stranded loop called the R loop that is thought to be sensitive to lesioning. Recent work in yeast suggests that transient R loop formation may occur during transcription but is kept in check by the helicase SEN1 (Mischo et al., 2011).

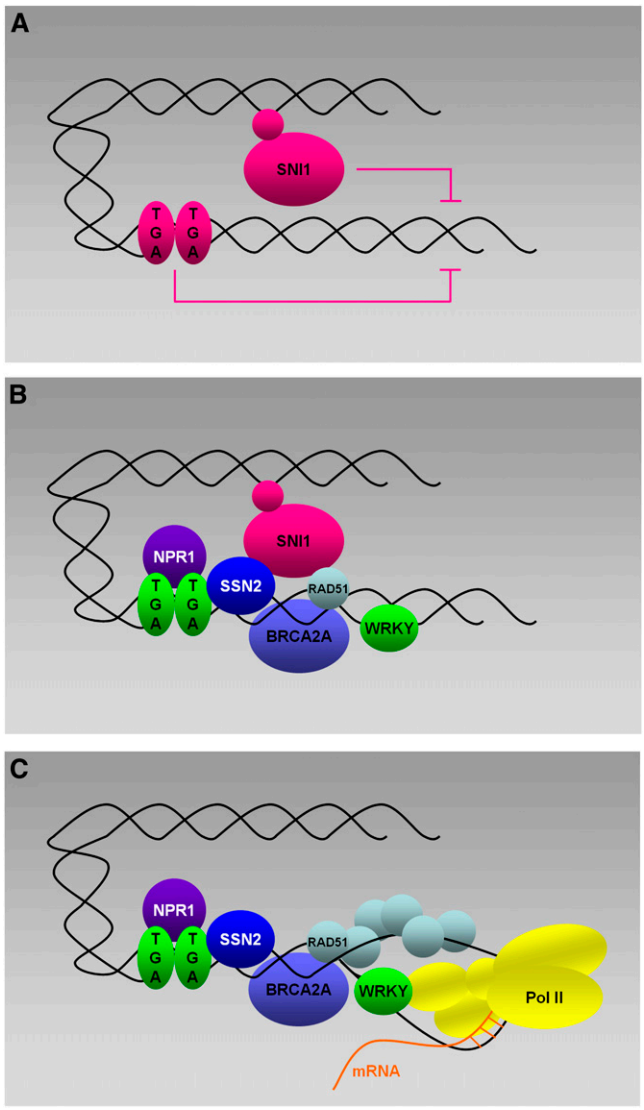

Figure 2. Simplified Model for Transcription Initiation of the SA-Responsive *PR-1* Promoter in *Arabidopsis*.

(A) In resting cells, both TGA transcription factors and the corepressor SNI1 are associated with the *PR-1* promoter and suppress its activation.
(B) Upon pathogen infection, SA induces the recruitment of NPR1 to the *PR-1* promoter, resulting in the transactivation of TGA factors (illustrated as a switch from magenta to green in **[A]** and **[B]**, respectively). Transactivated TGA factors recruit SSN2, which physically interacts with SNI1. Concomitantly, SA independently recruits BRCA2A and associated RAD51, the latter of which also physically interacts with SNI1. Additionally, as a result of transcription cascading (see text for details), WRKY transcription activators are also hypothesized to be recruited to the promoter.
(C) The activities of SSN2 and RAD51 lead to the expulsion of SNI1 from the promoter, allowing the recruitment of RNAPII to the promoter. Initiation of transcription exposes the nontemplate strand, which may be protected against single-stranded DNA damage by BRCA2A-coordinated RAD51 filament formation.

Interestingly, *SEN1* genetically interacted with genes involved in homologous recombination and DNA repair. Thus, it is plausible that in plants RAD51D, BRCA2A, and SSN2 function to limit R loop formation during high cellular demands for transcription and thereby prevent transcription-associated genomic instability (Figure 2).

Transcription Initiation by (Co)activators

Historically, transcription initiation has been presented as a static model in which transcription (co)activators bind promoters and simply activate transcription. However, this leaves many unanswered questions for a process that depends on dynamic and repeated deployment of RNAPII. For example, how do (co) activators thermodynamically establish repeated recruitment of RNAPII and can they regulate the rate at which mRNA is produced? Excitingly, studies to answer these questions are now under way in a range of eukaryotes and some progress has been made in understanding transcription initiation by immune activators in plants.

Infection by the necrotrophic fungus *Botrytis cinerea* leads to accumulation of the powerful antimicrobial camalexin, which is synthesized by the Cytochrome P450 enzymes CYP71A13 and PAD3. The pathogen-induced expression of both the *CYP71A13* and *PAD3* genes is dependent on the MAP kinases MPK3 and MPK6 (Ren et al., 2008). Recently, it was reported that high level camalexin accumulation in a genetic background with constitutively active MAPK kinase signaling requires the transcription factor WRKY33 (Mao et al., 2011). The authors went on to show that WRKY33 is a substrate of MPK3 and MPK6 in vitro and that WRKY33 is phosphorylated in vivo. Mutation of five Ser residues in WRKY33 abolished phosphorylation but did not affect DNA binding activity to its cognate binding motif. Instead, it was shown that while WRKY33 directly binds to the *PAD3* and likely *CYP71A13* promoters, loss of phosphorylation decreased its ability to initiate transcription. These findings suggest that phosphorylation of immune-related transcription activators may be a key step in transcription initiation.

Phosphorylation was also reported to regulate the SA-responsive coactivator NPR1. Upon SA induction, nuclear NPR1 was subject to site-specific phosphorylation of two N-terminal Ser residues. In this case, phosphorylation was surprisingly found to recruit a Cullin 3–based ubiquitin ligase that targeted NPR1 for degradation by the proteasome (Spoel et al., 2009). Importantly, failure to phosphorylate and thus degrade NPR1 abolished the expression of direct target genes, indicating that NPR1 coactivator instability is intimately linked to its ability to initiate transcription. Similar scenarios in which (co)activator destruction is necessary for gene activation have been uncovered in yeast and mammalian cells (Collins and Tansey, 2006; Kodadek et al., 2006; Spoel et al., 2010). In fact, many transcription activators contain overlapping sequences that activate transcription and signal for their degradation (Salghetti et al., 2000). This paradox may be reconciled by the finding that RNAPII-associated kinases may be responsible for phosphorylating activators to signal their degradation (Lipford et al., 2005). This implies that activators recruit RNAPII before being destroyed by the proteasome. Therefore, continuous clearance of activators that have already recruited RNAPII may facilitate the release of RNAPII (to progress to the transcription elongation step) and reset the promoter for reinitiation of transcription (Spoel et al., 2010). Notably, activation by activator destruction may be common in plants: in addition to NPR1, it was recently proposed that the transcription factor FER-like Iron deficiency-induced Transcription factor, an activator of iron deficiency response genes, is regulated by similar means (Sivitz et al., 2011).

TRANSCRIPTION REGULATORS FUNCTION WITHIN LARGE TRANSCRIPTION NETWORKS

To produce a physiologically relevant response, transcriptional regulators must function within large gene networks. In plants, immunity appears to be established by gene networks that consist of cascading rounds of transcription in which regulators must fine-tune their activity to cooperate with or antagonize other regulators. Although pathogens have evolved to hijack this highly interconnected network of regulators to promote their virulence (Grant and Jones, 2009), emerging evidence suggests that crosstalk between immune regulators provides plants with the potential to fine-tune its defense responses.

Transcription Cascading

Comparison of genes directly induced by NPR1 in the absence of de novo protein synthesis and the total set of NPR1-dependent genes (Wang et al., 2005, 2006) reveals that this coactivator may regulate many loci indirectly. Indeed, NPR1 has been shown to facilitate the activities of other downstream transcription factors. The nuclear translocation of an unknown transcription factor that regulates genes involved in the secretion of defense compounds was shown to be dependent on NPR1 (Wang et al., 2005). Moreover, using global expression profiling and bioinformatics tools, it was demonstrated that eight WRKY transcription factor genes (WRKY18, 38, 53, 54, 58, 59, 66, and 70) are direct targets of NPR1 (Wang et al., 2006). Individually, WRKY18, 53, 54, and 70 all act as positive regulators of SA-mediated resistance, while WRKY58 appears to attenuate the defense response. Comparison of wild-type, *npr1*, and *wrky18* mutant transcription profiles following induction of the SA-dependent pathway indicated that the expression of nearly 20% of NPR1-dependent genes is also regulated by WRKY18. Significantly, WRKY18 predominantly functioned as an auxiliary factor that amplified the responsiveness of NPR1-dependent genes. Additionally, independent work indicates that WRKY38 and WRKY62 are induced in an NPR1-dependent manner and function to suppress basal defenses (Kim et al., 2008), while supporting NPR1-dependent systemic acquired resistance (SAR), a broad-spectrum systemic immune response that is acquired after an initial pathogen attack (Spoel et al., 2009). Thus, NPR1 activates a network of primary response genes, including many WRKY transcription factors that fine-tune and amplify downstream transcriptional responses of secondary response genes (Figure 3A). This is further supported by a recent elaborate dissection of the NPR1-dependent secondary response promoter *PR-1*, which showed WRKY transcription factors coregulate this promoter at three or four different sites

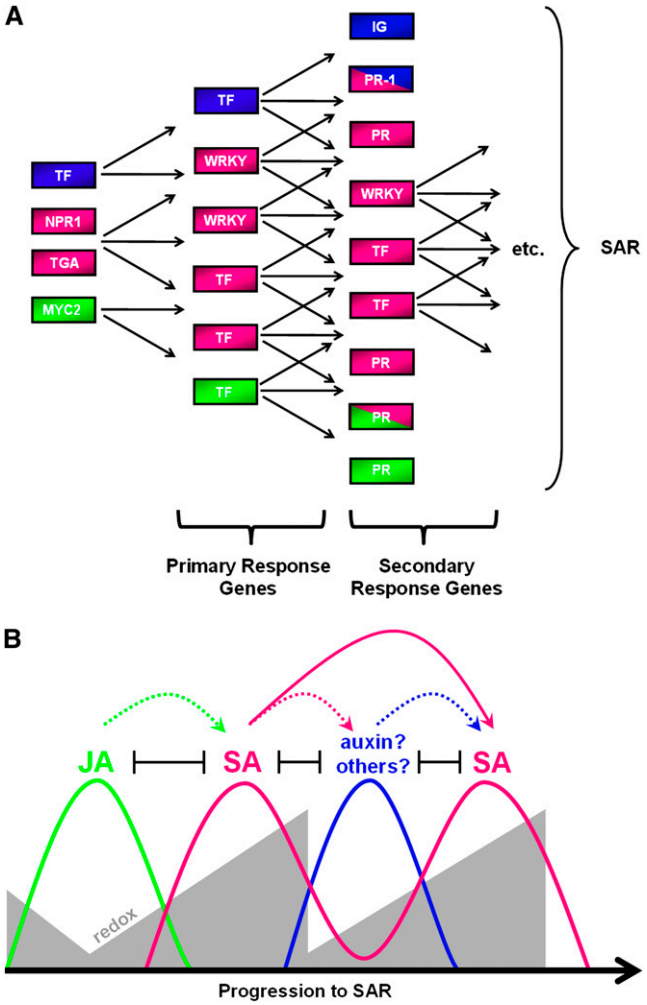

Figure 3. Transcription Cascades and Pulses May Establish Immunity.

(A) Transcription cascades involving different hormone signaling pathways lead to plant immunity. Immune activation induces the activity of a set of transcription (co)factors (TF) from different hormone signaling pathways that activate the transcription of primary response genes. Genes encoding for these activated TFs are among the primary response genes, providing an amplification step. However, many primary response genes encode for other, additional transcription (co)factors that go on to activate secondary response genes. While further transcription (co) factors may be among the secondary response genes, these also consist of genes that encode immune-related compounds, including proteins with antimicrobial activity. Importantly, the color scheme indicates that extensive crosstalk between hormone networks may occur during transcription cascading. SA (magenta), JA (green), auxin, and other (blue) hormone signaling components are shown. The transcription (co)factors NPR1, TGA, WRKY, and MYC2 have been specifically indicated as well as *PR* genes and genes involved in synthesis of indole glucosinolate (IG) defense compounds. Note that this figure shows temporal transcription hierarchies within but not between signaling cascades. Thus, it shows that multiple interacting signaling cascades require each other's activities, but as further explained in **(B)**, these cascades are not necessarily active simultaneously.

(B) Speculative model for how transcription pulses may establish SAR. We propose that changes in redox within 24 to 48 h after activation of SAR dictate transcription pulses of different hormone signaling pathways in systemic tissues. As these pathways are frequently antagonistic, the transcription pulses may be mutually exclusive within single cells. Nonetheless, feedforward loops may exist (indicated by arrows at the top) in which multiple hormone signals control the next transcription pulse through transcription cascading.

(Pape et al., 2010). As WRKY factors have been reported to form homo- and heterodimers (Xu et al., 2006), multiple promoter binding sites may embed further specificity and combinatorial control in transcriptional cascades.

Gene Transcription Pulses May Establish Immunity

If transcription regulators function in large cascading transcription networks, how are their activities fine tuned to each other? An important clue to answer this question comes from studies on temporal activity measurements of transcription activators. Contrary to expectation, many transcription activators are not continuously active upon signal perception, but they rather act in short bursts. For example, the animal immune activator NF-κB exhibits oscillatory nuclear translocation. This oscillatory behavior is largely the result of NF-κB-activated gene expression of its cytoplasmic inhibitor IκB, constituting a delayed negative feedback loop. Importantly, activation of NF-κB–dependent genes appears to be differentially regulated by the persistence, period, and amplitude of oscillations (Hoffmann et al., 2002; Nelson et al., 2004; Ashall et al., 2009). In plants, the activity of the immune coactivator NPR1 may also be regulated by nuclear fluctuations. In response to attempted pathogen infection, NPR1 localizes to the nucleus to initiate target gene transcription after which it is immediately turned over by the proteasome. Surprisingly, however, proteasome-mediated turnover of NPR1 occurs in cyclical bursts, allowing the protein level to recover in between bursts. Consequently, NPR1 target genes are only activated during these degradation bursts, resulting in transient pulses of target gene transcription (Spoel et al., 2009). Bursts of NPR1 degradation are probably controlled by fluctuations in cellular redox. Transient cellular reduction and oxidation allows NPR1 to switch between its nuclear monomer and cytosolic oligomer states, thereby regulating the influx of NPR1 into the nucleus and, thus, its availability to the proteasome (Tada et al., 2008; Spoel et al., 2010).

Transient transcription pulses as a result of negative feedback loops have also been described in JA signaling. JAZ and TOPLESS repressors and corepressors maintain JA-responsive genes in an inactive state by suppressing the activity of the activator MYC2 (Chini et al., 2007; Thines et al., 2007; Pauwels et al., 2010). As described earlier, upon perception of JA-Ile, the F-box protein COI1 forms a SCF^{COI1} E3 ligase that targets JAZ repressors for 26S proteasome-mediated degradation. Consequently, the transcription activation capacity of MYC2 is derepressed, allowing rapid activation of JA-responsive genes. Interestingly, included among the myriad genes induced by MYC2 are JAZ genes, creating a self-limiting negative feedback loop (Chico et al., 2008). Computational modeling of this negative

feedback loop indeed reproduced the experimentally observed pulses in JA-activated gene transcription (Banerjee and Bose, 2011). Similar negative feedback loops are also at the center of the auxin signaling pathway. Auxin-induced degradation of AUX/indole-3-acetic acid repressors liberates ARF activators, which activate the transcription of auxin-responsive genes, including those that encode for AUX/indole-3-acetic acid repressors (Gray et al., 2001). Thus, negative feedback loops between repressors and corepressors may be a common mechanism by which plants establish transcription pulses.

Remarkably, evidence suggests that self-limiting feedback loops may be fine-tuned by transcription regulators from external signaling pathways. It was shown that DELLA repressors, important regulators of genes responsive to the developmental hormone gibberellin, compete with MYC2 for binding to JAZ. As a result, DELLA proteins may sequester JAZ proteins to allow JA-responsive, MYC2-dependent gene transcription. Upon accumulation of gibberellin, however, DELLA proteins are targeted for degradation by the 26S proteasome, permitting inhibitory JAZ-MYC interaction (Hou et al., 2010). Moreover, abscisic acid and ethylene, two important hormones in local immune responses, are also able to modulate the stability of DELLA proteins (reviewed in Grant and Jones, 2009), potentially widening the web of signaling pathways that could fine-tune the MYC2-JAZ feedback loop.

Recent findings indicate that oscillatory pulses in transcription programs are necessary for proper development of eukaryotes. In *Arabidopsis*, for example, repeated formation of lateral roots is regulated by two different transcription programs that exhibit oscillating pulses in opposite phase (Moreno-Risueno et al., 2010). Importantly, the periodicity of these pulses is regulated by a set of oscillating transcription regulators that regulate different developmental pathways. Establishment of plant immunity may also depend on pulse activation of multiple distinct hormone signaling pathways. The broad-spectrum immune response SAR was initially thought to be regulated solely by SA. However, studies using ethylene-insensitive tobacco plants indicated that ethylene is required in infected tissues that generate the SAR signal (Verberne et al., 2003), while studies in *Arabidopsis* demonstrated that ethylene functions synergistically with SA to potentiate the expression of SA-dependent defense genes (Lawton et al., 1994; De Vos et al., 2006). More recently, it was suggested that components of the JA signaling pathway surprisingly also are required for SAR development (Truman et al., 2007). Not only did establishment of SAR require a component of the JA biosynthesis pathway, it also required the JA-responsive transcription activator MYC2. JA signaling components were notably only required in the early phase of SAR establishment. However, these findings contradict with another report that suggests SAR development is independent of JA (Attaran et al., 2009). This disparity may be the result of differences in the type of pathogen, pathogen dosage, and environmental conditions used to establish SAR. Further work has indicated that there may also be a role for the developmental hormone auxin in SAR (Truman et al., 2010). Induction of SAR was associated with increased expression of auxin biosynthetic genes, and auxin receptor or transport mutants failed to induce SAR. Taken together, these data suggest that in a large network the activity of transcription regulators may be fine-tuned by temporally separated, hormone-driven pulses in specific transcription programs (Figure 3).

Signal Crosstalk May Control Transcription Pulses and Immunity

Studies of the mouse segmentation clock, which establishes the segmental patterns of the spine during development, indicate that transcription programs may oscillate in opposite phases due to mutual antagonism (Dequéant et al., 2006). In plants, antagonistic and synergistic signal crosstalk has been a subject of intense investigation. Transcription programs coordinated by SA have been reported to show extensive cross communication with other hormone-mediated transcription programs. While most studies found crosstalk between SA and JA signaling to be mutually antagonistic, concentration-dependent synergism has also been reported (Mur et al., 2006; Beckers and Spoel, 2006; Pieterse et al., 2009). Kinetic studies indicate that SA suppresses JA-responsive genes only within a specific time frame that is linked to transient cellular redox changes (Koornneef et al., 2008), indicating that pathway prioritization occurs in a specific temporal window. The redox changes associated with this specific window are known to activate NPR1, the cytosolic form of which was previously identified as a key regulator of SA-triggered suppression of JA-responsive genes (Spoel et al., 2003). Moreover, nuclear NPR1 activates expression of the *WRKY70* transcription factor, an activator of SA-responsive genes but a potent repressor of JA-responsive genes (Li et al., 2004). NPR1 also activates the expression of *Glutaredoxin 480*, the corresponding protein of which was shown to interact with various TGA transcription factors to suppress the expression of JA-responsive genes (Ndamukong et al., 2007). A small collection of other nontranscription (co)factor proteins have also been identified as SA-JA crosstalk regulators (reviewed in Pieterse et al., 2009); hence, it is becoming clear that many different nodes of interaction exist between SA- and JA-responsive gene networks.

Besides JA signaling, SA has been reported to suppress auxin signaling. As a pivotal developmental hormone, auxin was only recently recognized to play an important role in plant defense. Many plant pathogens produce auxin-like molecules to promote virulence (Robert-Seilaniantz et al., 2007). Indeed, exogenous application of auxin to plants enhances susceptibility to the bacterial pathogen *Pseudomonas syringae*, while auxin signaling mutants display enhanced resistance to this pathogen (Navarro et al., 2006; Chen et al., 2007; Wang et al., 2007). The virulence-promoting effect of auxin depends in part on suppression of SA signaling. Conversely, SA is a potent suppressor of many auxin-related genes, including those that encode components of an auxin-inducible ubiquitin ligase that degrades auxin-responsive transcription repressors (Wang et al., 2007).

Many plant pathogens have evolved to manipulate and mimic plant hormones to promote their virulence. Manipulation of hormone homeostasis has striking effects on disease resistance and as such has received a lot of attention (Grant and Jones, 2009). Why plants have evolved to employ extensive crosstalk networks to control hormone balances is a less explored

question. Crosstalk has been proposed to provide the plant with the regulatory potential to favor immune response pathways over pathways that regulate normal cellular development (Spoel and Dong, 2008; Pieterse et al., 2009). In light of the emerging evidence described above, it is likely that crosstalk also provides plant cells with the ability to launch multiple hormone-driven transcription programs without cross-interference to establish immunity. This would explain why activation of the plant immune response SAR is associated with pulses in SA-induced transcription events that are probably separated by mutually exclusive pulses in JA, auxin, and perhaps other hormone signaling events (Truman et al., 2007, 2010; Spoel et al., 2009) (Figure 3B).

PERSPECTIVES

Emerging evidence indicates transcription dynamics of the plant immune response are incredibly complex. The mechanisms by which transcription (co)factors perceive cellular signals and modulate their transcriptional activity are diverse, but general commonalities are materializing, including the regulation by Ca^{2+} waves, cellular redox, site-specific phosphorylation, and ubiquitin-mediated degradation. Moreover, to advance our understanding of how transcription (co)factors function in larger networks, we must not see studies of transcription regulation and signal crosstalk as separate works. Instead, we should assess the transcription dynamics of plant immunity as a regulatory circuit that wires together multiple hormone-induced transcription programs necessary to acquire immunity. This dynamic circuit likely consists of many feedback and feedforward loops that allow fine-tuning of hormone-induced transcription programs. Although many feedback loops have been identified in plant immune signaling, feedforward loops are less well characterized. While the feedback loop is able to transmit downstream information to upstream regulators and fine-tune responses accordingly, the feedforward loop provides a mechanism to coordinate the amplitude, pulse length, and response time of gene expression (Yosef and Regev, 2011). In a transcriptional feedforward loop, an upstream factor (X) directly regulates a downstream gene (Z), while also modulating the activity of a distinct factor (Y) that regulates the same downstream gene (Z). Key genes in plant immunity, such as SA biosynthesis genes, may be regulated by this sophisticated mechanism. For example, regulation of SA biosynthetic genes is essential for correct activation of many immune response genes, which require rapid SA accumulation and sustained presence of SA for an extensive period of time. Conversely, overaccumulation of SA is toxic to the cell and must therefore be carefully managed. How are these integral adjustments to the amount of SA made? It is well established that among the earliest cellular changes upon pathogen recognition involves a characteristic Ca^{2+} signature. Indeed, as discussed earlier, SR1 and CPB60g are Ca^{2+}/calmodulin binding transcription factors that appear to have a modulatory capacity at different levels in the transcriptional cascade essential for the production of SA, including transcription activation of the SA biosynthetic gene *ICS1*. Interestingly, pathogen-induced Ca^{2+} may also activate MAP kinase signaling, and the phosphorylative action of several MAP kinases has been shown to activate the ethylene-responsive transcription activator EIN3 (Yoo et al., 2008; Boudsocq et al., 2010). EIN3 and EIN3-Like1 (EIL1) were recently reported not only to regulate ethylene-responsive genes but also modulate SA synthesis. Microarray analysis comparing *ein3 eil1* double mutant versus wild-type plants identified differential expression of a large subset of SA-responsive genes. Chromatin immunoprecipitation combined with gel shift analysis demonstrated that EIN3 is recruited to a consensus binding site located within the *ICS1* promoter and repressed gene activity (Chen et al., 2009). Hence, the *ICS1* promoter appears to constitute a point of convergence for a potential Ca^{2+} and ET-mediated transcriptional feedforward loop. In analogy to the (X), (Y), and (Z) definitions used above to describe the feedforward loop model, factor (X) would be represented by Ca^{2+}/calmodulin activation of CBP60g, factor (Y) by Ca^{2+} activation of MAPK/EIN3, while the *ICS1* promoter would constitute downstream target (Z). Another example of a potential, markedly different feedforward loop is a report in which the cytokinin-activated transcription activator ARR2 was shown to physically interact with the SA-responsive transcription activator TGA3 to promote SA-responsive *PR* gene expression (Choi et al., 2010), indicating that transcription regulators from different hormone signaling pathways can directly support one another. Together, these data provide compelling indications that numerous independently regulated transcription factors may function in combination to modulate key immune genes through potential feedforward loops. Future studies should elucidate the exact nature of these potential feedforward loops and integrate these into the dynamic behavior of transcription regulators and chromatin remodeling at immune gene promoters.

ACKNOWLEDGMENTS

We thank Xinnian Dong for fruitful discussions and apologize to colleagues whose work we did not review due to space limitations. This work was supported by grants from The Royal Society (UF090321) to S.H.S. and the Biotechnology and Biological Science Research Council (BB/D011809/1) to G.J.L.

Received May 13, 2011; revised July 21, 2011; accepted July 28, 2011; published August 12, 2011.

REFERENCES

Andreasson, E., et al. (2005). The MAP kinase substrate MKS1 is a regulator of plant defense responses. EMBO J. **24:** 2579–2589.

Ashall, L., et al. (2009). Pulsatile stimulation determines timing and specificity of NF-kappaB-dependent transcription. Science **324:** 242–246.

Attaran, E., Zeier, T.E., Griebel, T., and Zeier, J. (2009). Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in *Arabidopsis*. Plant Cell **21:** 954–971.

Banerjee, S., and Bose, I. (2011). Transient pulse formation in jasmonate signaling pathway. J. Theor. Biol. **273:** 188–196.

Beckers, G.J.M., and Spoel, S.H. (2006). Fine-tuning plant defence signalling: Salicylate versus jasmonate. Plant Biol (Stuttg) **8:** 1–10.

Bethke, G., Unthan, T., Uhrig, J.F., Pöschl, Y., Gust, A.A., Scheel, D., and Lee, J. (2009). Flg22 regulates the release of an ethylene

response factor substrate from MAP kinase 6 in *Arabidopsis thaliana* via ethylene signaling. Proc. Natl. Acad. Sci. USA **106:** 8067–8072.

Boudsocq, M., Willmann, M.R., McCormack, M., Lee, H., Shan, L., He, P., Bush, J., Cheng, S.H., and Sheen, J. (2010). Differential innate immune signalling via $Ca^{(2+)}$ sensor protein kinases. Nature **464:** 418–422.

Boyle, P., Le Su, E., Rochon, A., Shearer, H.L., Murmu, J., Chu, J.Y., Fobert, P.R., and Després, C. (2009). The BTB/POZ domain of the *Arabidopsis* disease resistance protein NPR1 interacts with the repression domain of TGA2 to negate its function. Plant Cell **21:** 3700–3713.

Butterbrodt, T., Thurow, C., and Gatz, C. (2006). Chromatin immunoprecipitation analysis of the tobacco *PR-1a*- and the truncated *CaMV 35S* promoter reveals differences in salicylic acid-dependent TGA factor binding and histone acetylation. Plant Mol. Biol. **61:** 665–674.

Chen, H., Xue, L., Chintamanani, S., Germain, H., Lin, H., Cui, H., Cai, R., Zuo, J., Tang, X., Li, X., Guo, H., and Zhou, J.M. (2009). ETHYLENE INSENSITIVE3 and ETHYLENE INSENSITIVE3-LIKE1 repress *SALICYLIC ACID INDUCTION DEFICIENT2* expression to negatively regulate plant innate immunity in *Arabidopsis*. Plant Cell **21:** 2527–2540.

Chen, Z., Agnew, J.L., Cohen, J.D., He, P., Shan, L., Sheen, J., and Kunkel, B.N. (2007). *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. Proc. Natl. Acad. Sci. USA **104:** 20131–20136.

Cheng, Y.T., Germain, H., Wiermer, M., Bi, D., Xu, F., García, A.V., Wirthmueller, L., Després, C., Parker, J.E., Zhang, Y., and Li, X. (2009). Nuclear pore complex component MOS7/Nup88 is required for innate immunity and nuclear accumulation of defense regulators in *Arabidopsis*. Plant Cell **21:** 2503–2516.

Chico, J.M., Chini, A., Fonseca, S., and Solano, R. (2008). JAZ repressors set the rhythm in jasmonate signaling. Curr. Opin. Plant Biol. **11:** 486–494.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J.M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L., and Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. Nature **448:** 666–671.

Choi, J., Huh, S.U., Kojima, M., Sakakibara, H., Paek, K.H., and Hwang, I. (2010). The cytokinin-activated transcription factor ARR2 promotes plant immunity via TGA3/NPR1-dependent salicylic acid signaling in *Arabidopsis*. Dev. Cell **19:** 284–295.

Chou, J.J., Li, S., Klee, C.B., and Bax, A. (2001). Solution structure of $Ca^{(2+)}$-calmodulin reveals flexible hand-like properties of its domains. Nat. Struct. Biol. **8:** 990–997.

Collins, G.A., and Tansey, W.P. (2006). The proteasome: A utility tool for transcription? Curr. Opin. Genet. Dev. **16:** 197–202.

Dequéant, M.L., Glynn, E., Gaudenz, K., Wahl, M., Chen, J., Mushegian, A., and Pourquié, O. (2006). A complex oscillating network of signaling genes underlies the mouse segmentation clock. Science **314:** 1595–1598.

Després, C., Chubak, C., Rochon, A., Clark, R., Bethune, T., Desveaux, D., and Fobert, P.R. (2003). The *Arabidopsis* NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. Plant Cell **15:** 2181–2191.

Després, C., DeLong, C., Glaze, S., Liu, E., and Fobert, P.R. (2000). The *Arabidopsis* NPR1/NIM1 protein enhances the DNA binding activity of a subgroup of the TGA family of bZIP transcription factors. Plant Cell **12:** 279–290.

De Vos, M., Van Zaanen, W., Koornneef, A., Korzelius, J.P., Dicke, M., Van Loon, L.C., and Pieterse, C.M.J. (2006). Herbivore-induced resistance against microbial pathogens in *Arabidopsis*. Plant Physiol. **142:** 352–363.

Du, L., Ali, G.S., Simons, K.A., Hou, J., Yang, T., Reddy, A.S.N., and Poovaiah, B.W. (2009). $Ca^{(2+)}$/calmodulin regulates salicylic-acid-mediated plant immunity. Nature **457:** 1154–1158.

Durrant, W.E., Wang, S., and Dong, X. (2007). *Arabidopsis* SNI1 and RAD51D regulate both gene transcription and DNA recombination during the defense response. Proc. Natl. Acad. Sci. USA **104:** 4223–4227.

Fan, W., and Dong, X. (2002). In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in *Arabidopsis*. Plant Cell **14:** 1377–1389.

García, A.V., Blanvillain-Baufumé, S., Huibers, R.P., Wiermer, M., Li, G., Gobbato, E., Rietz, S., and Parker, J.E. (2010). Balanced nuclear and cytoplasmic activities of EDS1 are required for a complete plant innate immune response. PLoS Pathog. **6:** e1000970.

García, A.V., and Parker, J.E. (2009). Heaven's Gate: Nuclear accessibility and activities of plant immune regulators. Trends Plant Sci. **14:** 479–487.

Grant, M.R., and Jones, J.D. (2009). Hormone (dis)harmony moulds plant health and disease. Science **324:** 750–752.

Gray, W.M., Kepinski, S., Rouse, D., Leyser, O., and Estelle, M. (2001). Auxin regulates $SCF^{(TIR1)}$-dependent degradation of AUX/IAA proteins. Nature **414:** 271–276.

Guo, H., and Ecker, J.R. (2003). Plant responses to ethylene gas are mediated by $SCF^{(EBF1/EBF2)}$-dependent proteolysis of EIN3 transcription factor. Cell **115:** 667–677.

Hager, G.L., McNally, J.G., and Misteli, T. (2009). Transcription dynamics. Mol. Cell **35:** 741–753.

Hayden, M.S., and Ghosh, S. (2004). Signaling to NF-kappaB. Genes Dev. **18:** 2195–2224.

Hoffmann, A., Levchenko, A., Scott, M.L., and Baltimore, D. (2002). The IkappaB-NF-kappaB signaling module: Temporal control and selective gene activation. Science **298:** 1241–1245.

Hou, X., Lee, L.Y.C., Xia, K., Yan, Y., and Yu, H. (2010). DELLAs modulate jasmonate signaling via competitive binding to JAZs. Dev. Cell **19:** 884–894.

Johnson, C., Boden, E., and Arias, J. (2003). Salicylic acid and NPR1 induce the recruitment of *trans*-activating TGA factors to a defense gene promoter in Arabidopsis. Plant Cell **15:** 1846–1858.

Kaminaka, H., Näke, C., Epple, P., Dittgen, J., Schütze, K., Chaban, C., Holt III, B.F., Merkle, T., Schäfer, E., Harter, K., and Dangl, J.L. (2006). bZIP10-LSD1 antagonism modulates basal defense and cell death in *Arabidopsis* following infection. EMBO J. **25:** 4400–4411.

Kesarwani, M., Yoo, J., and Dong, X. (2007). Genetic interactions of TGA transcription factors in the regulation of pathogenesis-related genes and disease resistance in Arabidopsis. Plant Physiol. **144:** 336–346.

Kim, K.C., Lai, Z., Fan, B., and Chen, Z. (2008). *Arabidopsis* WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. Plant Cell **20:** 2357–2371.

Kinkema, M., Fan, W., and Dong, X. (2000). Nuclear localization of NPR1 is required for activation of *PR* gene expression. Plant Cell **12:** 2339–2350.

Kodadek, T., Sikder, D., and Nalley, K. (2006). Keeping transcriptional activators under control. Cell **127:** 261–264.

Koornneef, A., Leon-Reyes, A., Ritsema, T., Verhage, A., Den Otter, F.C., Van Loon, L.C., and Pieterse, C.M.J. (2008). Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. Plant Physiol. **147:** 1358–1368.

Lawton, K.A., Potter, S.L., Uknes, S., and Ryals, J. (1994). Acquired resistance signal transduction in *Arabidopsis* is ethylene independent. Plant Cell **6:** 581–588.

Lebel, E., Heifetz, P., Thorne, L., Uknes, S., Ryals, J., and Ward, E. (1998). Functional analysis of regulatory sequences controlling *PR-1* gene expression in Arabidopsis. Plant J. **16:** 223–233.

Lecourieux, D., Ranjeva, R., and Pugin, A. (2006). Calcium in plant defence-signalling pathways. New Phytol. **171:** 249–269.

Li, J., Brader, G., and Palva, E.T. (2004). The WRKY70 transcription factor: A node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell **16:** 319–331.

Li, X., Zhang, Y., Clarke, J.D., Li, Y., and Dong, X. (1999). Identification and cloning of a negative regulator of systemic acquired resistance, SNI1, through a screen for suppressors of npr1-1. Cell **98:** 329–339.

Lindermayr, C., Sell, S., Müller, B., Leister, D., and Durner, J. (2010). Redox regulation of the NPR1-TGA1 system of *Arabidopsis thaliana* by nitric oxide. Plant Cell **22:** 2894–2907.

Lipford, J.R., Smith, G.T., Chi, Y., and Deshaies, R.J. (2005). A putative stimulatory role for activator turnover in gene expression. Nature **438:** 113–116.

Ma, W., and Berkowitz, G.A. (2007). The grateful dead: Calcium and cell death in plant innate immunity. Cell. Microbiol. **9:** 2571–2585.

Mao, G., Meng, X., Liu, Y., Zheng, Z., Chen, Z., and Zhang, S. (2011). Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in *Arabidopsis*. Plant Cell **23:** 1639–1653.

Mateo, A., Funck, D., Mühlenbock, P., Kular, B., Mullineaux, P.M., and Karpinski, S. (2006). Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. J. Exp. Bot. **57:** 1795–1807.

Mischo, H.E., Gómez-González, B., Grzechnik, P., Rondón, A.G., Wei, W., Steinmetz, L., Aguilera, A., and Proudfoot, N.J. (2011). Yeast Sen1 helicase protects the genome from transcription-associated instability. Mol. Cell **41:** 21–32.

Moreno-Risueno, M.A., Van Norman, J.M., Moreno, A., Zhang, J., Ahnert, S.E., and Benfey, P.N. (2010). Oscillating gene expression determines competence for periodic *Arabidopsis* root branching. Science **329:** 1306–1311.

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

Mou, Z., Fan, W., and Dong, X. (2003). Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell **113:** 935–944.

Mur, L.A., Kenton, P., Atzorn, R., Miersch, O., and Wasternack, C. (2006). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol. **140:** 249–262.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O., and Jones, J.D. (2006). A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science **312:** 436–439.

Ndamukong, I., Abdallat, A.A., Thurow, C., Fode, B., Zander, M., Weigel, R., and Gatz, C. (2007). SA-inducible Arabidopsis glutaredoxin interacts with TGA factors and suppresses JA-responsive *PDF1.2* transcription. Plant J. **50:** 128–139.

Nelson, D.E., et al. (2004). Oscillations in NF-kappaB signaling control the dynamics of gene expression. Science **306:** 704–708.

Pape, S., Thurow, C., and Gatz, C. (2010). The Arabidopsis *PR-1* promoter contains multiple integration sites for the coactivator NPR1 and the repressor SNI1. Plant Physiol. **154:** 1805–1818.

Pauwels, L., et al. (2010). NINJA connects the co-repressor TOPLESS to jasmonate signalling. Nature **464:** 788–791.

Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S., and Van Wees, S.C.M. (2009). Networking by small-molecule hormones in plant immunity. Nat. Chem. Biol. **5:** 308–316.

Potuschak, T., Lechner, E., Parmentier, Y., Yanagisawa, S., Grava, S., Koncz, C., and Genschik, P. (2003). EIN3-dependent regulation of plant ethylene hormone signaling by two *arabidopsis* F box proteins: EBF1 and EBF2. Cell **115:** 679–689.

Qiu, J.L., et al. (2008). *Arabidopsis* MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. EMBO J. **27:** 2214–2221.

Ren, D., Liu, Y., Yang, K.Y., Han, L., Mao, G., Glazebrook, J., and Zhang, S. (2008). A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in Arabidopsis. Proc. Natl. Acad. Sci. USA **105:** 5638–5643.

Robert-Seilaniantz, A., Navarro, L., Bari, R., and Jones, J.D. (2007). Pathological hormone imbalances. Curr. Opin. Plant Biol. **10:** 372–379.

Rochon, A., Boyle, P., Wignes, T., Fobert, P.R., and Després, C. (2006). The coactivator function of *Arabidopsis* NPR1 requires the core of its BTB/POZ domain and the oxidation of C-terminal cysteines. Plant Cell **18:** 3670–3685.

Salghetti, S.E., Muratani, M., Wijnen, H., Futcher, B., and Tansey, W.P. (2000). Functional overlap of sequences that activate transcription and signal ubiquitin-mediated proteolysis. Proc. Natl. Acad. Sci. USA **97:** 3118–3123.

Santner, A., and Estelle, M. (2009). Recent advances and emerging trends in plant hormone signalling. Nature **459:** 1071–1078.

Sivitz, A., Grinvalds, C., Barberon, M., Curie, C., and Vert, G. (2011). Proteasome-mediated turnover of the transcriptional activator FIT is required for plant iron-deficiency responses. Plant J. **66:** 1044–1052.

Song, J., Durrant, W.E., Wang, S., Yan, S., Tan, E.H., and Dong, X. (2011). DNA repair proteins are directly involved in regulation of gene expression during plant immune response. Cell Host Microbe **9:** 115–124.

Spoel, S.H., and Dong, X. (2008). Making sense of hormone crosstalk during plant immune responses. Cell Host Microbe **3:** 348–351.

Spoel, S.H., et al. (2003). NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell **15:** 760–770.

Spoel, S.H., and Loake, G.J. (2011). Redox-based protein modifications: The missing link in plant immune signalling. Curr. Opin. Plant Biol. **14:** 358–364.

Spoel, S.H., Mou, Z., Tada, Y., Spivey, N.W., Genschik, P., and Dong, X. (2009). Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity. Cell **137:** 860–872.

Spoel, S.H., Tada, Y., and Loake, G.J. (2010). Post-translational protein modification as a tool for transcription reprogramming. New Phytol. **186:** 333–339.

Subramaniam, R., Desveaux, D., Spickler, C., Michnick, S.W., and Brisson, N. (2001). Direct visualization of protein interactions in plant cells. Nat. Biotechnol. **19:** 769–772.

Tada, Y., Spoel, S.H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., and Dong, X. (2008). Plant immunity requires conformational changes [corrected] of NPR1 via *S*-nitrosylation and thioredoxins. Science **321:** 952–956.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A., and Browse, J. (2007). JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. Nature **448:** 661–665.

Truman, W., Bennett, M.H., Kubigsteltig, I., Turnbull, C., and Grant, M. (2007). *Arabidopsis* systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. Proc. Natl. Acad. Sci. USA **104:** 1075–1080.

Truman, W.M., Bennett, M.H., Turnbull, C.G., and Grant, M.R. (2010). Arabidopsis auxin mutants are compromised in systemic acquired resistance and exhibit aberrant accumulation of various indolic compounds. Plant Physiol. **152:** 1562–1573.

Verberne, M.C., Hoekstra, J., Bol, J.F., and Linthorst, H.J.M. (2003). Signaling of systemic acquired resistance in tobacco depends on ethylene perception. Plant J. **35:** 27–32.

Wang, D., Amornsiripanitch, N., and Dong, X. (2006). A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. PLoS Pathog. **2:** e123.

Wang, D., Pajerowska-Mukhtar, K., Culler, A.H., and Dong, X. (2007). Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr. Biol. **17:** 1784–1790.

Wang, D., Weaver, N.D., Kesarwani, M., and Dong, X. (2005). Induction of protein secretory pathway is required for systemic acquired resistance. Science **308:** 1036–1040.

Wang, L., Tsuda, K., Sato, M., Cohen, J.D., Katagiri, F., and Glazebrook, J. (2009). Arabidopsis CaM binding protein CBP60g contributes to MAMP-induced SA accumulation and is involved in disease resistance against *Pseudomonas syringae*. PLoS Pathog. **5:** e1000301.

Wang, S., Durrant, W.E., Song, J., Spivey, N.W., and Dong, X. (2010). *Arabidopsis* BRCA2 and RAD51 proteins are specifically involved in defense gene transcription during plant immune responses. Proc. Natl. Acad. Sci. USA **107:** 22716–22721.

Weerapana, E., Wang, C., Simon, G.M., Richter, F., Khare, S., Dillon, M.B.D., Bachovchin, D.A., Mowen, K., Baker, D., and Cravatt, B.F. (2010). Quantitative reactivity profiling predicts functional cysteines in proteomes. Nature **468:** 790–795.

Xu, X., Chen, C., Fan, B., and Chen, Z. (2006). Physical and functional interactions between pathogen-induced *Arabidopsis* WRKY18, WRKY40, and WRKY60 transcription factors. Plant Cell **18:** 1310–1326.

Yoo, S.D., Cho, Y.H., Tena, G., Xiong, Y., and Sheen, J. (2008). Dual control of nuclear EIN3 by bifurcate MAPK cascades in C_2H_4 signalling. Nature **451:** 789–795.

Yosef, N., and Regev, A. (2011). Impulse control: Temporal dynamics in gene transcription. Cell **144:** 886–896.

Zhang, Y., Fan, W., Kinkema, M., Li, X., and Dong, X. (1999). Interaction of NPR1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the *PR-1* gene. Proc. Natl. Acad. Sci. USA **96:** 6523–6528.

Zhang, Y., Tessaro, M.J., Lassner, M., and Li, X. (2003). Knockout analysis of *Arabidopsis* transcription factors *TGA2*, *TGA5*, and *TGA6* reveals their redundant and essential roles in systemic acquired resistance. Plant Cell **15:** 2647–2653.

Zhang, Y., Xu, S., Ding, P., Wang, D., Cheng, Y.T., He, J., Gao, M., Xu, F., Li, Y., Zhu, Z., Li, X., and Zhang, Y. (2010). Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. Proc. Natl. Acad. Sci. USA **107:** 18220–18225.

Zhou, J.M., Trifa, Y., Silva, H., Pontier, D., Lam, E., Shah, J., and Klessig, D.F. (2000). NPR1 differentially interacts with members of the TGA/OBF family of transcription factors that bind an element of the *PR-1* gene required for induction by salicylic acid. Mol. Plant Microbe Interact. **13:** 191–202.