Tansley review

Transcriptional networks in plant immunity

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Summary

Next to numerous abiotic stresses, plants are constantly exposed to a variety of pathogens within their environment. Thus, their ability to survive and prosper during the course of evolution was strongly dependent on adapting efficient strategies to perceive and to respond to such potential threats. It is therefore not surprising that modern plants have a highly sophisticated immune repertoire consisting of diverse signal perception and intracellular signaling pathways. This signaling network is intricate and deeply interconnected, probably reflecting the diverse lifestyles and infection strategies used by the multitude of invading phytopathogens. Moreover it allows signal communication between developmental and defense programs thereby ensuring that plant growth and fitness are not significantly retarded. How plants integrate and prioritize the incoming signals and how this information is transduced to enable appropriate immune responses is currently a major research area. An important finding has been that pathogen-triggered cellular responses involve massive transcriptional reprogramming within the host. Additional key observations emerging from such studies are that transcription factors (TFs) are often sites of signal convergence and that signal-regulated TFs act in concert with other context-specific TFs and transcriptional co-regulators to establish sensory transcription regulatory networks required for plant immunity.

I. Introduction

Plants combat invading pathogenic microbes by employing a two-tier innate immune system. The first layer of plant immunity is triggered upon perception of conserved molecular structures called microbe/pathogen-associated molecular patterns (MAMPs) through plasma membrane-localized pattern recognition receptors (PRRs). Immunity triggered by this mode is designated PAMP/pattern-triggered immunity (PTI). Well-studied examples for PRRs are Arabidopsis thaliana AtFLS2 (FLAGELLIN-SENSING2) and AtEFR (EF-TU RECEPTOR) that recognize bacterial flagellin and elongation factor-Tu (EF-Tu), respectively.
Adapted pathogens have acquired a number of virulence mechanisms to suppress plant immunity including PTI, for instance by deploying effector proteins (Dou & Zhou, 2012). As a countermeasure, plants have evolved the second layer of the innate immune system to recognize – directly or indirectly – such effector proteins, resulting in initiation of effector-triggered immunity (ETI). Typical receptors for ETI are nucleotide-binding site/leucine-rich repeat (NLR) proteins which fall broadly into two major sub-classes that have distinct N-terminal domains. NLRs with a Toll-Interleukin1 Receptor (TIR) domain or coiled-coil (CC) domain are referred to as TNLs or CNLs, respectively (Bonardi et al., 2012).

During PTI and ETI, plants trigger a diverse array of immune responses such as reactive oxygen species (ROS) generation, cellular Ca^{2+} spikes, MAP kinase (MAPK) activation, production of phytohormones, and transcriptional reprogramming. These immune responses are integrated and collectively contribute to immunity both locally at the infection site, and systemically at distant uninfect ed sites. PTI and ETI share signaling components with distinct activation dynamics and amplitudes (Tsuda & Katagiri, 2010).

Single immune receptor activation triggers a number of immune responses that need to be carefully modulated as plants face multiple biotic and abiotic stresses in natural environments (Tsuda & Katagiri, 2010; Suzuki et al., 2014). In particular, plants need to coordinate their stress responses with growth to maximize their fitness (Denance et al., 2013). Recent advances suggest that signal integration is dictated by transcription factor (TF) regulatory networks. Transcriptional reprogramming is a major feature of plant immunity and is governed by TFs and co-regulatory proteins associated within discrete transcriptional complexes (Moore et al., 2011). Upon receptor activation and signal initiation, selected TFs and associated co-factors integrated within signaling pathways decode this information in various ways leading to diverse transcriptional changes.

In this review, we discuss properties of major TF families involved in plant immunity, regulation of transcriptional complexes, and convergence of different phytohormone signaling pathways at TFs forming sensory TF regulatory networks. We also point out difficulties associated with the analysis of such networks and measures to be taken for their analyses. Due to space limitations we do not address the role of pathogen-derived effectors that impact on transcriptional regulation in the host. Effector research has advanced enormously over the past few years and several excellent reviews on this topic exist (Feng & Zhou, 2012; Boch et al., 2014; Kazan & Lyons, 2014; Rovenich et al., 2014).

II. Major transcription factor families involved in plant immunity

TFs are major players in modulating transcriptional programs controlling plant development and response to internal and environmental cues. The repertoire of TFs within plants clearly varies from those of metazoans. Whereas the majority of metazoan TFs belong to members of the class of bHLH, homeodomain and C_{2}H_{2} zinc finger proteins, the predominant families in plants include, next to bHLH, homeodomain and C_{2}H_{2} zinc fingers, also AP2/ERF, bZIP, MADS box, MYB, NAC and WRKY (Weirauch & Hughes, 2011). Likely the sessile lifestyle and the lack of an adaptive immune system are reasons for the parallel expansion of the TF repertoires that include several larger plant-specific families. For a detailed discussion why plant TF genes show a higher degree of expansion relative to the metazoans see Shiu et al. (2005).

Over the past two decades molecular and genetic studies have uncovered numerous TF family members that are critical in regulating proper transcriptional responses when plants are confronted by phytopathogens. Our current knowledge on the role of distinct TFs involved in plant immunity is still mainly based on research employing a few model plants such as Arabidopsis and rice. Nevertheless, numerous isolated reports from other plant species have helped to broaden the spectrum of TFs that are vital for host defense (for a comprehensive list see Supporting Information Table S1).

It appears that certain TF families are particularly dedicated to regulating host immune responses. Although not exclusive, these comprise members of the AP2/ERF, bHLH, bZIP, MYB, NAC and WRKY TF families. We briefly introduce these TF families and mention selected members of each that are discussed during the course of this review.

1. AP2/ERF family

The APETALA2/ETHYLENE-RESPONSE ELEMENT BINDING FACTOR (AP2/ERF) family constitutes a large plant-specific TF family with over 140 members in Arabidopsis and 160 in rice (Licausi et al., 2013). All members share a common c. 60 amino acid long DNA binding domain. Within the large ERF family, the DREB protein subfamily binds to a DNA motif, A/GCCGAC, termed Dehydration-Response-Element (DRE), and regulates expression of target genes under abiotic stress conditions including freezing, drought and high salinity. By contrast, ERF subfamily members show the greatest affinity to the GCC sequence (AGCCG GCC), and participate in the regulation of genes responsive to biotic stress, in particular to genes related to the jasmonic acid (JA) and ethylene hormone signaling pathways. Members of this subfamily include AtORA59, AtERF1, AtERF6 and AtERF104.

2. bHLH family

The basic-helix-loop-helix (bHLH) family was estimated to consist of 162 and 167 members in Arabidopsis and rice, respectively, but numerous additional atypical bHLH proteins have recently been described (Carretero-Paulet et al., 2010). The DNA binding domain of bHLH proteins comprises 50–60 amino acids, and this domain allows for homo- or heterodimerization to their DNA consensus hexamer sequence CANNTG. Very few members of this TF family have been identified as being critical for plant immunity. However one factor, AtMYC2/JAI1/JIN1, along with its closely related proteins AtMYC3 and AtMYC4 are key master regulators coordinating JA-mediated defense responses and in mediating crosstalk with other phytohormones such as salicylic acid (SA),
abscisic acid (ABA), gibberellins (GA), and auxin (Kazan & Manners, 2013).

3. TGA-bZIP family

The basic domain leucine zipper (bZIP) family has 74 members in Arabidopsis and 89 in rice. In Arabidopsis bZIP proteins are classified into 10 groups, two of which (Groups C and D) contain members that are implicated in plant immunity (Jakoby et al., 2002). Generally bZIPs bind as homo- or heterodimers to DNA sequences with an ACGT core. Particularly the TGA factors of group D that are further subdivided into three clades, AtTGA1 and AtTGA4 (Clade I), AtTGA2, AtTGA5 and AtTGA6 (Clade II), and AtTGA3 and AtTGA7 (Clade III), are central players of the defense system especially within the SA-signaling pathway conferring resistance toward biotrophic pathogens. TGA-bZIPs bind to the palindromic DNA sequence TGAC/GTCA with an intact TGACG motif being the minimal requirement (Gatz, 2013). AtTGA2, 5 and 6 also have vital roles in establishing systemic acquired resistance (SAR), a form of long-term resistance throughout the entire plant following local infection by a pathogen. Moreover, these three TFs also regulate host detoxification pathways as well as being essential activators of certain ethylene-induced defense responses (Zander et al., 2014).

4. MYB family

MYBs constitute another large family comprising >160 genes within the Arabidopsis and rice genomes (Dubos et al., 2010). In contrast to animals, plants contain a MYB-protein subfamily characterized by the R2R3 MYB domain (Stracke et al., 2001). R2R3-MYB proteins are separated into two types that can bind the distinct DNA sequence elements, (T/C)AAC(T/G)G and G(G/T)T(A/T)G(G/T)T. Several reported MYB proteins have functions in plant immunity with the most prominent members being AtMYB30, AtMYB44, AtMYB108/BOSI1 from Arabidopsis, and HvMYB6 from barley (Buscaill & Rivas, 2014).

5. NAC family

The NAC family consists of c. 100 genes in Arabidopsis and 150 genes in rice. NAC proteins bind to the DNA motif CATGTG (Nakashima et al., 2012). Although a major role assigned to this family is coping with abiotic stresses such as drought and high salinity, the barley factor HvATAF1 and several Arabidopsis members – AtANAC019, AtANAC055 and AtANAC072 – have also been identified as important immune components (Jensen et al., 2008; Nuruzzaman et al., 2013).

6. WRKY family

The WRKY family of TFs consists of >70 members in Arabidopsis and >100 in rice. This family is mainly restricted to plants but their existence within the slime mold Dictyostelium discoideum and the unicellular protist Gardia lamblia genomes have been documented (Rushton et al., 2010). Common to all members is the c. 60 amino acid long WRKY domain that binds to the DNA motif C/TGTAC/T, termed the W-box. Extensive research has firmly established a major role of numerous distinct WRKY members in host immunity, in Arabidopsis, barley and rice (Pandey & Somssich, 2009).

III. Regulation of transcriptional complexes

TF expression is controlled both transcriptionally and post-transcriptionally, and activities of TFs are regulated in multiple ways, such as phosphorylation/dephosphorylation, altered subcellular localization and targeted degradation (Moore et al., 2011). Regulation of TFs is key for mounting successful transcriptional reprogramming and immunity. Some pathogen-derived factors subvert host transcriptional complexes to enhance their virulence, highlighting the importance of transcriptional regulation of host defenses (Kazan & Lyons, 2014). In this section, we discuss recent advances on how activities of TFs are molecularly regulated by plants.

1. Direct regulation of transcription factors by immune receptors

Activation of NLRs triggers a diverse array of immune responses including transcriptional reprogramming. For several cases of nucleocytoplasmic NLRs, nuclear accumulation is required for their full functions in ETI, suggesting that these NLRs directly regulate transcription (Bhattcharjee et al., 2013). Indeed, emerging evidence shows that certain NLRs exert their functions through physical interactions with TFs.

The barley nucleocytoplasmic CNI MLA10 (MILDEW LOCUS A10) interacts through its CC domain with the WRKY transcriptional repressors HvWRKY1 and HvWRKY2 that are negative regulators of immunity against the powdery mildew fungus Blumeria graminis (Shen et al., 2007). The MLA10 CC domain also interacts with the MYB transcriptional activator HvMYB6, which positively regulates immunity against this fungus (C. Chang et al., 2013). HvWRKY1 interacts with HvMYB6 thereby suppressing its function. MLA10 modulates the transcriptional complex by enhancing HvMYB6 DNA binding activity and releasing HvMYB6 from HvWRKY1 suppression (C. Chang et al., 2013). Thus, MLA10 directly regulates transcription through modulation of the transcriptional complex comprising HvMYB6 and HvWRKY1/2 in the nucleus, resulting in activation of immunity (Fig. 1a).

The Nicotiana benthamiana nucleocytoplasmic TNL N specifically recognizes the Tobacco mosaic virus (TMV) replicase (Burgh-Smith et al., 2007). Upon activation, N interacts in the nucleus with the SQUAMOSA PROMOTER BINDING PROTEIN (SBP)-domain TF NbsPL6, which is also essential for N-mediated immunity (Padmanabhan et al., 2013).

The Arabidopsis nucleocytoplasmic TNLs RPS4 (RESISTANCE TO PSEUDOMONAS SYRINGE4) and RRS1 (RESISTANCE TO RALSTONIA SOLANACEARUM1) form a hetero complex and cooperate to recognize multiple effectors including AvrRps4 and PopP2 from the bacterial pathogens, Pseudomonas.
**Fig. 1** Direct interaction of immune receptors with transcription factors. (a) Barley plants expressing the intracellular immune receptor MLA are resistant towards isolates of the powdery mildew fungus *Blumeria graminis* expressing the corresponding Avr (Avirulence) gene. In uninfected cells, HvWRKY1 forms a nuclear complex with HvMYB6 thereby suppressing defense gene activation. Upon infection, the fungal-derived Avr effector is perceived in the cytosol by the CC-domain of MLA resulting in MLA translocation to the nucleus. Within the nucleus MLA associates with both HvWRKY1/2 and HvMYB6 allowing HvMYB6 to contact DNA and to activate gene expression. (b) The rice PRR XA21 is kept in an inactive state by association with the ATPase OsXB24 at the plasma membrane. Upon perception of *Xanthomonas oryzae* pv. *oryzae* (Xoo), probably via a bacteria-derived ligand, OsXB24 is released, thereby activating XA21. Activation results in cleavage of the C-terminal intracellular domain of XA21 and its translocation to the nucleus where it interacts with the transcription factor OsWRKY62. Following activation OsXB15, a protein phosphatase 2C, associates with XA21 leading to its dephosphorylation and inactivation. OsWRKY62 and OsWRKY76 are negative regulators of XA21-mediated resistance whereas OsWRKY71 is a positive regulator. Potential interactions between the WRKY factors are currently unknown. Solid arrows indicate experimentally verified events whereas dashed arrows indicate potential unverified events.

**syringae** and *Ralstonia solanacearum*, respectively (Gassmann et al., 1999; Deslandes et al., 2003; Narusaka et al., 2009; Williams et al., 2014). Interestingly, the Arabidopsis homolog of NbSPL6, AtSPL6, also contributes to RPS4/RRS1 ETI against *P. syringae* (Padmanabh et al., 2013), although the underlying molecular mechanisms remain unknown. In addition, the bHLH TF *Ab*HLH84 and its homologs interact with RPS4 as well as with the Arabidopsis nucleo-cytoplasmic TNL SNC1, and are involved in ETI (Xu et al., 2014).

RRS1 possesses a C-terminal WRKY domain that can bind to the W-box in vitro (Noutoshi et al., 2005). The RRS1/RPS4 TNL pair employs the WRKY domain for effector recognition and possibly for positioning of a RRS1/RPS4 pre-activation complex at specific chromatin sites (Williams et al., 2014). RRS1 also contributes to transcriptional reprogramming triggered by an RPS4 autoimmune response. Interestingly, the W-box is enriched in the promoters of RRS1-regulated genes (Heidrich et al., 2013). Therefore, it is conceivable that RRS1 has an additional post-activation signaling role, perhaps in repositioning RPS4/RRS1 complexes to W-boxes at certain chromatin sites.

The rice nucleo-cytoplasmic CNL Pb1 (Panicle blast1) interacts through the CC domain with OsWRKY45, which is also required for Pb1-mediated immunity against the rice blast fungus *Magnaporthe oryzae* (Hayashi et al., 2010; Inoue et al., 2013). In nonactivated rice cells, OsWRKY45 is subject to proteasome-mediated degradation (Matsushita et al., 2013). Nuclear Pb1 protects OsWRKY45 from such degradation, thereby enhancing OsWRKY45 levels and thus presumably contributing to transcriptional reprogramming and enhanced immunity (Inoue et al., 2013).

These examples illustrate that NLR immune receptors directly regulate the activities of TFs. How activated NLRs modulate transcriptional reprogramming and other immune responses remains a highly attractive research area.

The Rice PRR XA21 confers immunity against the rice blast pathogen *Xanthomonas oryzae* pv. *oryzae* (Song et al., 1995). As with other PRRs, XA21 localizes at the plasma membrane but is subsequently cleaved to release the intracellular kinase domain. This domain localizes in the nucleus and interacts with OsWRKY62. Cleavage of XA21 and nuclear localization of the domain is required for XA21-mediated immunity (Park & Ronald, 2012). It remains unclear how this cleavage is regulated and how the nuclear-localized kinase domain influences OsWRKY62-mediated transcription. Whether other PRRs translocate to the nucleus is unknown. Nevertheless, this example demonstrates the potential of membrane-localized immune receptors to translocate to the nucleus and to engage in direct transcriptional regulation through TF interactions (Fig. 1b).

2. Regulation of transcriptional complexes by MAPK cascades

MAPK cascades are important signaling pathways that link stimuli to downstream responses via phosphorylation of substrates including TFs, and many studies show their implications in plant immunity (Meng & Zhang, 2013). For instance, Arabidopsis AtMPK3 and AtMPK6 are activated by the upstream MAPK kinases AtMKK4 and AtMKK5, whereas AtMPK4 and likely AtMPK11 are regulated by AtMKK1 and AtMKK2, which are activated by the upstream MAPK kinase AtMEKK1 (Asai et al., 2002; Ichimura et al., 2006; Meszaros et al., 2006; Nakagami et al., 2006; Suarez-Rodriguez et al., 2007; Qiu et al., 2008b; Bethke et al., 2012). Although the mechanisms by which these MAPK cascades are activated during immunity remain to be
binding to its target genes. Thus, the MAPK-WRKY modules are tightly regulated core signaling components for immune responses.

MAPK cascades regulate other TFs during plant immunity. AtMPK3 and AtMPK6 phosphorylate AεR6 (ETHYLENE RESPONSE FACTOR6), and this phosphorylation increases AεR6 protein stability, leading to enhanced expression of target genes such as the defensin gene, AεPDF1.2. This MAPK-AεR6 module appears to be physiologically important as the phosphor-mimic AεR6 confers immunity against B. cinerea (Meng et al., 2013). AεMPK6 phosphorylates AεR104 (ETHYLENE RESPONSE FACTOR104), and the complex is dissociated in response to the MAMP flg22. Complex dissociation requires AεMPK6 activity and ethylene signaling (Bethke et al., 2009). These results suggest that ethylene signaling acts on the AεMPK6-AεR104 complex to allow AεR104 to access specific chromatin sites.

AεMPK3 phosphorylates AεVIP1 (VirE2 interacting protein 1), resulting in repositioning of AεVIP1 from the cytosol to the nucleus (Djamei et al., 2007). Nuclear AεVIP1 regulates gene promoters containing the AεVIP1 response DNA element (Pitzschke et al., 2009). For instance, AεMYB44 is an AεVIP1 target gene and directly regulates expression of AεWRKY70, which regulates SA- and jasmonic acid (JA)-mediated transcription (Li et al., 2004; Pitzschke et al., 2009; Shim et al., 2013). Interestingly, AεMYB44 is also phosphorylated by AtMPK3 and AtMPK6 (Nguyen et al., 2012; Persak & Pitzschke, 2013), suggesting a feed-forward regulation of AεMYB44 by AtMPK3/6 through phosphorylation and by theAtMPK3-AεVIP module through induced expression. These results illustrate that the AtMPK3-AεVIP1-AεMYB44-AεWRKY70 module is an important immune component.

Activation of MAPKs is transient during PTI but sustained during ETI triggered by Arabidopsis plasma membrane-associated CNLs RPS2 (RESISTANCE TO PSEUDOMONAS SYRINGE2) and RPM1 (RESISTANCE TO PSEUDOMONAS SYRINGAE PV. MACULICOLA1), or upon Botrytis infection (Underwood et al., 2007; Ren et al., 2008; Tsuda et al., 2013). Such quantitative differences in the duration of MAPK activation may produce qualitatively distinct transcriptional responses. For instance, prolonged MAPK activation in RPS2- and RPM1-mediated ETI regulates specific gene expression. During PTI, when MAPK activation is transient, regulation of a subset of genes is dependent on SA. During ETI, when MAPK activation is extended, the same genes become less dependent on SA, and defects in SA signaling are compensated for by prolonged MAPK activation (Tsuda et al., 2013). How this quantitative MAPK activation leads to qualitatively different transcriptional outputs remains to be determined. One may hypothesize that differential MAPK activation leads to activation of distinct TFs. For example, prolonged MAPK activation may bypass SA accumulation to specifically activate TFs involved in SA-mediated transcription. Future research needs to address the mechanisms by which immune receptors such as NLRs and PRRs activate MAPKs and their substrates, and whether MAPKs themselves shuttle between cell compartments during pathogen infection.
3. Regulation of transcriptional complexes by Ca\textsuperscript{2+} signaling

Calcium ion is an important second messenger in many physiological processes including plant immunity. The concentration of Ca\textsuperscript{2+} in the cytosol transiently increases, and this transient Ca\textsuperscript{2+} elevation triggers a number of immune responses (Poobaugh et al., 2013). There are no canonical Ca\textsuperscript{2+}-selective voltage-gated ion channels in higher plants, but the involvement of cyclic nucleotide-gated ion channels (CNGCs), glutamate receptors and annexins have recently been proposed (Swarbreck et al., 2013). Ca\textsuperscript{2+} signatures produced through Ca\textsuperscript{2+} channels are decoded by Ca\textsuperscript{2+} sensor proteins binding to Ca\textsuperscript{2+}. In plants, the Ca\textsuperscript{2+} sensors are divided into three classes: calmodulin (CaM), calcineurin B-like (CBL) and Ca\textsuperscript{2+}-dependent protein kinases (Poovaiah et al., 2013; Steinhorst & Kudla, 2013). Ca\textsuperscript{2+} sensor proteins relay Ca\textsuperscript{2+} signatures to a multitude of immune responses including nitric oxide (NO) and ROS generation, as well as transcriptional reprogramming. Systemic regulation and amplification of immune responses governed by a feedback regulation of Ca\textsuperscript{2+} signaling, ROS and NO have been proposed (for excellent reviews see Jeandroz et al., 2013; Steinhorst & Kudla, 2013; Romeis & Herde, 2014). Here, we discuss regulation of transcriptional complexes by Ca\textsuperscript{2+} sensor proteins in plant immunity (Fig. 3).

CaM is a conserved eukaryotic Ca\textsuperscript{2+} sensor protein that modulates target protein activities through physical interactions, thereby relaying Ca\textsuperscript{2+} signatures to downstream responses. CaM interacts with a diverse array of TFs (Poovaiah et al., 2013). The CAMTA (CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR) TFs interact with CaM, and their functions are modulated by Ca\textsuperscript{2+}/CaM (Finkler et al., 2007). Mutants of AtCAMTA3/5 (SIGNAL RESPONSIVE) show elevated SA concentrations and enhanced immunity against \textit{P. syringae} and \textit{B. cinerea} (Galton et al., 2008). AtCAMTA3/5R1 binds to the promoter of \textit{AtEDS1}, encoding a central regulator of the positive feedback loop of SA accumulation and TNL signaling (Wierner et al., 2005; Heidrich et al., 2012), and this leads to suppression of \textit{AtEDS1} expression (Du et al., 2009). Combinatorial mutant analysis indicates that \textit{AtCAMTA3/5R1} and its homologs \textit{AtCAMTA1/2} also suppress expression of the SA biosynthesis gene \textit{AtSID2} and TFs involved in \textit{AtSID2} transcription (Kim et al., 2013). Thus, the three CAMTA homologs coordinately suppress SA accumulation but target genes other than \textit{AtEDS1} remain unknown. Recently an \textit{AtCAMTA3/5R1}-interacting protein was identified that links \textit{AtCAMTA3/5R1} to ubiquitin-mediated protein degradation, thereby enhancing \textit{AtEDS1} expression and immunity against \textit{P. syringae} (L. Zhang et al., 2014). Transcription of \textit{AtSID2} is also controlled by the CaM-binding TF \textit{AtCBP60g} and its homolog \textit{AtSARD1} (Wang et al., 2009, 2011; Zhang et al., 2010). Although \textit{AtCBP60g} has been shown to interact with CaM and CaM-binding is required for its function (Wang et al., 2009), \textit{AtSARD1} does not appear to be a CaM-binding protein (Zhang et al., 2010; Wang et al., 2011). Despite this difference, \textit{AtCBP60g} and \textit{AtSARD1} are partially redundant for \textit{AtSID2} expression and SA accumulation during immunity (Zhang et al., 2010; Wang et al., 2011). Another close homolog of \textit{AtCBP60g} and \textit{AtSARD1}, \textit{AtCBP60a}, negatively regulates \textit{AtSID2} expression and SA accumulation, and \textit{AtCBP60a} CaM-binding is required for its function (Truman et al., 2013). Thus, SA biosynthesis is regulated by multiple CaM-binding TFs that positively or negatively regulate proper SA accumulation, although direct evidence showing that these TFs are indeed regulated by calcium is lacking. It is proposed that distinct Ca\textsuperscript{2+} signatures are decoded to initiate specific transcriptional responses (McAinsh & Pittman, 2009). Conceivably, distinct Ca\textsuperscript{2+} channels are activated in different tissue or subcellular locations or by explicit stimuli to produce varying Ca\textsuperscript{2+} signatures that lead to activation of selected TFs. The Arabidopsis bZIP TF \textit{AtTGA3} involved in SA responses interacts with CaM, and its CaM-binding enhances its DNA-binding activity (Szymbanski et al., 1996). An Arabidopsis CaM-binding NAC TF, \textit{ACBNAC}, directly binds to the promoter of the SA marker gene \textit{AtPR1} (PATHOGENESIS-RELATED PROTEIN1), and suppresses its expression (Kim et al., 2012). In summary, Ca\textsuperscript{2+}/CaM signaling modules are critical modulators of SA biosynthesis and SA-mediated transcriptional reprogramming.

Unlike CaM and CBLs, CDPKs (CALCIUM-DEPENDENT PROTEIN KINASES) have both intrinsic Ca\textsuperscript{2+} sensing and responding sites, thereby allowing individual CDPK proteins to relay Ca\textsuperscript{2+} signatures to downstream components via phosphorylation events. In Arabidopsis, there are 34 CDPK members and many of them have been demonstrated or predicted to be membrane-associated proteins (Romeis & Herde, 2014). Consistent with this, multiple CDPKs interact with and phosphorylate membrane-localized NADPH oxidase \textit{AtRBOHD} (RESPIRATORY BURST OXIDASE HOMOLOGUE D), which regulate ROS generation during immunity (Dubiel et al., 2013; Gao et al., 2015).
Activation of CDPKs also triggers transcriptional reprogramming, and this process is synergistically linked in some cases with MAPK cascades (Boudsocq et al., 2010). Recently, Arabidopsis CDPKs, ACPK4, 5, 6 and 11, were shown to re-localize to the nucleus and to interact with and to phosphorylate the WRKY factors, AtWRKY8, 28 and 48, which in turn regulate expression of AtWRKY46 during ETI mediated by RPS2 or RPM1. Mutants in AtWRKY8 or AtWRKY48 fail to induce full expression of AtWRKY46 and AtSID2 (Gao et al., 2013). AtWRKY28 directly interacts with the promoter of AtSID2 (van Verk et al., 2011). Thus, during RPS2- or RPM1-mediated ETI, these CDPKs relay Ca2+ signatures to generate downstream responses including AtSID2 expression and SA accumulation through the action of WRKY transcription complexes. Whether this also occurs during ETI mediated by nucleocytoplasmic NLRs will be of interest to test.

4. Additional factors contributing to the immune transcriptional network

Apart from the direct consequences of TF binding to regulatory regions of immune genes, several other factors influence the complexity and fine gradations of transcriptional outputs. Certain components of the mediator complex play selective roles in plant immunity (Kidd et al., 2009; Ou et al., 2011; An & Mou, 2013). Among these, AtMED18 and AtMED25 have been identified to interact with the TFs YY1 and AtERF1 (ETHYLENE RESPONSE FACTOR1), respectively, and specifically impact JA signaling (Ou et al., 2011; Lai et al., 2014). In addition, several components of the post-transcriptional gene silencing pathway have been shown to modulate PTI and ETI (Pumpkin & Voinnet, 2013; Boccarda et al., 2014). In one case it was shown that siRNA-directed DNA methylation of the AtWRKY22 promoter precludes transcription. Signal information decoded from changes in the chromatin state of the host during pathogen infection can have clear consequences on the ultimate immune response (Alvarez et al., 2010; Berr et al., 2012). The histone modifying enzyme AtHDA19 (HISTONE DEACETYLASE19) of Arabidopsis interacts with AtWRKY38 and AtWRKY62, thereby influencing disease resistance to P. syringae (Kim et al., 2008). AtHDA19 has also been shown to interact with AtTPR1 (TOPLESS-RELATED1), a transcriptional co-repressor that is required for resistance mediated by the TNL SNC1. Altered methylation states of the chromatin at specific promoter sites have also been identified that may modulate expression of the immune response. Treatment of Arabidopsis plants with the SA analog BTH resulted in elevated levels of histone 3 acetylation (AcH3) and dimethylation of lysine-4 of histone 3 (MeH3K4) at the AtPR1 promoter (Mosher et al., 2006). Infestation of Arabidopsis plants by P. syringae or upon application of BTH, resulted in increased H3K4 trimethylation at the AtWRKY6, AtWRKY29 and AtWRKY53 promoters, which correlated with augmented expression of the genes following subsequent application of a secondary stress (Jaskiewicz et al., 2011). Similarly, altered H3K4 methylation by the histone methyltransferase AtATX1 (Arabidopsis homolog of trithorax) was observed at the AtWRKY70 promoter in wild-type Arabidopsis plants challenged with P. syringae, and maximal transcriptional activation of AtWRKY70 was shown to require AtATX1 function (Alvarez-Venegas et al., 2007).

Other levels of regulation can also affect transcriptional immune responses including ubiquitination (Marino et al., 2012), sumoylation (Park et al., 2011), alternative mRNA splicing (Yang et al., 2014) and alternative polyadenylation (Tsuchiya & Eulgem, 2013), but discussion on these are beyond the scope of this review.

IV. Transcription factor networks in phytohormone signaling

Phytohormones are signaling molecules that regulate major portions of plant immunity (reviewed by Pieterse et al., 2012; Kazan & Lyons, 2014). Literally, all phytohormones including SA, JA, ethylene, ABA, GA, auxin, cytokinin and brassinosteroid (BR) are involved in plant immunity, and they form complex interwoven phytohormone signaling networks to coordinate diverse stress responses and growth. Among them, SA, JA and ethylene are core immune phytohormones (Pieterse et al., 2012). In general, SA is a positive regulator of immunity against biotrophic and hemibiotrophic pathogens, whereas JA and ethylene are positive regulators of immunity against necrotrophic pathogens. Other phytohormones modulate the core phytohormone signaling network and their interactions. Phytohormone biosynthesis is regulated during immunity, and the produced phytohormones are perceived by dedicated receptors that transduce signals to transcriptional complexes. These processes are governed by the core TFs in each signaling cascade and, furthermore, hormonal crosstalk occurs at the level of TFs (Fig. 4). We discuss the significance of phytohormone transcriptional networks with a focus on phytohormone crosstalks regulated by TFs.

1. Salicylic acid (SA)

SA is mainly synthesized in chloroplasts and exported to the cytosol through chloroplast membrane-localized ADE55 (Verberne et al., 2000; Mauch et al., 2001; Wildermuth et al., 2001; Ishihara et al., 2008; Serrano et al., 2013). Induced SA accumulation is sensed by the central regulator AnNPR1 (NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1), which is required for the majority of the SA analog BTH-mediated transcriptional outputs (Wang et al., 2006). AnNPR1 interacts with Clade I (AtTGA1 and 4), II (AtTGA2, 5 and 6) and III (AtTGA3 and 7) bZIP TFs at least in yeast two-hybrid assays, which led to a model that NPR1 coordinates SA-mediated transcription with TGA factors (Zhang et al., 1999; Zhou et al., 2000; Despres et al., 2003; Song et al., 2011). Although this is a simple and attractive model, regulation of SA-mediated transcriptional reprogramming by NPR1 appears to be much more complex. We refer readers to recent reviews for the details (Pajerowska-Mukhtar et al., 2013; Yan & Dong, 2014).

2. Jasmonic acid (JA)

JA biosynthesis is initiated in chloroplasts and JA appears to be converted to the bioactive molecule JA-isoleucine (JA-Ile) in the cytosol (Mosblech et al., 2009). Although JA accumulation often
increases upon pathogen infection, it remains unresolved whether JA biosynthesis is controlled at the transcriptional level during immunity. Perception of JA-Ile by the E3 ubiquitin ligase AtCOI1 (CORONATINE-INSENSITIVE1) triggers degradation of AtJAZ (JASMONATE ZIM-DOMAIN) proteins that act as negative regulators of JA signaling by interacting with the bHLH TF AtMYC2 and its homologs (Chini et al., 2007; Thines et al., 2007). Next to JAZ, the repressor complex associated with AtMYC2 at JA-response gene promoters includes AtNINJA (NOVEL INTERACTOR OF JAZ) and the Groucho/Tup1-type co-repressor TPL (AtTOPLESS; Pauwels et al., 2010). JA-Ile perception releases AtMYC2 from the repressor complex, thereby enabling AtMYC2 to regulate JA-mediated transcription (Chini et al., 2007; Pauwels et al., 2010). In addition to JAZ proteins, additional negative regulators of JA signaling have been identified. For instance, AtJAV1 (JASMONATE-ASSOCIATED VQ MOTIF GENE1) negatively regulates JA signaling and is degraded by the 26S proteasome pathway. This degradation appears to promote JA signaling mechanisms leading to immunity against B. cinerea and the insect Spodoptera exigua. AtJAV1 interacts with AtWRKY28 and AtWRKY51 that regulate JA signaling positively and negatively, respectively, although the regulatory mechanism remains unknown (Hu et al., 2013).

3. Ethylene

The production of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthases (ACS) is the first committed and very likely the rate-limiting step in ethylene biosynthesis (Broekaert et al., 2006). Ethylene production is enhanced upon perception of MAMPs such as flg22 (Liu & Zhang, 2004). In Arabidopsis, stabilization of AtACS2 and AtACS6 proteins through phosphorylation by AMPK3 and AMPK6 is a critical step for enhanced ethylene production during

Fig. 4 Transcription factor sub-networks in phytohormone signaling. This figure describes several examples in which multiple signaling pathways converge at transcriptional complexes (a, d) and at gene promoters (b, c). However, for simplicity, not all associated components and pathways are shown. (a) Convergence of multiple signaling pathways at transcriptional complexes. The Arabidopsis transcription factors AtEIN3/EIL1 and AtMYC2 inhibit each other, representing antagonism between ethylene and jasmonic acid (JA) signaling. AtMYC2 also directly suppresses AtORA59 transcription. AtEIN3/EIL1 are stabilized by ethylene signaling and released from JAZ (JASMONATE ZIM-DOMAIN) protein suppression upon JA signaling, representing synergy between ethylene and JA signaling. The small transcriptional regulators, DELLAs, compete with AtMYC2 for binding to AtJAZ1, providing the mechanism for antagonism between JA and gibberellin (GA) signaling. (b, c) Convergence of multiple signaling pathways at gene promoters. (b) JA- and abscisic acid (ABA)-activated AtMYC2 positively regulates JA-responsive genes containing G-boxes in their promoters, and the ABA-inducible transcription factor AtIAM1 competes with AtMYC2 for G-box binding to repress gene expression. (c) Ethylene-activated AtEIN3/EIL1 and the JA-inducible transcription factors AtNAC019/055/072 suppress AtSID2 transcription whereas Ca2+/CalM-regulated AtCBP60g activates it. These transcription factors bind different DNA sequences. (d) Shared transcription factors by two signaling pathways. The transcription factors AtTGA2, 5, and 6 control both ethylene- and salicylic acid (SA)-mediated transcription. AtTGA2, 5, and 6 are required for SA-mediated activation of AtPR1 and for SA-mediated suppression of AtORA59 induction by ethylene. Solid red and gray arrows indicate experimentally verified interactions, whereas dashed arrows indicate potential unverified interactions.
PT1 and immunity against B. cinerea (Liu & Zhang, 2004; Joo et al., 2008; Han et al., 2010). Thus, control of ACS enzymes at the protein level is critical, although ACS transcription is also controlled during immunity. In Arabidopsis, AWRKY33, which is phosphorylated by AtMPK3 and AtMPK6, directly binds to the promoters of AtACS2 and AtACS6, thereby regulating their transcription and ethylene production (Li et al., 2012). Produced ethylene binds to its receptors such as AtETR1 (ETHYLENE RESPONSE1) that predominantly localize to the membrane of the endoplasmic reticulum. In the absence of ethylene, the active receptors negatively regulate the key signaling component AtEIN2 (ETHYLENE INSENSITIVE2) through phosphorylation via the protein kinase AACKR1 (CONSTITUTIVE TRIPLE RESPONSE1) (Merchant et al., 2013). In this state, the key TFs AtEIN3 (ETHYLENE INSENSITIVE3) and AtEIL1 (EIN3-LIKE1) are constantly being degraded by two F-box proteins, AtEBF1 (EIN3 BINDING F-BOX1) and AtEBF2, through the 26S proteasome (Guo & Ecker, 2003; Potuschak et al., 2003; Gagne et al., 2004). Upon ethylene perception, the receptors are inactivated, which in turn facilitate AtEIN2 activation, thereby initiating subsequent ethylene signaling. Upon activation, the C-terminal part of AtEIN2 is cleaved and translocates to the nucleus to stabilize AtEIN3 and AtEIL1 and to induce degradation of AtEBF1 and AtEBF2, thus further stabilizing AtEIN3 and AtEIL1 (Ju et al., 2012; Qiao et al., 2012; Wen et al., 2012). AtEIN3 and AtEIL1 regulate the expression of ethylene-responsive genes such as the TF genes AtERF1 and AtOR59 (OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF59, Solano et al., 1998; Zandet et al., 2012). These TFs regulate the expression of defense genes such as AtPDF1.2.

4. JA with other phytohormones

Synergy between JA and ethylene signaling for immune transcriptional reprogramming has often been observed (Xu et al., 1994; Penninckx et al., 1998; Thomma et al., 1999; Lorenzo et al., 2003). For example, expression of AtERF1 is induced by ethylene, JA or synergistically by both hormones and requires both Arab1 and AtEIN2. Overexpression of AtERF1 rescues immune response defects in coi1 and ein2 mutants (Lorenzo et al., 2003). Similar observations were made for AtOR59 (Pro et al., 2008). These results indicate that induced expression of AtERF1 and AtOR59 is the consequence for synergy between ethylene and JA signaling. Moreover, the activities of the key TFs of ethylene signaling AtEIN3 and AtEIL1 are also suppressed by JAZ proteins through the co-repressor AtHD1A6 (HISTONE DEACETYLASE6; Zhu et al., 2011). Thus, AtEIN3 and AtEIL1 are interaction sites for synergy between ethylene and JA signaling; with ethylene stabilizing and JA de-repressing AtEIN3 and AtEIL1, resulting in high expression of AtERF1 and AtOR59 (Fig. 4a). However, ethylene and JA signaling can also act antagonistically. AtMYC2 physically interacts with AtEIN3 to inhibit its DNA binding activity and conversely, AtEIN3 represses AtMYC2 function (Fig. 4a; Song et al., 2014; X. Zhang et al., 2014). AtMYC2 also directly binds the promoter of AtOR59 to suppress its expression (Fig. 4a) and myc2 mutants are resistant to B. cinerea infection (Zhai et al., 2013).

Thus, coordinated transcription and accumulation of the TFs AtERF1 and AtOR59 by ethylene and JA are important for immunity against necrotrophic pathogens, and ethylene and JA signaling interlink at key transcriptional regulators such as AtMYC2 and AtEIN3/EIL1, resulting in synergistic or antagonistic outcomes.

AtMYC2 is also the central convergent point for other signaling pathways. GA is perceived by the receptors GA INSENSITIVE DWARF1 (GID1) and its homologs, and GA-binding to GID1 enables the interaction with the negative regulators of GA signaling, the DELLA proteins, and subsequent destruction of DELLAs through the 26S proteasome pathway. DELLA proteins modulate GA-mediated transcription by interacting with multiple regulatory proteins including a set of TFs (Shan et al., 2012). Interestingly, DELLAs compete with AtMYC2 for binding to AtJAZ1. In the absence of GA, DELLA proteins bind to AtJAZ1, resulting in release of AtMYC2 to promote JA signaling, whereas GA triggers degradation of DELLAs, allowing AtJAZ1 to inhibit AtMYC2 function (Fig. 4a; Hou et al., 2010). Therefore, AtMYC2 represents a convergence site for the antagonism observed between GA and JA signaling. Like AtMYC2, AtJAM1/1/AIB (JA-ASSOCIATED MYC2-LIKE1/ABA-INDUCIBLE BHLH-TYPE TRANSCRIPTION FACTOR) is a bHLH TF and regulates ABA-mediated responses (Li et al., 2007; Nakata et al., 2013). AtJAM1 and likely its homologs AtJAM2 and 3 compete with AtMYC2 for DNA binding to target sequences, the likely mechanism for negative regulation of JA signaling (Nakata et al., 2013; Sasaki-Sekimoto et al., 2013). Thus, the TFs AtMYC2 and AtJAM1/2/3 provide a platform for JA–ABA antagonism through transcriptional regulation (Fig. 4b).

Various strains of P. syringae produce the phytotoxin coronatine, which is a molecular mimic of JA-Ile (Browse, 2009). Like JA-Ile, coronatine is perceived by COI1 complexes and this perception triggers AtMYC2 release, thereby contributing to coronatine-mediated transcription (Chini et al., 2007; Katsir et al., 2008; Melotto et al., 2008; Yan et al., 2009). AtMYC2 directly interacts with the promoters of three homologous NAC TFs AtANAC019, AtANAC055 and AtANAC072 and activates their transcription. These ANACs bind to the promoter of AtSIS1 and negatively regulate its expression (Fig. 4c; Zheng et al., 2012). In addition, they also directly interact with the promoters of AtBSMT1 (SA METHYL TRANSFERASEI) and AtSAGT1 (SA GLUCOSYL TRANSFERASE GENE1) and positively regulate expression of these genes. AtBSMT1 and AtSAGT1 convert SA to the inactive forms (Dean & Delaney, 2008). Therefore, the action of these ANAC proteins results in reduced SA accumulation, providing a mechanism for the virulence of the coronatine-producing bacterial pathogen. Because expression of these ANAC genes is induced by JA (Tran et al., 2004), it is likely that JA activates the same signaling cascades as coronatine does to suppress SA accumulation.

5. SA with other phytohormones

ABA mediates stomatal closure (Laanemets et al., 2013). Stomata are the main routes for bacterial entry into plants, and therefore ABA signaling can be considered a positive contributor to
immunity against bacterial pathogens (Melotto et al., 2006). In pathogen-induced stomata closure, SA and ABA function coordinately (Melotto et al., 2006; Zeng & He, 2010). However, ABA signaling suppresses SA signaling, thereby negatively regulating post-entry immunity against bacterial pathogens (Yasuda et al., 2008; de Torres Zabala et al., 2009). Interestingly, ABA induces expression of *AtANAC019*, *AtANAC055* and *AtANAC072*, and ABA accumulation is required for corona-triggered expression of the ANAC genes. Furthermore, these ANACs are involved in ABA-mediated responses (Zheng et al., 2012). Thus, these NAC TFs may act at the interface of SA suppression by both JA and ABA. Consistent with negative regulation of post-entry immunity by ABA, the type III effector AvrPtoB from *P. syringae* induces ABA accumulation through upregulation of the ABA biosynthesis gene *AtNCED3* (9-CIS-EPoxyCAROTENOID DIOXYGENASE3) by a currently undefined modus (de Torres-Zabala et al., 2007). Ethylene signaling also suppresses SA signaling. *AtEIN3* directly binds the AtSID2 promoter to repress its expression (Fig. 4c; Chen et al., 2009). Consistently, the *ein3 eil1* double mutant shows constitutive SA accumulation.

The bZIP TFs *AtTGA2*, *AtTGA5* and *AtTGA6* are essential for SA-mediated transcription of genes such as *AtPR1* (Zhang et al., 2003). Interestingly, the same TGA factors are also essential for ethylene-mediated *AtPDF1.2* induction (Fig. 4d; Zander et al., 2010). Although both JA and ethylene accumulation lead to *AtERF1* and *AtORAS9* expression thereby contributing to *AtPDF1.2* induction, JA-mediated *AtPDF1.2* expression does not require these three TGA factors (Zander et al., 2010). SA signaling suppresses both JA and ethylene-mediated gene expression including induction of *AtPDF1.2*. Suppression of JA- and ethylene-dependent *AtPDF1.2* expression by SA is mediated by the same TGA factors (Ndumukong et al., 2007; Zander et al., 2014). The TGA factors directly interact with the *AtORAS9* promoter and activate its expression in the presence of ethylene, whereas these TGA factors are required for suppression of *AtORAS9* expression by SA (Zander et al., 2014). In Arabidopsis, recognition of cytokinins by receptors initiates phosphorylation of type-B Arabidopsis response regulators (ARRs), which function as TFs (Gruhn & Heyl, 2013), and cytokinin-mediated signaling interacts with immune responses also involving SA (Naseem et al., 2014). For instance, cytokinin signaling synergistically acts on SA signaling. *AtTGA3* forms a complex with *AtARR2*, and this complex provides the basis of synergism between SA and cytokinin signaling (Choi et al., 2010). Thus, TGA TFs are convergent points for multiple signaling pathways.

V. Discussion and perspectives

Over the past decade enormous progress has been made in identifying key components, regulatory nodes and individual TFs involved in various signaling pathways associated with plant immunity. Combined with global expression and chromatin immunoprecipitation studies, along with computational approaches, we are beginning to define the transcription regulatory networks governing plant immunity. Transcription regulatory networks describe the interaction of TFs and the genes that they regulate. Although this sounds straightforward, the truth of the matter is that precisely defining specific transcriptional networks remains extremely challenging. There are several reasons for this. For one, our understanding on how diverse upstream signaling pathways prioritize diverse environmental and cellular cues and relay the appropriate signal information to the downstream transduction pathways remain very limited. Based on work mostly in the animal field it is obvious that various factors will determine the selective response of signaling pathways and how this response translates into distinct transcriptional outputs (Purvis & Lahav, 2013). Such factors include intensity, duration and fluctuation of signaling pathway stimulation, inter-communication between different signaling pathways (crosstalk), the topology of the signaling network, and, in particular, the dynamics of signaling (Housden & Perrimon, 2014). Thus, to understand transcriptional networks in their entirety we will need to dissect the complex mechanisms that integrate spatial and temporal information to generate the diversity observed in signaling outputs.

Currently molecular components regulating and decoding signaling dynamics remain unknown but their elucidation is of utmost interest as TF dynamics can also control gene expression (Purvis & Lahav, 2013). Indeed, K. N. Chang et al. (2013) recently characterized the dynamic ethylene transcriptional response in Arabidopsis by determining the targets of *AtEIN3*, the master regulator of ethylene signaling. A major unforeseen finding of these studies was that ethylene-induced transcription occurs in temporal waves regulated by *AtEIN3*, thereby revealing distinct layers of transcriptional control.

Presently our picture of the transcriptional networks underlying plant immunity is extremely fragmentary and mostly relies on isolated cases of TFs impacting selective signaling pathways, and the expression of a limited number of individual target genes as outlined above. Numerous genetic and global expression studies have revealed that the plant immune response to diverse pathogens involves simultaneous activation of multiple hormonal signaling sub-pathways that often share common TFs. Thus, the complexity of gene regulation circuitry and the technical challenges associated with analyzing host–microbe interactions in complex plant tissue under *in vivo* conditions limit our current knowledge on the molecular mechanisms involved in prioritizing and regulating appropriate transcriptional outputs. In fact, to date there has been no reports on the genome-wide binding of TFs in the context of plant immunity using, for example, methods such as ChIP-seq, and what the consequences of such binding are with respect to the expression of identified immune-response target genes. Nevertheless, certain general principles have been uncovered that are helping to understand how certain TFs act on the defense gene regulatory network. For example, many gene members of the Arabidopsis WRKY TF family are themselves transcriptionally activated during immune signaling (Pandey & Somsich, 2009). Experimental and bioinformatics analyses have revealed that WRKY factors form a complex and highly interconnected regulatory network containing several recurring regulation patterns, called ‘network motifs’ (Alon, 2007). The motif structure of this WRKY network, which includes both positive and negative feedback and feedforward loops (Fig. 5), probably ensures rapid and efficient signal amplification but at the
same time allows for tighter control in limiting the extent of the host immune response (Llorca et al., 2014).

No eukaryotic TF operates on its target genes in isolation but instead acts in a combinatorial manner with other TFs, co-regulators and auxiliary factors that are required for proper RNA polymerase II-dependent gene expression. Unfortunately the number of well-characterized genetically verified DNA–protein complex associations involved in immunity is still currently limited (An & Mou, 2013; see also Table S1). Although numerous additional plant TF-TF and TF-cofactor interactions have been discovered, mainly by yeast-two-hybrid screens and via in vitro protein–protein studies, their biological significance remains to be experimentally validated and their in vivo DNA targets identified.

The availability of ever more affordable high-throughput technologies (ChIP-seq, RNA-seq, miRNA-seq, DNasel-seq, FAIRE-seq, high-resolution mass spectrometry), large-scale data generation projects and sophisticated computational resources will certainly have a strong positive impact on elucidating the fundamental structure of transcriptional networks associated with plant immunity. These advances will allow us to construct testable gene regulatory network models to address how plants cope with the diverse set of phytopathogens within their environment. Detailed understanding of the fundamental principles underlying the regulatory circuit is not only of academic interest, but is also of vital importance for the design of future strategies aimed at crop improvement. For example, we are beginning to shed light into the complex regulatory mechanisms responsible for the often-observed trade-off between plant growth and immunity (De Bruyne et al., 2014; Huot et al., 2014). The Arabidopsis bHLH transcription factors AtBZR1 and AtBE1/AtBZR2 are key players in shaping BR signaling allowing proper plant growth and development. BR was found to suppress immune signaling by the interaction of AtBZR1 with AtWRKY40, a co-repressor of PTI (Lozano-Duran et al., 2013). The Arabidopsis bHLH TF AtHBI1 was identified as another central component in this process acting downstream of AtBZR1 (Fan et al., 2014). AtHBI1 negatively regulates a subset of immune response genes while positively affecting genes in other hormone signaling pathways utilized for plant growth. Additionally, the DELLA repressors of GA signaling also interact with AtBZR1, but also with the JAZ repressors associated with the JA immune response (De Bruyne et al., 2014). The lessons learned from these and similar studies will guide us in our endeavor to optimize plant performance towards pathogens without a substantial loss in yield.

Still, despite the advances, there are several foreseeable problems intrinsic to plant research that will continue to pose a major challenge for rapid progress. As highlighted by the excellent achievements of the international ENCODE project aimed at identifying all functional elements within the human genome (Encyclopedia of DNA Elements; http://www.genome.gov/encode/), the methodologies now appear to be in place to tackle similar questions in plants. However, research in the animal field often benefits from its potential application in medicine and human health. Thus, commercial vendors are often actively involved in providing valuable resources such as the production of vital antibodies required for methods such as ChIP-seq, co-IPs and western detection. Even so, one must keep in mind that currently antibodies for some 230 protein isoforms are available to this project (Bolouri, 2014). Although impressive, this represents still only c. 10% of all human TFs (estimated to be 1500–3000). In plants the number of antibodies available for such studies is dramatically smaller despite the number of TFs in most plant genomes being similar to that in humans. An additional problem is that most experiments need to be performed in intact plant tissue using various pathogens having diverse and often discreet temporal and spatial infection strategies. Almost no specialized cell lines that can simplify experimentation are available for plants. Nevertheless, technologies such as DNasel-seq that do not require specific antibodies, and the diverse genetic tools available particularly for Arabidopsis should at least partly help to compensate for such deficiencies. Furthermore, the apparent applicability of transcription Activator-Like Effector Nucleases (TALENs) and the CRISPR-CAS systems in plants for diverse genome editing and targeting approaches (Voytas & Gao, 2014) will certainly prove beneficial to significantly advance this research area in the future. Finally, there is an urgent need to improve and broaden the molecular toolkit to monitor signaling pathway dynamics at high resolution in intact tissue in order to understand the spatial and temporal dynamics of the regulatory network in planta. Only by incorporating such dynamic information will we be able to fully understand the transcriptional network governing plant immunity.
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References


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A new type of TGA transcription factor that interacts with jasmonate and ethylene signaling pathways has been identified. This factor, which we have named JAZ5, plays a crucial role in regulating plant defense responses.

JAZ5 is a member of the TIR-NBS-LRR family of disease-resistance genes and is involved in the regulation of ethylene signaling. It contains a conserved domain that is required for its function in planta. The expression of JAZ5 is induced by jasmonate treatment, suggesting a role in mediating the cross-talk between the jasmonate and ethylene pathways.

The JAZ5 gene is activated upon treatment with jasmonate and ethylene, and its expression is regulated by the transcription factor JAZ5. The JAZ5 protein is localized to the nucleus, suggesting that it is involved in the regulation of gene expression.

In summary, the discovery of JAZ5 provides new insights into the molecular mechanisms that underlie plant defense responses. These findings may have implications for the development of new strategies to control plant pathogens and improve crop productivity.


**Supporting Information**

Additional supporting information may be found in the online version of this article.

**Table S1 List of TFs and cofactors associated with plant immunity**

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