CHARACTERIZATION OF NPR1 SUPPRESSORS AND THEIR ROLE IN PLANT IMMUNITY

By

CHRISTOPHER DEFRAIA

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
UNIVERSITY OF FLORIDA
2010

This dissertation is dedicated to those who seek the truth.

ACKNOWLEDGMENTS

First and foremost, I thank Zhonglin Mou for his inspiration, guidance, and patience. Sharing heated scientific discussions and exciting results was a pleasure and a privilege. I thank all the members, past as present, of his laboratory including Yuqing Xiong and Yongsheng Wang. I would especially like to thank Xudong Zhang for providing much of my training. Mrs. Zhang generated the data for the characterization of the *35S::ELP2-GFP* plants and the determination of avirulent pathogen growth. I thank George Marek for mapping the *anac1* mutant, and for counting me worthy to be his teacher. I thank Mary Wildermuth for providing the primers for *ICS1* expression analysis, and Mieke Van Lijsebettens for the generous gift of the *elo* seeds. I thank Keelnatham Shanmugam, William Gurley, Eric Triplett, and David Oppenheimer for their advice and keen analysis. I thank my colleagues in the Department of Microbiology and Cell Science for technical assistance, use of equipment, and helpful discussions. I thank my parents, Gary and Isabelle and my brother, Dan for their love and support. I thank my great friends Maxim Salganik, Matthew Desarle, Matthew Reis, and Helen Mcguirk, for their encouragement and counsel. I thank all my teachers, who were a candle in the dark to me. Finally, I thank the National Science Foundation, Margaret Davidson, and the University of Florida for funding my work.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

CHARACTERIZATION OF NPR1 SUPPRESSORS AND THEIR ROLE IN PLANT IMMUNITY

Christopher DeFraia

May, 2010

Chair: Zhonglin Mou
Major: Microbiology and Cell Science

Plants have evolved inducible immune responses to pathogen infection. Pathogen-induced, isochorismate synthase-dependent salicylic acid (SA) biosynthesis promotes immunity to biotrophic pathogens, which keep the host alive as a long-term food source, partially through NPR1 (non-expresser of pathogenesis-related 1) activation. NPR1 also prevents harmful SA hyperaccumulation and SA cytotoxicity through an unknown mechanism. In this study, mutation of three genes was found to restore SA tolerance to *npr1*. Overexpression of one of these genes, the transcription factor ANAC1, was associated with increased pathogen resistance, but this gene was not essential for immunity. The other two genes, *ELP2* (Elongator subunit 2) and *ELP3* (Elongator subunit 3), encode subunits of the histone acetyltransferase Elongator, which is conserved in eukaryotes and functions in RNA polymerase II-dependent transcription, as well as in tRNA modification, exocytosis, and tubulin modification. Mutation of human ELP1 (Elongator subunit 1), causes the neural disorder familial dysautonomia. This study shows Elongator functions both upstream and downstream of SA to positively regulate biotrophic pathogen resistance, and does so in an NPR1-independent manner. Plants lacking ELP2 were susceptible to avirulent pathogen infection, possibly due to

the delayed induction of defense genes including *ICS1*. Plants lacking both ELP2 and NPR1 were highly susceptible to avirulent pathogen infection compared to the single mutants, suggesting ELP2 and NPR1 act synergistically in plant immunity. However, pre-activation of defense genes during systemic acquired resistance (SAR) restored pathogen resistance to *elp2* plants. In light of these results, a model is proposed where Elongator promotes immunity through the acceleration of defense gene activation.

CHAPTER 1
INTRODUCTION

Why Study Plants and Their Immunity?

Human Nutrition

Approximately one sixth of people worldwide are malnourished due to the uneven distribution of resources. According to one study, six million people die each year from malnutrition, and ~800 million are malnourished. Most of the food supply comes directly from plants, and the rest is indirectly derived. Although enough food is produced to provide each person on earth with an adequate diet, poverty, plant disease, environmental disasters, and political strife cause local food shortages (Leathers and Foster, 2004). Understanding the factors that affect our food supply is essential for devising strategies to promote food security and fight malnutrition.

Food supply is largely affected by agricultural productivity. In the last century, agricultural production has greatly increased due to the use of industrial machinery and fertilizer. This increase has mostly been limited to industrialized countries, where agricultural problems affect profits more than nutrition. In developing countries, increases in agricultural production and farming cost reduction would increase access to cheaper food while keeping for-profit farmers in business and subsistence farmers nourished (Leathers and Foster, 2004).

Non-Nutritional Applications

Other plant applications include biofuel production, phytoremediation, and phytochemical production (the plant kingdom can produce >100,000 metabolites).

(McGuinness and Dowling, 2009; Turner, 2009) (Trethwewny, 2004). Plants are also sought after for their natural beauty.

Impacts of Plant Disease

One major factor affecting plant growth is disease. Disease results in lower crop yield and quality, and in most cases occurs after investments of water, fertilizer, and labor (Alam and Rolfe, 2006). The adoption of crop monoculture also contributes to the spread of disease. Monoculture of crops is an efficient method of production. However, it exacerbates disease outbreaks because non-host barriers do not interrupt large swaths of potential hosts (Zhu et al., 2000). The Irish potato famine, caused by *Phytopthora infestans*, was responsible for the deaths of between two hundred thousand and 1.2 million people, though as is usually the case with famine, political, cultural, and economic conditions were also major factors (Gráda, 2006). More locally, citrus canker (*Xanthomonas axonopodis*) and citrus greening (likely caused by *Liberibacter* species) are damaging the Florida citrus industry (Parnell et al., 2009).

Disease Management

Currently, most disease management practices focus on prevention. Once a diseased plant is detected, it is often destroyed to prevent dissemination (Fry, 1982). Disease “treatment” in the clinical sense is rare. While knowledge of the mammalian immune system has allowed the invention and widespread use of medical drugs, the lack of understanding of plant diseases and plant immunity has limited such advancements for plants. Antibiotic therapies have been tried, but cost and other factors have limited their use (Daniels, 1982).

Pesticides are used to contain disease and pests, but these applications can be detrimental to the environment and human health. The fact that these costs are not borne by the farmer, combined with the effectiveness of pesticides at increasing crop yield and quality, contributes to their widespread usage (Phipps, 1989). Chemical companies have designed crops around tolerance to pesticides and herbicides (Funke et al., 2006). Recent advancements in the understanding of plant immunity have led to the introduction of compounds that activate the plant's innate defenses. Though effective, the biological consequences of these treatments are not fully understood. A better understanding of the plant immune system will facilitate the use of these treatments, and allow the rational design of disease treatments and disease-resistant crops, reducing the need for toxic and costly pesticides. In the developing world, plant immunity research can inform crop selection and management practices, while reducing the cost of farming, provided advances are effectively communicated and distributed.

Understanding Cell Biology Through Plant Immunity

Investigation of plant-pathogen interactions has brought insights into diverse biological processes. Upon pathogen infection, significant transcriptional reprogramming occurs (Tao et al., 2003) (Schenk et al., 2000). Knowledge of defense gene regulation has led to a greater understanding of how genes are regulated generally. This is also the case for other cellular processes, as the entire cell works in concert to combat the infection. Many cellular processes have been implicated in disease resistance. A few examples are apoptosis, metabolism, photosynthesis, transport, cell wall synthesis, stomata closure, and signal transduction. Plant-pathogen interactions also provide an interesting case study of co-evolution (Katagiri et al., 2002). The reproductive success

of a pathogen often depends on its ability to infect its host, just as the plant will be less successful if infected. Thus, plants and their pathogens are locked into an evolutionary arms race (Thompson and Burdon, 1992) (Bergelson et al., 2001). As discussed below, the race alternates between the pathogen suppressing defense activation, and the plant countering this suppression.

Pathogenesis

Initial Contact

Plants possess an innate immune system that protects them from microbial pathogens, but lack specialized immune cells and long-term immunological memory of mammals (Nurnberger et al., 2004). Due to the sessile nature of plant life, plants must quickly and effectively respond to changes in their environment or perish. Most often, when a microbe contacts the plant, entry is prevented by preformed and constitutive defenses such as the waxy cuticle, the cell wall, antimicrobial enzymes, and secondary metabolites (Martin, 1964) (Hahn et al., 1989) (Broglie et al., 1991) (Nicholson and Hammerschmidt, 1992). Elimination or exclusion of the microbe occurs without activation of costly defenses. However, plant pathogens may gain entry by injuries to the plant tissue, enzymatic degradation of cell walls, or through stomata (Romantschuk and Bamford, 1986) (Vidal et al., 1998) (Davis et al., 1984).

Lifestyles of Plant Pathogens

Pathogens utilize two general strategies to gain access to the photosynthate inside plant cells. Necrotrophic pathogens rapidly kill host cells using toxic molecules and lytic enzymes, resulting in rapid tissue necrosis. The necrotroph then grows saprophytically on the dead tissue (van Kan, 2006). Biotrophic pathogens persist and

multiply in live plant tissue, eventually resulting in starvation of the plant cells. Chlorosis and water soaking are followed by complete collapse of the tissue. If successful, both kinds of pathogens eventually kill the infected tissue (Staskawicz et al., 2001) (Glazebrook, 2005).

Model Organisms for the Study of Plant/Pathogen Interactions

The *Arabidopsis thaliana*/*Pseudomonas syringae* pathosystem has emerged as a model for the study of plant/biotrophic pathogen interactions (Katagiri et al., 2002). Genome sequences from both organisms are available, and a large fraction of their genes have been characterized or at least assigned putative functions (The Arabidopsis Genome Initiative, 2000) (Buell et al., 2003). Arabidopsis is also an ideal system for genetics due to its relatively small diploid genome, short generation time, high fertility, ease of growth, and small size. The availability of many polymorphic markers between two ecotypes greatly facilitates the identification of induced mutations, allowing forward genetic screens to be carried out efficiently (Jander et al., 2002). *Pseudomonas syringae* pv. *maculicola* ES4326 (*Psm*) is a hemibiotrophic, plant-pathogenic bacterum that is Gram-negative, rod-shaped, and has polar flagella. Pathovars of this species are assigned based on host range. *Psm* typically invades plants through wounds or stomata (Romantschuk, 1992) (Romantschuk and Bamford, 1986) (Kreig and Holt, 1984). It persists and multiplies in the apoplast, eventually causing “leakage” of water and photosynthate, which it uses to multiply and eventually overwhelm plant defenses (Katagiri et al., 2002).

Infection Outcome Depends on Signal Potentiation and Suppression

Recognition

If the pathogen does invade the plant, it will likely be recognized by its pathogen associated molecular patterns (PAMPs), which are essential and conserved structural motifs that are present in pathogens but not in plants. Examples include the peptide flg22 and elongation factor (EF) Tu, which are components of flagella and the ribosome, respectively. PAMPS are recognized by transmembrane pattern recognition receptors (PRRs), each specific for its cognate PAMP. flg22 is recognized by the plant receptor FLS2 (flagellin sensitive 2), while EF-Tu is recognized by EFR (EF-Tu receptor) (Felix et al., 1999) (Gomez-Gomez et al., 1999) (Kunze et al., 2006). After recognition, the pathogen signal is amplified through a MAP kinase cascade(s); eventually affecting the activity of transcription factors in the nucleus and activating resistance (Nuhse et al., 2000) (Asai et al., 2002). PAMP recognition results in PAMP-triggered immunity (PTI), which limits pathogen growth (Nimchuk et al., 2003).

Immune Responses

PTI includes diverse immune responses. Following pathogen infection, antimicrobial, low molecular weight compounds known as phytoalexins are produced, and cell walls are reinforced by callose deposition (Donofrio and Delaney, 2001) (Skou, 1985) (Keen and Bruegger, 1977) (Smith, 1982). Additionally, antimicrobial pathogenesis-related (PR) proteins accumulate. These are low-molecular weight, thermostable, protease-resistant proteins; some of which degrade microbial cell walls and membranes (Edreva, 2005). Examples include the β-1,3-glucanase PR-2 that breaks down fungal cell walls, and the thaumatin-like PR-5 protein, which has

membrane permeabilizing activity. The PR-1 protein has an unknown function, but can comprise 1-2% of all protein during an infection. During the presence of certain pathogenic effector proteins, plants cells may also undergo a form of programmed cell death, depriving the pathogen of nutrients and water (Holliday et al., 1981) (Goodman and Novacky, 1994). Since pathogens may enter the plant through stomata, stomatal closure also occurs during infection (Melotto et al., 2008). Collectively, these responses limit the growth and spread of the pathogen. The importance of these responses is emphasized by the discovery that they may be directly suppressed by pathogens to promote pathogenesis (Glawischnig, 2007) (Melotto et al., 2008). Additionally, the pathogens attempt to short-circuit the PAMP-generated signal, preventing the activation of these responses.

Signal Suppression

Pathogens may attempt to suppress PTI signaling by injecting effector proteins into the plant cell via a type III secretion system (TTSS). These effector proteins promote virulence and pathogen growth by reducing the plant defense response (Wei et al., 1999) (van Dijk et al., 1999) (Gopalan et al., 1996). An effector protein may, in turn, be recognized by its cognate plant resistance (R) protein, present in resistant cultivars of a given species (Belkhadir et al., 2004). In this case, the effector protein is referred to as an avirulence (Avr) factor, and activates effector-triggered immunity (ETI). Transcriptionally, ETI is qualitatively similar to PTI, but with faster and stronger defense gene activation (Tao et al., 2003). *Psm* and another pathovars, *Pseudomonas syringae* pv. *tomato (Pst)*, secretes over 30 effector proteins into plant cells, and can cause disease in Arabidopsis. Examples of effector proteins secreted by *Pst* DC3000 are

AvrRpm1, which is recognized by the plant R protein RPM1; AvrRps2, which is recognized by RPS2; and AvrRpt2, also recognized by RPS2 (Kunkel et al., 1993) (Boyes et al., 1998) (Ritter and Dangl, 1995) (Chen et al., 2000) (Yu et al., 1993). Recognition of AvrRpm1 by RPM1 is mediated by RIN4. AvrRpt2 is a cysteine protease that cleaves RIN4, preventing AvrRpm1 recognition by RPM1 (Mackey et al., 2003). RIN4 can also bind to RPS2 and prevents its inappropriate activation. AvrRpt2 cleavage of RIN4 triggers activation of RPS2 and resistance (Mackey et al., 2002). These observations have led to a "Guard Hypothesis," which states that R proteins function to detect changes in the targets (i.e. RIN4) of bacterial effector proteins (Jones and Dangl, 2006). The absence of either Avr or R protein results in disease if PTI defenses are insufficient (Jones and Takemoto, 2004; Nurnberger et al., 2004). Addition of an *avr* gene to virulent pathogens renders them avirulent, and addition of *R* genes to plants confers resistance to pathogens containing the cognate *avr* gene (Ronald et al., 1992) (Hammond-Kosack et al., 1998).

Salicylic Acid-Mediated Immunity

Hormones Modulate Plant Immune Responses

Plants can customize their defense response based on the type of pathogen they encounter. This is accomplished by modulation and crosstalk of hormone-dependent signaling pathways. Salicylic acid (SA), jasmonates and jasmonic acid (JA) and ethylene (ET) are the main signaling molecules acting during disease resistance, although hormones like abscisic acid (ABA), auxin, and brassinosteroids also play a role (White, 1979) (Jacobs, 1952) (Jensen et al., 1998) (Whenham et al., 1985) (Gundlach et al., 1992) (Lund et al., 1998) (Spoel and Dong, 2008). These hormones play

significant roles in other physiological processes as well (Roberts et al., 1980) (Hildman et al., 1992). For example, SA regulates germination, flowering, and heat generation (Raskin et al., 1989) (Hayat and Ahmed, 2007). It should be noted that salicylic acid is not a hormone in the strictest sense, as it apparently does not travel from its site of origin to elicit a response in another part of the plant (Vernooij et al., 1994). Nonetheless it is a central signaling molecule in plant immunity (Gaffney et al., 1993) (Lawton et al., 1995). Defense responses involving JA and ET are generally more effective against necrotrophic pathogens and chewing insects (Ton et al., 2002) (Glazebrook et al., 2003). JA- and ET-dependent responses antagonize those of SA and vice versa, although these responses may also act synergistically in some cases (Rojo et al., 1999) (van Wees et al., 2000) (Spoel et al., 2003) (Kunkel and Brooks, 2002).

Salicylic Acid Metabolism

During infection, SA accumulates in cells and promotes pathogen resistance (Métraux et al., 1990) (Durrant and Dong, 2004). Exogenous SA treatment or expression of ectopic SA biosynthesis genes results in resistance, while removal of SA causes susceptibility (Kauss et al., 1992) (Verberne et al., 2000) (Nawrath and Métraux, 1999). In plants, SA is made through two metabolic pathways. Starting with phenylalanine, the phenylpropanoid pathway converts this amino acid into SA. Additionally, chorismate is converted to SA via isochorismate through the action of a chloroplastic isochorismate synthase (ICS1), and a putative isochorismate pyruvate lyase (IPL). ICS1 is the rate-limiting enzyme for pathogen-induced SA biosynthesis during the immune response (Strawn et al., 2006).

SA can be glucosylated through the activity of SA-glucosyltransferase and stored in the vacuole as 2-*O*-β-D-glucosylbenzoic acid (SAG) (Lee et al., 1995). SAG is biologically inert and accumulates to high levels during pathogen infection (Malamy et al., 1992).

Regulation of SA Biosynthesis and Accumulation

SA biosynthesis is amplified by positive-feedback regulation. The interacting lipases PAD4 (phytoalexin deficient 4) and EDS1 (enhanced disease susceptibility 1) are upstream inducers of SA synthesis. In *eds1 pad4* plants, *ICS1* induction is diminished following infection (Falk et al., 1999) (Wiermer et al., 2005) (Zhou et al., 1998) (Jirage et al., 1999). In *pad4* plants, *EDS1* expression is lowered, while in *eds1* plants, *PAD4* expression is lowered, forming a positive feedback loop between PAD4 and EDS1, both of which promote SA accumulation. In turn, SA increases *EDS1* and *PAD4*, expression, suggesting the existence of a positive feedback loop between EDS1/PAD4 and SA (Wiermer et al., 2005) (Delaney et al., 1995; Feys et al., 2001). EDS1 and PAD4 are also essential for PTI and ETI, suggesting they act to amplify the immune response (Durrant and Dong, 2004). SA biosynthesis is also subject to negative feedback regulation by NPR1 (nonexpresser of PR genes 1) (Shah et al., 1997). This protein and its immune functions are discussed in detail below. Conversion of SA to methyl-SA and SAG may also modulate SA levels during infection. Overexpression of the SA glucosyltransferase SGT1 or an SA methyltransferase reduced SA accumulation and resistance during pathogen infection (Koo et al., 2007) (Song et al., 2008). The *Psm* virulence factor coronatine is an analog of JA, and induces

methyl-SA production, reducing free SA levels and increasing susceptibility (Song et al., 2008).

Although conversion of SA to biologically inert compounds and negative feedback regulation by NPR1 serve to prevent SA hyperaccumulation during pathogen infection, *ICS1* must also be repressed during normal growth to avoid wasteful immune activation. Basal repression is accomplished by Ethylene Insensitive 3 (EIN3) and ethylene insensitive 3-like (EIL1). These transcription factors bind to the *ICS1* promoter and prevent transcription (Chen et al., 2009).

Fitness Costs of SA Hyperaccumulation

Constitutive accumulation of SA is detrimental to the plant. Indeed, plants may have evolved an inducible and tightly regulated system of immunity to forgo the cost of constitutive defense activation. The SA analogues benzothiadiazole (BTH), and isonicotinic acid (INA) induce defense activation in plants without cytotoxic effects (Friedrich et al., 1996) (Métraux et al., 1991). However, in the absence of pathogens, BTH incurs a fitness cost in the form of reduced seed yield and fresh weight (Heil et al., 2000) (Heil, 2002). Several *Arabidopsis* mutants that have elevated SA levels and constitutive defense responses also exhibit reduced seed yield and dwarf phenotypes, indicating that unregulated defense activation reduces fitness. In some cases, reduced fitness in these mutants requires SA (Heidel et al., 2004) (Kirik et al., 2001) (Silva et al., 1999).

Systemic Acquired Resistance

SA is essential for systemic acquired resistance (SAR), a long lasting, broad-spectrum resistance to pathogens in systemic tissue following an infection in distal

tissue (Wildermuth et al., 2001) (Gaffney et al., 1993) (Métraux, 2002) (Durrant and Dong, 2004). SAR, like ETI, is qualitatively similar to PTI, employing similar and overlapping signaling pathways (Maleck et al., 2000). However, defense activation is induced more slowly and to a lower level than during PTI or ETI (Ryals et al., 1996). PAMPs, avirulent and virulent pathogens, and high doses of non-pathogenic bacteria all induce SAR (Mishina and Zeier, 2007).

An SAR-inducing mobile signal that moves from local infected tissue to systemic tissue has been proposed (Ross, 1961) (Durrant and Dong, 2004). The identity of the signal(s), and the molecular mechanism underlying its generation and perception has been the subject of intense study. Mutational analysis has revealed several genes that are required for the generation of the mobile signal. DIR1 (defective in induced resistance 1), a putative lipid transfer protein, lacks SAR but displays normal local pathogen resistance, suggesting DIR1 is involved in the generation and/or transmission of the mobile signal (Maldonado et al., 2002). FAD7, a fatty acid desaturase, the lipases EDS1, PAD4, and SA-binding protein 2 (SABP2), as well as the dihydroxyacetone phosphate reductase *SFD1*, are essential for SAR (Chaturvedi et al., 2008) (Forouhar et al., 2005). These data suggest that a lipid or lipid-derived molecule may be a mobile signal in SAR. The aspartic protease CDR1 (constitutive disease resistance 1) is required for both local and systemic resistance, suggesting the possible involvement of a peptide signal in SAR (Xia et al., 2004). Although local SA synthesis and signaling are essential for SAR signal generation, SA and methyl-SA are probably not long-distance

signals (Attaran et al., 2009). Following signal recognition, de-novo SA biosynthesis in systemic leaves occurs and activates resistance (Métraux et al., 1990).

Chromatin Remodeling is Associated with SA-Responsive Gene Activation

DNA encodes all the information needed to build a cell. Further programming instructions in response to environmental cues can also be found on the histones associated with DNA that form chromatin. These ancillary instructions come in the form of post-translational modifications to the N-terminal regions of histones 2, 3, and 4 (H2, H3, and H4). These largely reversible modifications include mono, di, and trimethylation, acetylation, phosphorylation, ubiquitination, and sumoylation (Loidl, 2004). Some of these modifications are associated with actively transcribed chromatin, while others are more closely associated with condensed and relatively silent chromatin. This plant histone "code" for influencing gene expression via histone modifications is still being elucidated. These efforts are complicated by the fact that the effects of histone modifications can depend upon other modifications.

Basal repression of defense genes is mediated by several factors that are thought to recruit chromatin-modifying factors that condense defense gene chromatin. SNI1 (suppressor of *npr1* inducible 1) represses basal expression of defense genes and H3 di-acetylation at the *PR-1* promoter (Li et al., 1999) (Mosher et al., 2006). HDA19 (histone deacetylase 19), a putative histone deacetylase, also represses basal expression of defense genes, possibly by removing histone acetylations that are normally associated with "open" chromatin. However, HDA19 positively regulates pathogen resistance, and interacts with and possibly deactivates the immune repressor transcription factors WRKY38 and WRKY62 (Kim et al., 2008). SIZ1, a small ubiquitin-

like modifier (SUMO) protein, is also a repressor of defense genes (Lee et al., 2007). The involvement of SNI1, HDA19, and SIZ1 in plant immunity suggests a role for histone modification in defense gene repression. However, genes that are directly targeted by these proteins have not been identified, with the exception of *WRKY70* by the histone methylase ATX1 [Alvarez-Venegas, 2007 #6678].

Changes in chromatin modification occur during plant defense induction. Treatment with exogenous SA leads to increased levels of H3Ac, H4Ac, H3K4me2, and H3K4me3 on the *PR-1* promoter (Mosher et al., 2006). These modifications are generally, but not always, associated with actively transcribed chromatin. This increase is NPR1-dependent and correlates positively with PR induction. NPR1 also prevents SA-dependent H3 deacetylation (Butterbrodt et al., 2006) (Koornneef et al., 2008). These results suggest that NPR1 or another protein recruits a histone acetyltransferase(s) and a histone methyltransferase(s) to defense genes during pathogen infection and/or SA treatment. A histone methylase, ATX1, activates the expression of the WRKY70 gene (itself an activator of defense genes), and directly establishes the H3K4me3 modification on WRKY70 nucleosomes, facilitating its transcription. A large number of genes (12%) depended on ATX for their expression, but only WRKY70 was shown to be a direct target of ATX (Alvarez-Venegas et al., 2007). The lack of characterized chromatin modifying enzymes that target defense genes represents a gap in the knowledge of how defense genes are repressed, primed, and induced. In particular, although histone acetylation is correlated with defense gene

expression, no histone acetyltransferases have been shown to regulate plant immunity or modify defense gene chromatin.

The NPR1 Protein

In order to identify components of the SA signaling pathway, researchers genetically screened for mutants lacking SA-inducible *PR* expression. One mutant, *npr1*, was deficient in *PR* expression, susceptible to *P. syringae* and *Hayaloperonospora parisitica*, and lacked SA-induced resistance and SAR (Ryals et al., 1997) (Shah et al., 1997) (Cao et al., 1994). These studies established NPR1 as a positive regulator of SA-dependant resistance that acts downstream of SA. NPR1 encodes a protein containing four ankyrin repeats and a BTB (Broad-Complex, Tramtrack and Bric-a-brac) domain, both of which are involved in protein-protein interactions (Cao et al., 1997).

Post-translational modification regulates NPR1 activity

During normal growth, NPR1 is present as an oligomer in the cytosol and is transcriptionally induced during infection (Mou et al., 2003) (Kinkema et al., 2000). During SAR, SA synthesized in the chloroplast is transported to the cytosol, which becomes more oxidized, followed by a reduction in redox potential and an increase in reduced glutathione. NPR1 is reduced by glutathione, causing NPR1 to form monomers (Mou et al., 2003). NPR1 conformation is also controlled by nitrosylation. Upon SAR induction, NPR1 is S-nitrosylated by S-nitrosoglutathione, facilitating oligomerization, and possibly preventing protein degradation. Both NPR1 oligomerization and monomerization are essential for pathogen resistance and PR expression. At the same time, thioredoxins catalyze the reduction of cysteine-156, disrupting intermolecular

disulfide bonds and promoting monomerization (Tada et al., 2008). Monomeric NPR1 localizes to the nucleus where it functions as a transcriptional co-activator of defense genes (Kinkema et al., 2000).

Spurious NPR1 nuclear accumulation is prevented by a CUL3-based ubiquitin ligase, which adds ubiquitin to NPR1, targeting it for degradation by the proteasome, and preventing PR expression in the absence of infection. NPR1 phosphorylation is essential for this degradation. Proteasome inhibition or mutation of the ubiquitin ligase also prevents the induction of SAR, suggesting NPR1 turnover is essential for its transcriptional co-activator activity (Spoel et al., 2009).

NPR1 coordinates the immune response through transcription factors

Nuclear NPR1 interacts with several members of the TGA transcription factor family (Zhang et al., 1999) (Després et al., 2003) (Johnson et al., 2003) (Després et al., 2000). The effects of these transcription factors are regulated at the level of NPR1 binding, protein turnover, and TGA factor disulfide reduction. Several TGAs have been shown to bind to SA-responsive *cis*-elements in the *PR-1* promoter. TGA factors include both positive and negative regulators of SAR, three of which function redundantly (Fan and Dong, 2002) (Pontier et al., 2001) (Niggeweg et al., 2000). The triple mutant *tga2/5/6* (but not single or double mutants) displayed normal local resistance but lacked SAR and had increased basal *PR-1* (Zhang et al., 2003b). These data suggest that these transcription factors act in concert to ensure *PR-1* is expressed at the appropriate time. NPR1 is also essential for the expression of several WRKY transcription factors, some of which control PR expression (Wang et al., 2006) (Kim et al., 2008) (Yang et al., 1999). Thus, PR expression depends upon SA accumulation and NPR1 activation.

Pathogen infection initiates transcriptional reprogramming, a fraction of which is SA-dependent. NPR1 controls a subset of these SA-dependent defense genes (Glazebrook et al., 2003) (Blanco et al., 2009). During infection, *PR-1*, *PR-2*, and *PR-5* are differentially dependent upon SA and NPR1 for their expression. *PR-1* almost completely depends upon NPR1 for its timely expression during pathogen infection. *PR-2* and *PR-5* are partially *npr1*-independant. *PR-2* requires SA accumulation for its expression, while *PR-5* is partially expressed independently of SA (Nawrath and Métraux, 1999) (Shah et al., 1997). The cellular machinery involved in the induction of PR genes, and the basis for differential PR activation by distinct pathways, is not fully understood.

During SAR, cytosolic oligomeric NPR1 is reduced to monomers which move into the nucleus to control both the expression of PR genes and genes involved in protein folding and transport, presumably for the folding and export of PR proteins (Kinkema et al., 2000) (Després et al., 2000) (Zhang et al., 1999; Després et al., 2000; Kinkema et al., 2000; Fan and Dong, 2002; Després et al., 2003; Johnson et al., 2003; Mou et al., 2003). Interruption of a protein chaperone results in leaf collapse and cell death following treatment with isonicotinic acid (INA), an SA analog, or tunicamycin, a protein misfolding agent (Wang et al., 2005). This suggests NPR1-regulated increases in protein folding and secretion capabilities are essential processes that must be coordinately regulated with defense responses for a non-toxic and effective resistance.

NPR1 prevents harmful hyper-activation of the immune response

Nuclear NPR1 alleviates the cytotoxic effects of high concentrations of SA (Zhang et al., 2009). NPR1 may accomplish this through its control of TGA factors, as

the *tga2/5/6* mutant lacks SA tolerance (Zhang et al., 2003b). Other proteins also likely contribute to SA tolerance. Identification of these proteins would lead to a better understanding of the underlying causes of SA toxicity, which are thought to include oxidative stress (Rao et al., 1997).

Nuclear NPR1 also prevents *ICS1* overexpression and SA hyperaccumulation during the immune response and moderates SA levels in mutants that constitutively accumulate SA (Zhang et al., 2009) (Clarke et al., 1998). This moderation of SA accumulation may serve to prevent SA toxicity and reduced plant fitness, and/or to shut off the immune response once the infection subsides (Durrant and Dong, 2004). Hence, SA accumulation is subject to both positive and negative feedback regulation. How NPR1 moderates *ICS1* expression is unknown.

Dissection of Plant Immunity Using Suppressor Screens

Suppressors of *npr1*

Researchers have previously examined the function of NPR1 by screening for mutations capable of suppressing *npr1* phenotypes. These studies have been instrumental in the identification of components of the SA-dependent defense signaling. Most *npr1* suppressor mutants were isolated based on their ability to restore SA-induced PR expression to *npr1*. One class of *npr1* suppressor mutants is the *ssi* (suppressor of SA insensitivity) group, which exhibit constitutive defense activation. *SSI2* encodes a stearoyl-ACP desaturase, and disruption of this gene in *ssi2* results in a ten-fold increase in 18:0 fatty acid content, suggesting involvement of a fatty acid signal molecule in plant immunity (Kachroo et al., 2001). A mutation in an R gene was responsible for the phenotypes of *ssi4*, which may be due to constitutive R protein

activation (Shirano et al., 2002). Activation of another R protein in *snc1* (suppressor of *npr1 constitutive 1)* resulted in constitutive PR expression and pathogen resistance in *npr1* (Zhang et al., 2003a). A screen for suppressors of *snc1* led to the identification of several genes that were shown to be essential for plant immunity (Zhang and Li, 2005) (Zhang et al., 2005). The *sni1* mutation, mentioned above, restored inducible PR expression and SAR to *npr1*, and selectively de-repressed NPR1-dependent genes (Li et al., 1999). The *sni1* phenotypes were rescued by disruption of Rad51d, a protein involved in homologous recombination (Durrant et al., 2007). These studies highlight the utility of suppressor screens in understanding the function of the suppressed mutant gene as well as in identifying new components of plant immunity.

Identification and Characterization of Three Novel *npr1* Suppressor Mutants

Although the transcriptional co-activator activity of NPR1 has been studied extensively, its role in regulating SA accumulation and SA tolerance is not well understood. To identify genes involved in SA tolerance and SA accumulation, this study screened for mutations that suppressed SA toxicity and SA hyperaccumulation in *npr1*. Thirteen unique suppressor mutants were isolated, and the affected genes in three of these mutants were identified. One mutation was found in the transcription factor ANAC1, which is induced during pathogen infection. Two mutants carried mutations in subunits 2 and 3 of the Elongator complex, which functions in histone acetylation, tubulin acetylation, and tRNA synthesis. *ELP2* and *ELP3* were found to regulate pathogen resistance independently of NPR1. The *elp2* mutants were characterized in detail, and ELP2 was found to function both upstream and downstream of SA by regulating the kinetics of defense gene expression. This study establishes Elongator as

an essential component of plant immunity. The mutant and transgenic plants characterized in this study will facilitate the dissection of Elongator's immune function. A greater understanding of plant immunity will aid in devising strategies for the management of plant disease and the rational design of disease-resistant crops.

CHAPTER 2
MATERIALS AND METHODS

Mutant Screen and Genetic Analysis

Ethylmethylsulfonate (EMS) mutagenesis was carried out as described previously (Weigel and Glazebrook, 2002). Approximately 250,000 M_2 seeds were surface sterilized, plated on ½ strength Murashige and Skoog (MS) [Murashige, 1962 #31] medium supplemented with 0.5 mM SA at a density of ~150 seeds/10 cm plate, and vernalized for three days. Seeds were then germinated with 16 hr light/8 hr dark cycles at 25°C. 10-day-old seedlings with green cotyledons were transferred to ½ strength MS medium without SA, grown for one week, and subsequently transplanted into soil. M3 seeds were tested for SA-tolerance by germinating seeds on ½ MS supplemented with 0.26 mM SA and visually scoring cotyledon color. The Mendelian inheritance of each mutant was determined by crossing to its parent, *npr1*, and scoring the F_1, F_2, and F_3 progeny for SA-tolerance and morphology. Only seeds that germinated were scored. Prior to further characterization, mutants were backcrossed three times.

Growth Conditions and Biological Materials

Plants were grown in Metromix 200 soil under 16 hr light/8 hr dark photoperiods at ~25°C. The Arabidopsis wild types used were *Arabidopsis thaliana* (L.) Heynh. Columbia (Col-0) ecotype and Landsberg erecta (Ler) ecotype. *Escherichia coli* XL-1 Blue was grown in LB broth or on LB agar plates with 50 μg/ml kanamycin. *Psm* was grown in King's B broth or on tryptic soy agar plates supplemented with 50 μg/ml streptomycin. The mutant *npr1-3* has been described previously (Cao et al., 1997).

Insertion mutant alleles were identified using the SIGnAL T-DNA Express Arabidopsis gene-mapping tool (http://signal.salk.edu/). All SALK lines were distributed by the NASC (http://arabidopsis.info/). All mutants in this study were identified by PCR using gene-specific markers as described in Table A-3.

Cloning and Transformation

For construction of 35S::*ELP2-GFP* transgenic plants, the *ELP2* cDNA was obtained from the RIKEN BRC and amplified with a 20:1 Taq/Pfu mixture (to ensure replication fidelity) and fused to the 5' end of the *GFP* gene in pRTL2-mGFP (Stacey et al., 1999) under control of the constitutive *35S* cauliflower mosaic virus promoter, and transformed into *Escherichia coli* XL-1 Blue by electroporation and subsequent selection with kanamycin. The plasmid was then transformed into *Agrobacterium tumefaciens* GV3101 (pMP90), and *elp2* and *elp2 npr1* plants were transformed using the floral dip method (Clough and Bent, 1998). T1 seeds were screened on soil by spraying a 1/100 dilution of Finale (Bayer Cropscience). T2 lines containing a single transgene insertion and T3 plants homozygous for the transgene were isolated based on segregation of Finale resistance. Homozygous T3 lines were subjected to western blot analysis to determine transgene expression. One line from each mutant background that displayed wild type morphology and expressed the transgene was selected for analysis of *elp2* phenotypes and ELP2-GFP subcellular localization.

Map-Based Cloning

For rough mapping, genomic DNA from ~100 F_2 homozygous plants was extracted by the CTAB method [Jander, 2002 #6536] and chloroform extraction and subjected to bulked segregant analysis using a collection of 22 simple sequence length

polymorphism (SSLP) markers (Table A-1) spaced roughly evenly over the entire genome (Lukowitz et al., 2000). Fine mapping was carried out with homozygous plants using various markers from The Arabidopsis Information Resource (TAIR) (http://arabidopsis.org/servlets/Search?action=new_search&type=marker), and markers generated in this study (Table A-2). DNA from genes within the mapping interval was amplified from the mutants by PCR, sequenced by Sanger sequencing (Sanger et al., 1977), and compared to the wild type sequence using CodonCode Aligner (CodonCode Corporation). Mutations were confirmed with derived CAPS markers in the original mutant plants and in 100 independent F_2 plants from the mapping populations.

Assessment of Pathogen Growth

For analysis of basal resistance, plants were infected with *Psm* (OD600 NM = 0.0001) and pathogen titers were determined as previously described (Clarke et al., 1998). For determination of SA-induced resistance, plants were soil-drenched with water or 1 mM SA solution 24 hours prior to infection with *Psm* (OD600 NM = 0.001). For SAR evaluation, three lower leaves were infected with *Psm* (OD600 NM = 0.002) or 10 mM $MgCl_2$ two days prior to secondary infection of two upper leaves with the *Psm* (OD600 NM = 0.001), which were then assayed for pathogen growth. For determination of ETI, leaves were infected with *Pst* DC3000 carrying *avrRpt2* gene (OD600 NM = 0.0001).

Analysis of Gene Transcripts

RNA extraction and RNA gel blot analysis were carried out as described previously (Cao et al., 1994). 150 mg leaf tissue was ground to a fine powder in liquid nitrogen and extracted with warm phenol and RAPD buffer (100mM LiCl, 100mM Tris

pH 8.0, 10mM EDTA, 1% SDS). The aqueous phase was extracted with chloroform, and the resulting aqueous phase ethanol-precipitated at -80°C overnight. RNA was pelleted by centrifugation and washed once with 70% ethanol, dried at room temperature, and resuspended in 40 μl DEPC-treated ddH_2O.

For RNA Blot analysis, 10 μg RNA was subjected to eletrophoresis on a formaldehyde-agarose gel, washed five times in five volumes ddH_2O, and transferred to a nylon membrane by vacuum transfer. Radioactive probes were synthesized by asymmetric PCR using P^{32}-labeled dCTP and isolated DNA fragments. Primers used to make probes are listed in Table A-4. Prehybridization and hybridization were performed at 55°C overnight in 0.5 M Na_2HP0_4, pH 7.2, 7% SDS, and 10 mg/mL BSA (Church and Gilbert, 1984). The blot was washed twice at 65°C for 20 min with 2x SSC (0.3 M NaCl, 0.03 M sodium citrate) and 1% SDS. The blots were then exposed to x-ray film and the film was then developed.

For reverse transcription (RT), total RNA was treated with DNase I (Ambion) at 37°C for 30 min. After inactivation of the DNase, RT was performed using the M-MLV Reverse Transcriptase first-strand synthesis system (Promega) with 5 μl of the DNase-treated RNA in a 20 μl reaction. The resulting cDNA products were diluted 20-fold with water, and 2.5 μl used for quantitative PCR. Quantitative PCR was performed in an Mx3000P qPCR system (Stratagene). All PCR reactions were performed in duplicate using the SYBR Green protocol (Applied Biosystems) under the following conditions: 94°C for 3 min, 40 cycles (94°C for 1 min, 56°C for 1 min, 72°C for 1 min with a 12.5 μl reaction volume and a 1 μM primer concentration. The primers used are listed in Table

A-4. The resulting Ct and E values were used to calculate the relative mRNA abundance according to the ΔCt method. The values were normalized to those for the reference gene *UBQ5*. Specific amplification was confirmed by examining melting curves after the run was completed.

Semi-quantitative RT-PCR was carried out as above, except diluted RT products were subjected to 28 cycles of conventional PCR and subjected to electrophoresis with primers specific to *UBQ5* as an internal control. *Psm*-treated samples were inoculated with an OD600 NM of 0.001 and leaves were harvested after one day.

Analysis of Protein Expression

Protein expression was determined by SDS-PAGE and western blot. For determination of protein expression of independent transgenic plant lines, 14-day-old seedlings were ground to a fine powder in liquid nitrogen and extracted in RIPA buffer (150 mM NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris, pH 8.0.and a protease inhibitor cocktail). Protein samples were loaded onto a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane by electroblotting. The blot was probed with a green fluorescent protein monoclonal antibody (1:1000 dilution, Santa Cruz Biotech). The antibody bound proteins were detected by using a horseradish peroxidase–conjugated anti-mouse secondary antibody (1:5000 dilution, Pierce) followed by chemiluminescence.

Salicylic Acid Measurement

Soil-grown plants (4-weeks old) were infected with *Psm* (OD600 NM of 0.001) to induce SA accumulation. 100 mg of tissue was harvested, frozen in liquid nitrogen, ground to a fine powder, and sequentially extracted with 90% and 100% methanol.

Samples were split into two tubes, sodium hydroxide was added to a final concentration of 2 mM, and the methanol/water mixture was evaporated to a final volume of ~20 μl, and hydrolysis buffer (0.1M sodium acetate buffer pH 5.5) was added to a final volume of 250 μl. Half the samples were incubated for 1.5 hr at 37°C with 10U of β-Glucosidase (EC3.2.1.21, from Sigma) for quantification of glucose-conjugated SA. 230 μl of 10% trichloroacetic acid was added to all the samples, followed by centrifugation. The supernatant was extracted with 1 ml of extraction buffer (50% cyclohexane, 50% ethyl acetate), and the organic phase transferred to a fresh Eppendorf tube containing 50 μl hydrolysis buffer, evaporated to a final volume of ~20 μl, and stored at -20°C. Prior to HPLC analysis, samples were brought to a final volume of 250 μl with hydrolysis buffer and centrifuged through a 0.2 μm filter. HPLC analysis of SA was performed with Agilent's Chemstation with a reverse phase C18 column. The eluent was 0.2 M sodium acetate buffer pH 5.5 (90%) with methanol (10%) at a flow-rate of 0.8 mL/min. SA was detected by fluorescence at an emission wavelength of 412 nm and an excitation wavelength of 301 nm. The retention time for SA was approximately 18 min. This procedure had an ~60% recovery rate, as estimated by extraction of known amounts of SA.

Confocal Microscopy

Confocal microscopy of live plant tissue was performed as described previously (Kinkema et al., 2000). Non-transgenic and *35S::ELP2-GFP* plants were imaged using identical settings, while *35S::GFP* plants were imaged using a lower exposure due to

the high expression of GFP in these plants. Images were processed identically using the ImageJ software (Abramoff et al., 2004).

Reproducibility of Experiments and Statistical Analysis

All experiments depicted in the Figures were conducted at least three times with similar results. Statistical analyses were performed using Student's *t* test for comparison of two data sets. A * indicates a statistically significant difference at the level of at least 95% confidence.

Locus Numbers

The locus numbers for the genes discussed in this article are as follows: *ELP2* (At1g49540), *ELP3* (At5g50320), *ANAC1* (At1g01010), *NPR1* (AT1G02450), *ICS1* (At1g74710), *UBIQUITIN* (At4g05320), *PR-1* (At2g14610), *PR-2* (At3g57260), *PR-5* (At1g75040.1) *GST11* (At1g02920), *LURP1* (At2g14560), *SAG21* (At4g02380), *EDR11* (At1g02930).

CHAPTER 3
ELP2 FUNCTIONS UPSTREAM AND DOWNSTREAM OF SALICYLIC ACID IN PLANT IMMUNITY

Isolation of *snt1*

The suppressor screen was designed to isolate mutations that restored SA tolerance to *npr1*. To screen for suppressors of SA toxicity, *npr1* seeds were mutagenized with ethyl-methanesulfonate (EMS), and M_2 seeds were germinated on ½ strength MS medium supplemented with 0.5 mM SA. Seedlings with green cotyledons were allowed to recover on MS medium for one week, then transferred to soil and allowed to self-fertilize. The SA-tolerance phenotype was then confirmed in the M3 generation. We identified 13 unique mutants, based on complementation tests and phenotype analysis, which were true breeding for SA-tolerance. One mutant that restored SA tolerance to *npr1* was *snt1* (for suppressor of npr1-mediated SA-toxicity-1) (Figure 3-1a).

Despite their increased disease susceptibility, *npr1* plants hyper-accumulate SA during pathogen infection. Since *snt1* restored SA tolerance to *npr1* plants, it may also suppress SA hyper-accumulation and pathogen susceptibility. We first determined SA levels in *snt1 npr1* after *Psm* inoculation. The *snt1* mutation reduced free and total *Psm*-induced SA accumulation in *npr1* (Figure 3-1b). To determine if *snt1* restores pathogen resistance, bacterial titers were determined after *Psm* infection of *snt1 npr1* plants. Surprisingly, pathogen growth was higher in the *snt1 npr1* double mutant than in *npr1* plants (Figure 3-1c). These results suggest that although *snt1* restores SA tolerance and normal SA accumulation to *npr1*, the *snt1* gene functions in plant immunity at least partially independently of NPR1. The *snt1 npr1* plants also exhibited a serrated and

curly leaf phenotype, and were a lighter shade of green than *npr1* (Figure 3-1d). These plants also displayed significantly reduced seed yield, short siliques, delayed silique senescence, and late flowering (not shown).

Genetic Analysis of *snt1*

When the *snt1 npr1* double mutant was backcrossed to the *npr1* parent, the F_1 progeny were not SA tolerant and had *npr1* morphology. Of 139 F_2 plants, 39, or roughly one quarter ($P > 0.1$, Figure 3-2a) had *snt1* morphology suggesting *snt1* is a single, recessive, nuclear mutation. The *snt1 npr1* mutant was backcrossed two more times to remove secondary-site mutations before further characterization. To determine if *snt1* morphology and SA tolerance are caused by the same mutation, co-segregation analysis was carried out in the segregating F_3 population. Nearly all of the progeny from parents with *snt1* morphology were SA tolerant, while only a fraction of the progeny from *npr1*-like plants were SA tolerant (Figure 3-2a and b). Therefore the suppression of SA-toxicity by *snt1* likely also causes abnormal development.

Map-based Cloning of *snt1*

The *snt1* mutation was identified using a map-based cloning approach. To map the *snt1* locus, we isolated a T-DNA insertion *npr1* mutant in the *Landsberg erecta* background (*npr1-L*), which displayed SA toxicity similar to *npr1* (Figure A-1). The *snt1 npr1* double mutant was crossed to *npr1-L* to generate an F_2 segregating population. This allowed rapid confirmation of *elp2* homozygosity in F_2 plants by analysis of SA-tolerance in the F_3 progeny. For rough mapping, ninety five plants homozygous for *snt1* were identified on the basis of morphology. The *snt1* allele was linked to the markers CIW1 and NGA280 on the lower arm of chromosome 1 (Figure 3-3a). Further three-

point mapping of *snt1* was carried out using a mapping population of 1198 homozygous *snt1* plants using various CAPS markers, and the mapping interval was narrowed to a region between markers CTD1 and CTD3. To identify the molecular lesion in *snt1*, the eleven open reading frames between these markers were amplified and sequenced in *snt1 npr1* and compared to the wild type genome sequence. One G→A transition, which formed a new stop codon, was found in the sixth exon of At1g49540, which was confirmed using a derived CAPS marker [Neff, 2002 #6677] specific for the wild type allele (Figure 3-3b). This gene encodes the ELP2 protein, the 93 kD second subunit of the Elongator complex. ELP2 contains several WD-40 domains, which are known to mediate protein-protein interactions (Fellows et al., 2000) (Smith et al., 1999) . The *snt1* mutation is a nonsense mutation halfway through the coding sequence, likely resulting in a truncated and non-functional protein.

To confirm that *SNT1* is *ELP2*, we isolated three loss-of-function T-DNA insertion *elp2* mutants (Figure 3-3c). These mutants were then crossed with *npr1* to obtain *elp2 npr1* double mutants. All *elp2 npr1* mutants restored SA tolerance and decreased SA accumulation, exacerbated pathogen susceptibility, and had *snt1*-like morphology (Figures 3-3d-g). The *snt1* mutation was therefore renamed *elp2-1*. This data suggests the *snt1* mutation destroys *ELP2* function, and that ELP2 is essential for SA toxicity, SA hyperaccumulation, and pathogen resistance.

ELP2 Functions in Defense Gene Expression and Pathogen Resistance

The Elongator complex has been shown to control the expression of stress-induced genes in yeast (Otero et al., 1999). ICS1 is the rate-limiting enzyme in SA

biosynthesis during the immune response and is highly induced by pathogen infection. Since *elp2* suppresses hyper-accumulation of SA in *npr1*, *ICS1* expression in the *elp2 npr1* mutants was examined. *ICS1* expression was significantly lower in the *elp2 npr1* double mutant than in *npr1* (Figure 3-4a), suggesting ELP2 is essential for *ICS1* overexpression and SA hyper-accumulation in *npr1*.

Induction of PR gene transcription also occurs during pathogen infection, and is partially dependent on NPR1 (Shah, 2003). To determine the contribution of ELP2 to PR expression in *npr1*, expression of these genes was examined in *elp2 npr1* plants during *Psm* infection. Compared to *npr1*, expression of *PR-2*, *PR-5*, and to a lesser extent *PR-1* was delayed and reduced (Figure 3-4b) This data, together with the observation that *elp2 npr1* plants are more susceptible than *npr1* plants, suggests ELP2 controls an additional layer of defense activation on top of the defenses controlled by NPR1.

Since ELP2 acts independently of NPR1, ELP2 may be essential for pathogen resistance when NPR1 is present. To determine the function of ELP2 in wild type plants, the *elp2 npr1* mutant was crossed to wild type, and *elp2* single mutants were isolated in the segregating F_2 population by CAPS marker genotyping. The *elp2* single mutant was morphologically indistinguishable from *elp2 npr1*. The *elp2* mutant exhibited less *ICS1* expression and SA accumulation than wild type (Figures 3-5a and 3-5b). These differences were modest, but statistically significant and reproducible. Plants lacking ELP2 also had delayed and reduced PR expression compared to wild type (Figure 3-5c). Interestingly, *PR-1* expression was delayed in *elp2* plants, but eventually reached

wild type levels. Pathogen resistance was also compromised in *elp2* plants (Figure 3-5d). Pathogen titers in *elp2* plants were typically 20-50-fold higher than in wild type, and *elp2* leaves were more chlorotic than those of wild type (not shown). These results show that ELP2 is essential for defense gene expression and pathogen resistance.

ELP2 is Essential for Salicylic Acid-Induced Immunity

Pathogen susceptibility in the *elp2* mutants may result from decreased SA accumulation, which in turn causes a reduction in SA-dependent gene expression. However, SA biosynthesis in *elp2* plants was only moderately less than in wild type (Figure 3-5b). It seemed unlikely that this moderate decrease in SA levels was responsible for the marked susceptibility of *elp2*. Therefore, the possibility that ELP2 regulates immune responses downstream of SA was explored by examining SA-inducible resistance in *elp2* plants. Exogenous SA provided *elp2* plants with less protection than wild type against *Psm* infection (Figure 3-6A). Additionally, SA-inducible expression of *PR-2* and *PR-5* was decreased in *elp2* plants (Figure 3-6B). These data suggest ELP2 acts downstream of SA as a positive regulator of defense responses. ELP2-independent SA-inducible resistance was also observed in the form of partial SA-inducible pathogen resistance and normal *PR-1* and residual *PR-2* and *PR-5* expression.

Initiation of Systemic Acquired Resistance Restores Pathogen Resistance to *elp2*

In order to explore the role of ELP2 in SAR, immune responses were pre-activated by SAR inducement before a second infection with *Psm*. The SAR-induced decrease in pathogen growth was similar in *elp2* and wild type plants, though pathogen growth in SAR-induced *elp2* plants was still somewhat greater than in wild type (Figure

3-7a). In the *npr1* negative control, high levels of pathogen growth were seen in both the mock-treated and SAR-induced plants. These results suggest ELP2, unlike NPR1, is not essential for SAR, and that SAR induction can partially rescue the susceptibility of *elp2* plants. SAR-induced gene expression was then examined in *elp2* plants. Of the six SAR-induced genes tested, three required ELP2 for their full expression, while all required NPR1. *PR-1* and *PR-2* were strongly NPR1-dependent, while the other genes were partially expressed in the absence of NPR1. Expression of all six genes was higher in *elp2* plants than in *npr1* plants. These results suggest SAR-induced gene expression in *elp2* plants is sufficient to limit pathogen growth, while in *npr1* this is not the case. Notably, the more highly expressed genes were ELP2-dependent, while genes expressed at lower levels were not (Figure 3-7b).

ELP2 is Essential for Effector-Triggered Resistance

ETI is considered a faster, stronger version of SAR and PTI. Since immune responses involving rapid transcriptional changes are deficient in *elp2*, the fastest and strongest immune response, ETI, may also be affected. Indeed, plants lacking ELP2 were susceptible to a low-dose infection of the avirulent pathogen *Pst* DC3000 *avrRpt2* (Figure 3-8a). The *npr1* plants were also susceptible. Removal of both NPR1 and ELP2 resulted in the complete elimination of ETI, suggesting NPR1 and ELP2 act synergistically in ETI. The fact that ELP2 is essential for basal resistance and ETI but not SAR may reflect the need for timely defense gene expression in locally infected naive tissues. If *elp2* plants are allowed sufficient time following SAR activation, defense gene expression and pathogen resistance are comparable to wild type, suggesting delayed induction of defense genes may contribute to compromised immunity in *elp2*

plants. To test this hypothesis, the expression profiles of several defense genes that are strongly induced early in infection were examined in *elp2* plants following infection with the ETI-inducing pathogen *Pst* DC3000 *avrRpt2*. *PR-1* expression was monitored as a marker for "late" defense gene induction. The *npr1* and *elp2 npr1* plants were also included to determine the specific contributions of NPR1 and ELP2 to early defense gene induction. Expression of these early defense genes in wild type plants peaked at 4-8 hpi, then rapidly decreased, suggesting a tight control of induction and suppression (Figure 3-8b). Induction of all genes except for WRKY18 was delayed in *elp2* plants, but in some cases eventually reached levels similar to wild type. This delay also occurred in the absence of NPR1. In contrast, gene induction in *npr1* plants was generally not delayed, but expression failed to reach wild type levels for some genes. At5g47230 was completely ELP2-dependent, and also showed the narrowest expression window, with expression returning to basal levels by 8 hpi. Surprisingly, defense gene expression in *elp2 npr1* plants remained high long after repression had occurred in wild type, *elp2*, and *npr1* plants, suggesting ELP2 and NPR1 act redundantly to repress gene expression at the appropriate time.

Subcellular Localization of the ELP2 Protein

To determine the subcellular localization of ELP2, the *ELP2* coding sequence was cloned downstream of the constitutive 35S promoter from cauliflower mosaic virus, and C-terminally tagged with green fluorescent protein. This construct was introduced into both *elp2-1* and *elp2-1 npr1* plants, and homozygous, single insertion transgenic plants that expressed the ELP2-GFP protein were isolated (Figure 3-9a). The *35S::ELP2-GFP* transgene complemented SA-induced PR expression and pathogen

susceptibility phenotypes of *elp2* (Figures 3-9b and 3-9c). The transgene also restored the SA toxicity, *ICS1* overexpression, and SA hyper-accumulation phenotypes to *elp2 npr1* plants (Figures 3-9d-f). The transgene also restored normal morphology to *elp2* and *elp2 npr1* plants (Figure 3-9g). These results suggest that ELP2-GFP can complement *elp2* phenotypes and is therefore a functional protein.

ELP2-GFP was localized to the cytosol in epidermal cells, guard cells, and root tip cells (Figure 3-10a and 3-10b). The absence of NPR1 or treatment with the SA analog INA failed to visibly alter ELP2-GFP localization (Appendix Figure A-2). These results suggest ELP2 is localized primarily to the cytosol of plant cells.

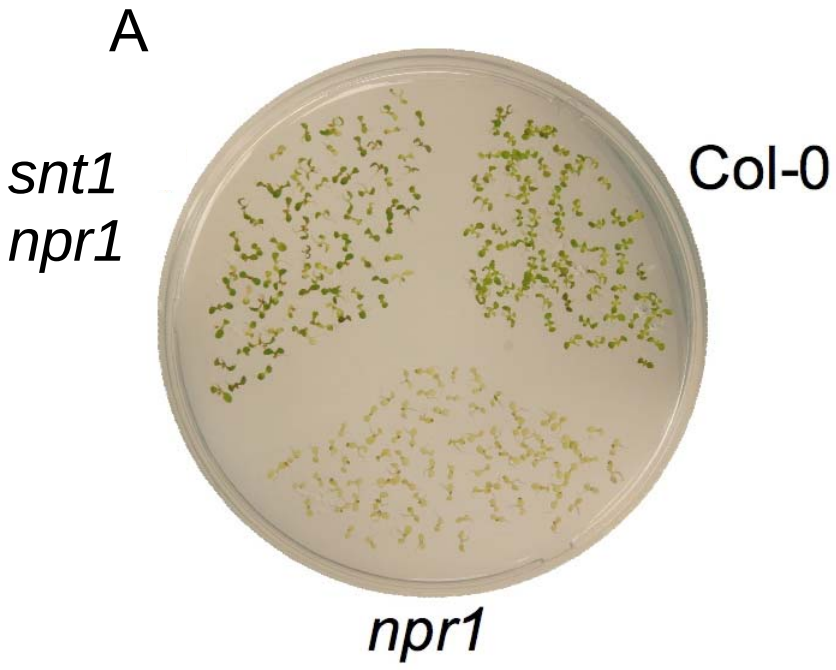


Figure 3-1. The *snt1* mutation suppresses SA toxicity and over-accumulation in *npr1.* (A) SA-tolerance of *snt1 npr1*. Plants were grown on $^1/_2$ MS medium containing 0.26 mM SA, and photographed 11 days after germination. (B) Accumulation of SA and SA + SAG in *snt1 npr1*. Leaves were infiltrated with *Psm* (OD600 NM = 0.001). SA levels were determined 24 hours after treatment. Values represent the average of three independent samples (± SD). (C) Morphology of the *snt1 npr1* mutants. Plants were grown under long-day conditions at 25°C and photographed 28 days after germination. (D) Growth of *Psm* in *snt1 npr1* plants. Leaves of 4-week-old plants were pressure-infiltrated with a bacterial suspension of OD600 NM = 0.0001 and bacterial numbers determined at 0 and 3 days after inoculation (dpi). Values represent the mean of 4-10 samples (± SD).

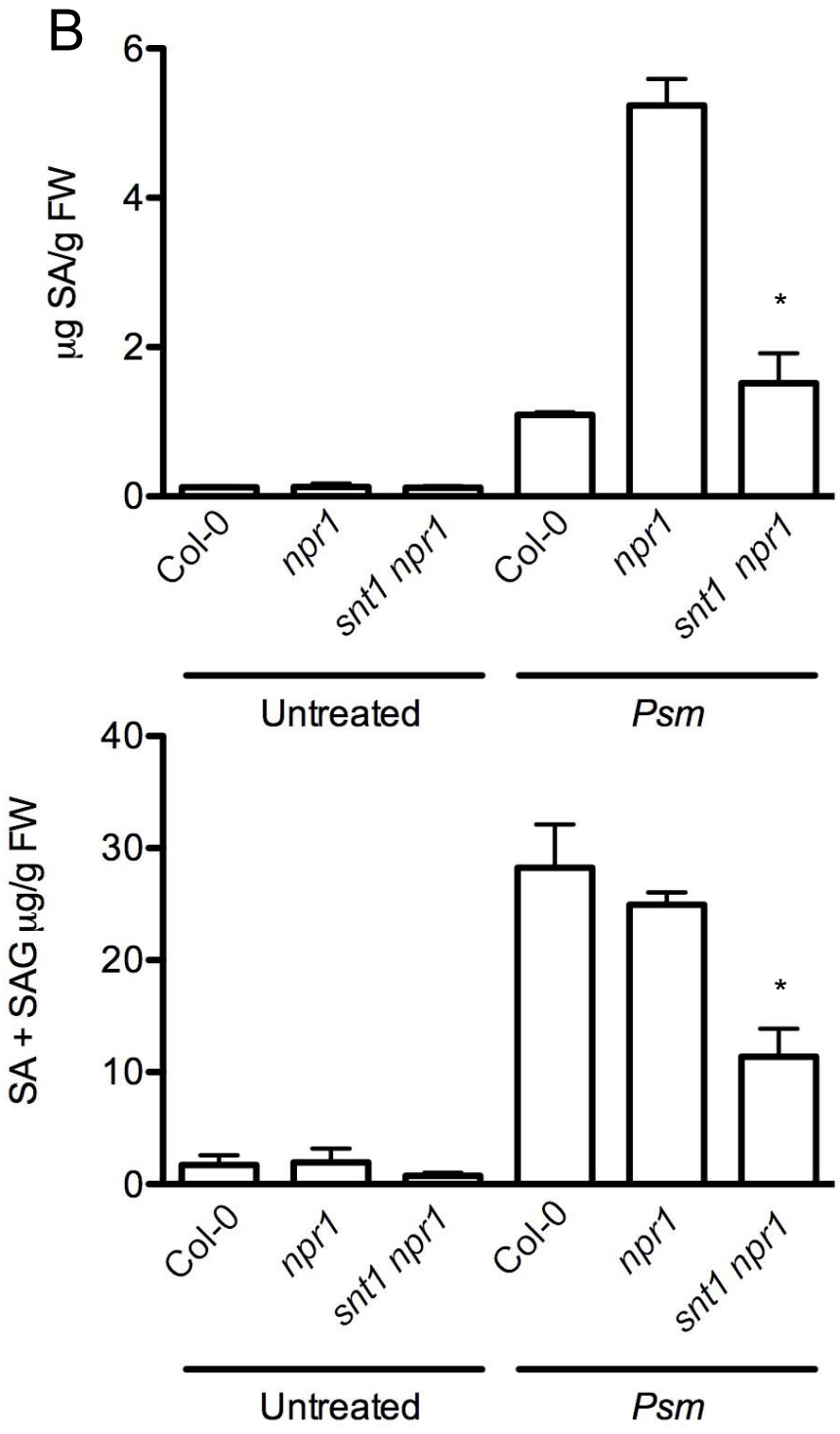


Figure 3-1. Continued

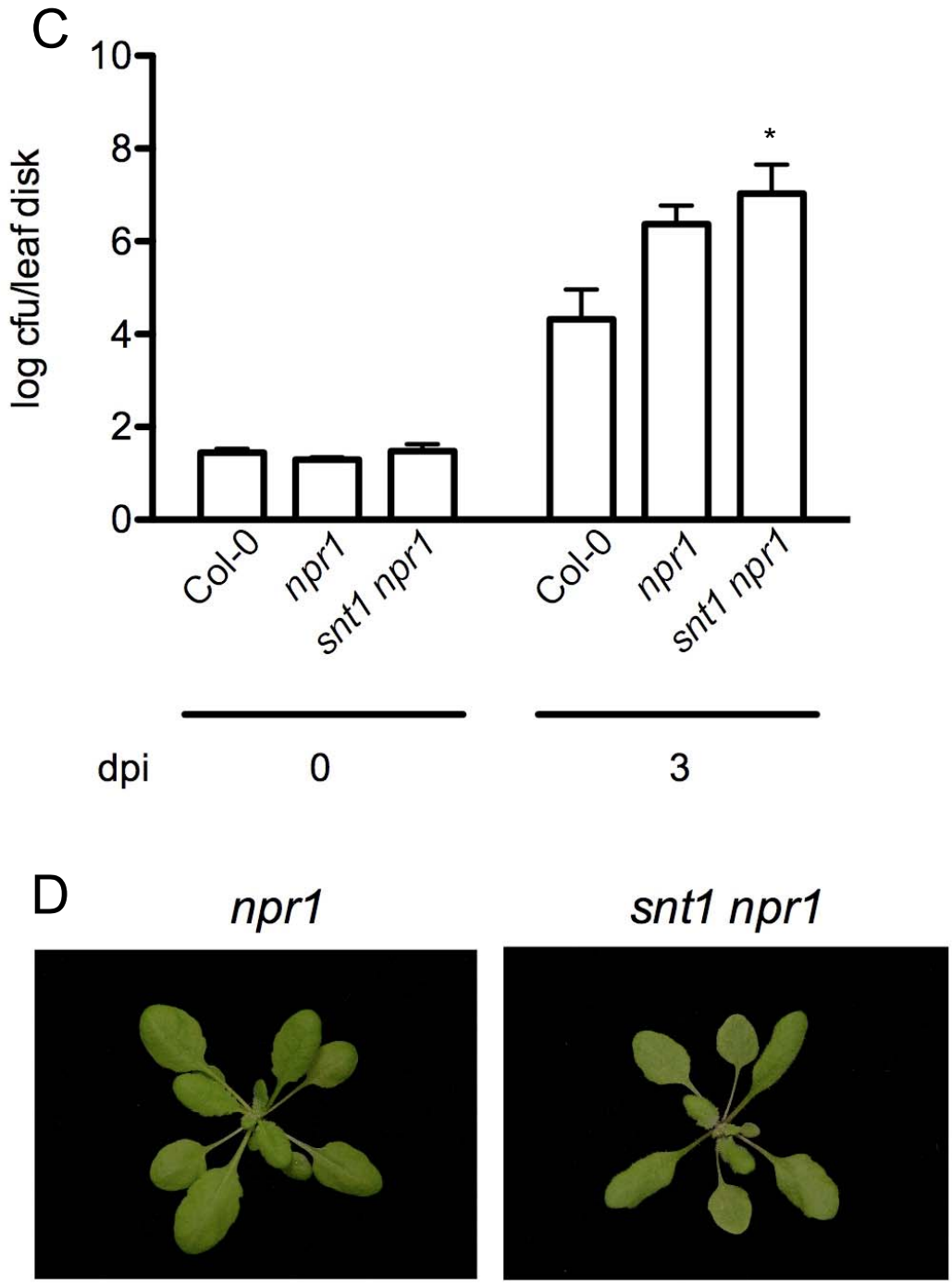


Figure 3-1. Continued

A

Generation	Narrow leaves	Normal leaves
F_1 *snt1*♀ x *npr1*♂	0	10
F_1 *snt1*♂ x *npr1*♀	0	10
F_2 *snt1* x *npr1*	39	139

B

F_2 Morphology	SA-tolerant	SA toxic
snt1	98.84 ± 3.69%	1.15 ± 3.69%
npr1	16.31 ± 10.74%	83.69 ± 10.74%

Figure 3-2. Genetic analysis of *snt1*. (A) Segregation of *snt1* morphology. (B) Co-segregation of *snt1* morphology and SA tolerance.

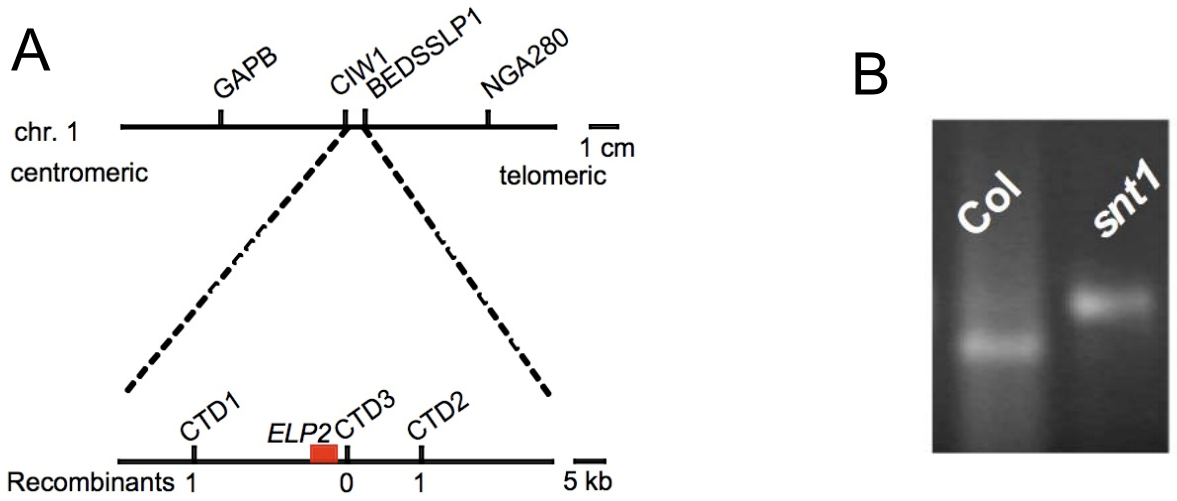


Figure 3-3. Disruption of *ELP2* confers *snt1* phenotypes. (A) Map-based cloning of *snt1*. Ninety-five F_2 progeny homozygous for *snt1* were used to determine the approximate position of the *snt1* mutation using bulked segregant analysis. *snt1* was linked to the markers CIW1 and NGA280. Out of a total mapping population of 1198 plants homozygous for *snt1*, 16 were heterozygous at BEDSSLP1 and 2 were heterozygous at CIW1. Plants that were heterozygotes at these two markers were mutually exclusive. Therefore *snt1* is flanked by these two markers. Of the CIW1 heterozygotes, one was heterozygous at CTD1. Of the BEDSSLP1 heterozygotes, one was heterozygous at CTD2. No heterozygotes were found at CTD3. Molecular markers used in this study are presented in Table A-2. (B) DNA polymorphism between *snt1* and wild type plants. Non-complementary primers were used to introduce a new BslI site unique to wild type. The DNA fragments flanking the BslI site were amplified from the wild type and *snt1* plants, digested with BslI, and separated on an agarose gel. (C) Structure of the *SNT1/ELP2* gene (At1g49540), the *snt1* mutation, and the insertion sites of three T-DNA insertion mutants. Boxes denote the coding sequence, and lines between denote introns. (D) SA-tolerance of *elp2 npr1* knockout mutants. Seedlings were grown on media supplemented with 0.26 mM SA and photographed after 10 days. (E) Accumulation of SA and SA + SAG in independent *elp2 npr1* mutants. Leaves were infiltrated with *Psm* (OD600 NM = 0.001). SA levels were determined 24 hours after inoculation. Values represent the average of three independent samples (± SD). (F) Growth of *Psm* in independent *elp2 npr1* mutants. Leaves of 4-week-old plants were pressure-infiltrated with a bacterial suspension of OD600 NM = 0.0001 and bacterial numbers determined at 0 and 3 days after inoculation (dpi). Values represent the mean of 4-10 samples (± SD). (G) Morphology of independent *snt1 npr1* mutants. Plants were grown under long-day conditions at 25°C and photographed 28 days after germination.

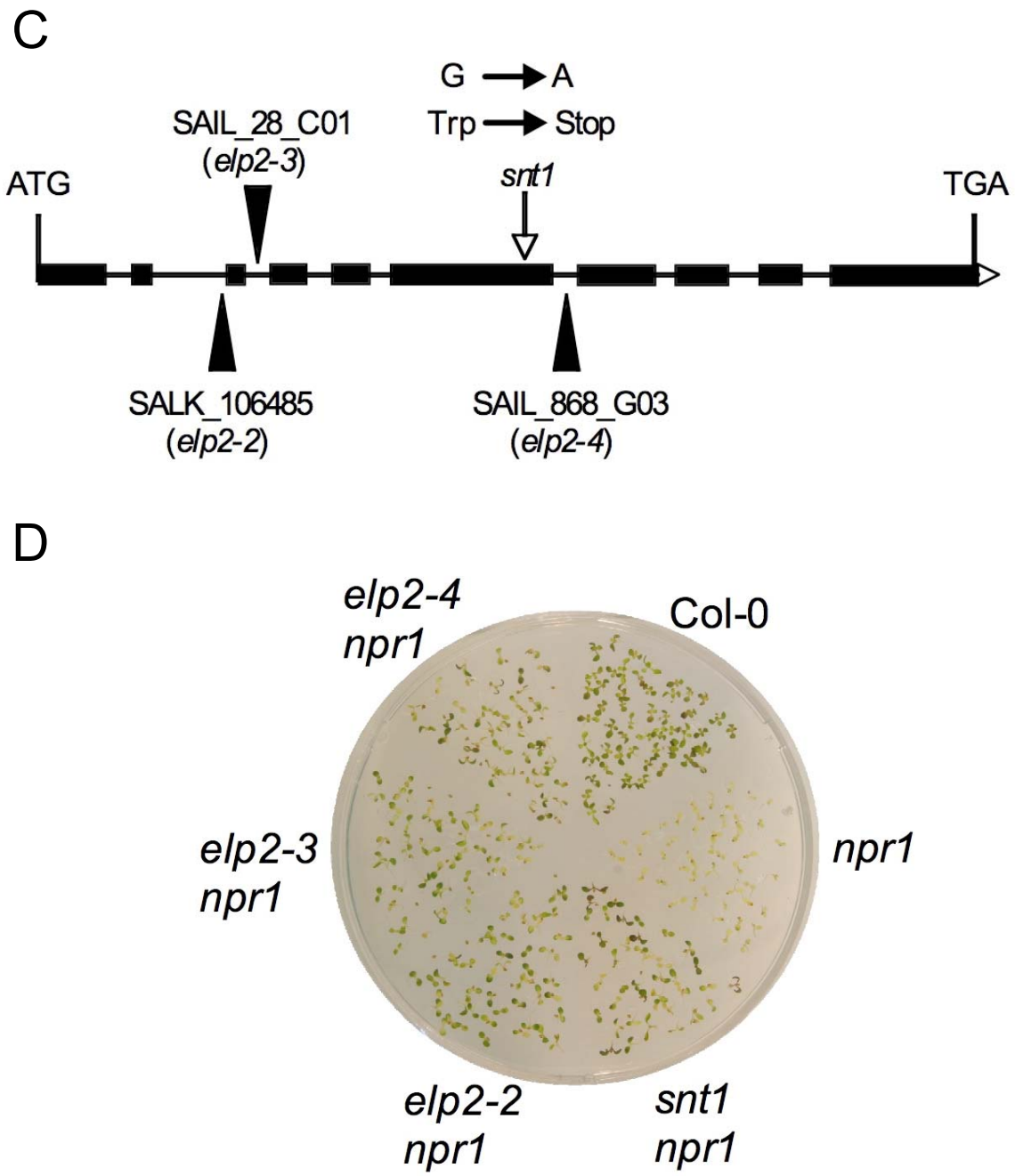


Figure 3-3. Continued

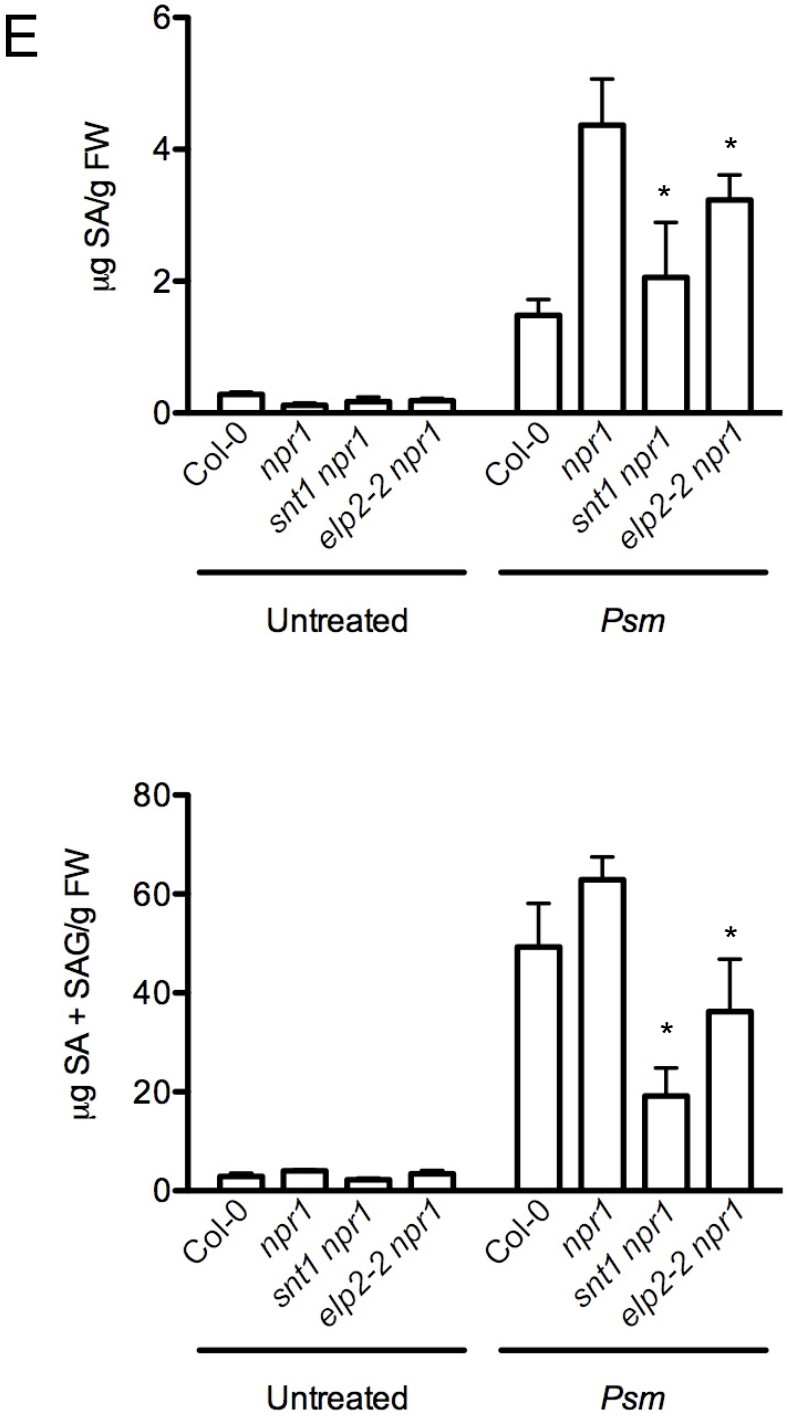
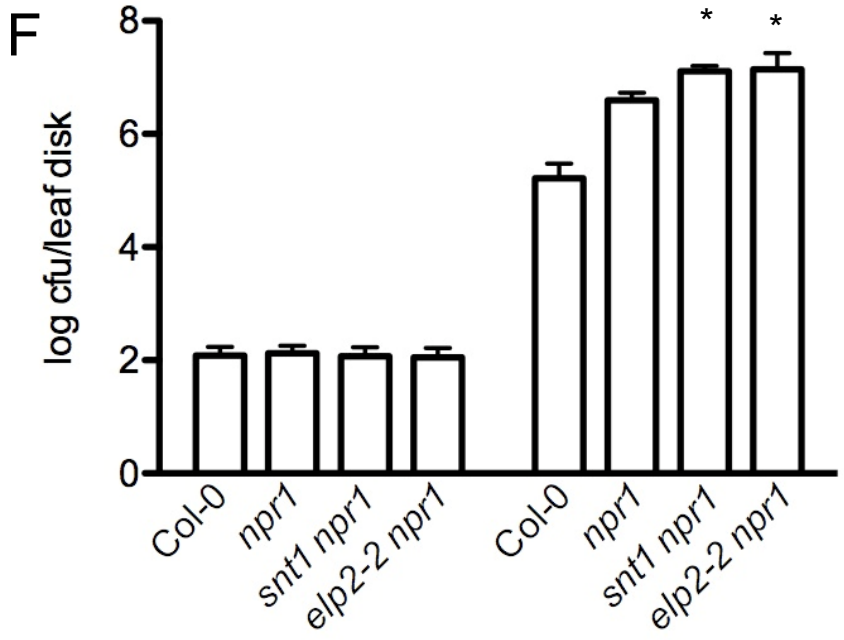


Figure 3-3. Continued



G

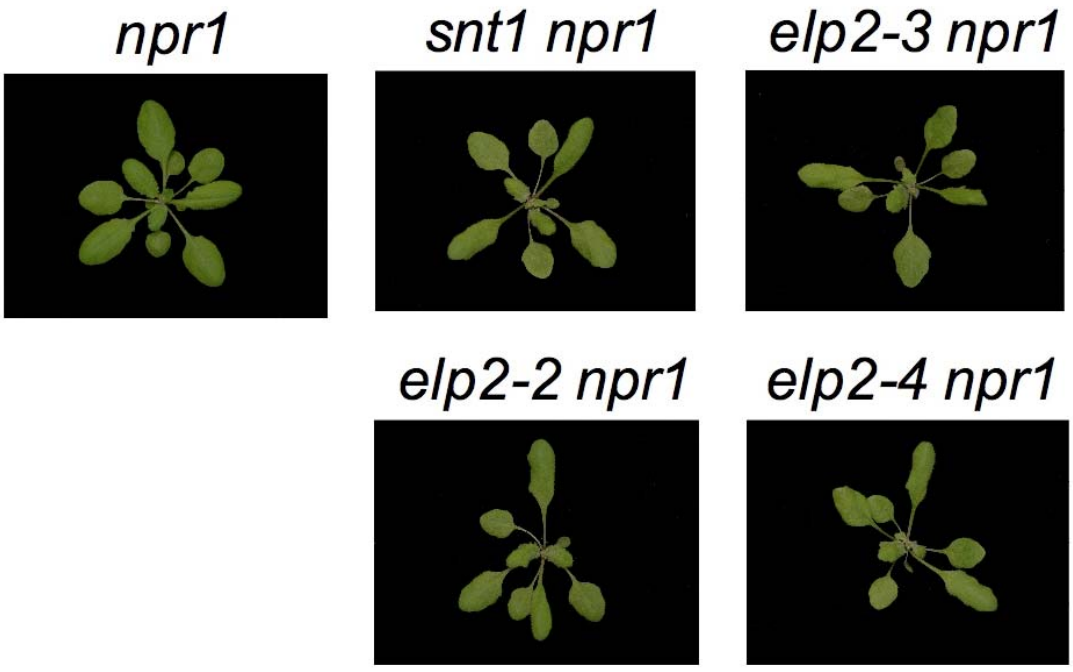


Figure 3-3. Continued

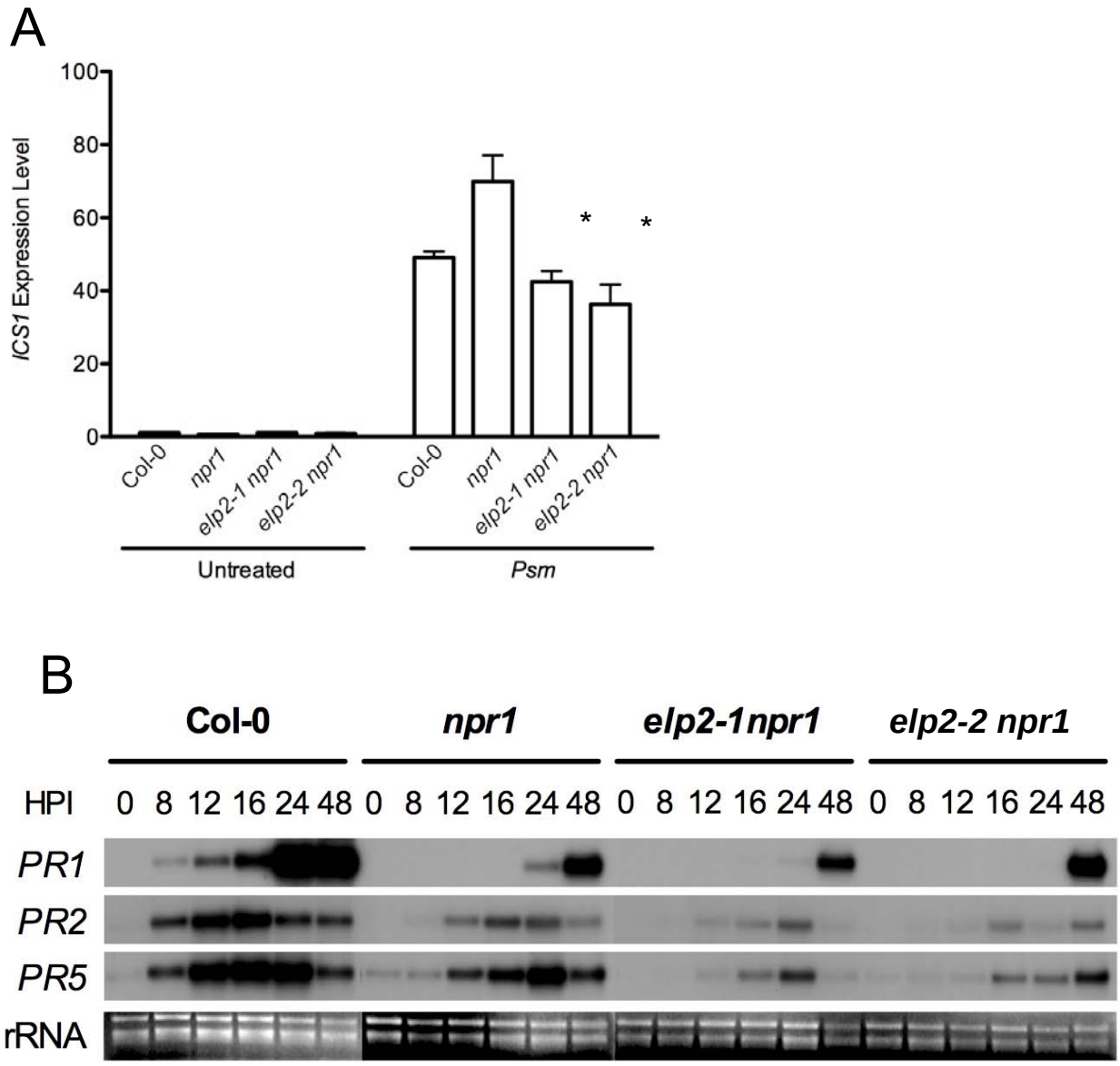


Figure 3-4. *ELP2* regulates *ICS1* and *PR* expression. (A) ICS1 expression in *elp2 npr1* plants. Leaves were infiltrated with *Psm* (OD600 NM = 0.001). Relative transcript levels were determined 24 hr after treatment by quantitative real-time PCR as described in Methods. Expression levels were normalized with respect to the internal control *UBQ5*. Expression levels are displayed relative to untreated samples. Values represent the average value from three independent samples (± SD). (B) *PR* expression in *elp2 npr1* plants. Leaves were infiltrated with *Psm* (OD600 NM = 0.001). Transcript levels were determined by RNA blot analysis. The 25s rRNA band in the ethidium bromide-stained gel was photographed as a loading control before transferring to a nitrocellulose membrane. Blots were sequentially probed for the indicated genes.

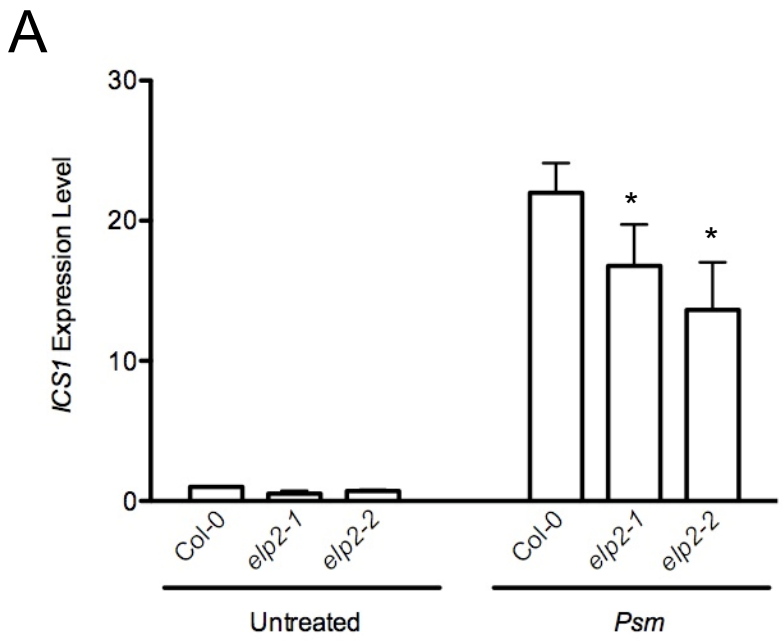


Figure 3-5. Characterization of *elp2* single mutants. (A) Growth of *Psm* in *elp2* plants. Leaves of 4-week-old plants were pressure-infiltrated with a bacterial suspension of OD600 NM = 0.0001 and bacterial numbers determined at 0 and 3 days post inoculation (dpi). Values represent the mean of 4-10 samples (± SD). (B) *PR* expression in *Psm*-infected *elp2* plants. Transcript levels were determined by RNA blot analysis. The 25s rRNA band in the ethidium bromide-stained gel was photographed as a loading control before transferring to a nitrocellulose membrane. Blots were sequentially probed for the indicated genes. (C) Accumulation of free and total SA in *elp2* plants. Leaves were infiltrated with *Psm* (OD600 NM = 0.001). SA levels were determined 24 hr after treatment. Values represent the average of three independent samples (± SD). (D) ICS1 expression in *elp2* plants. Leaves were infiltrated with *Psm* (OD600 NM = 0.001). Relative transcript levels were determined 24 hr after treatment by quantitative real-time PCR as described in Methods. Expression levels were normalized with respect to the internal control *UBQ5*. Expression levels are displayed relative to untreated samples. Values represent the average value from three independent samples (± SD).

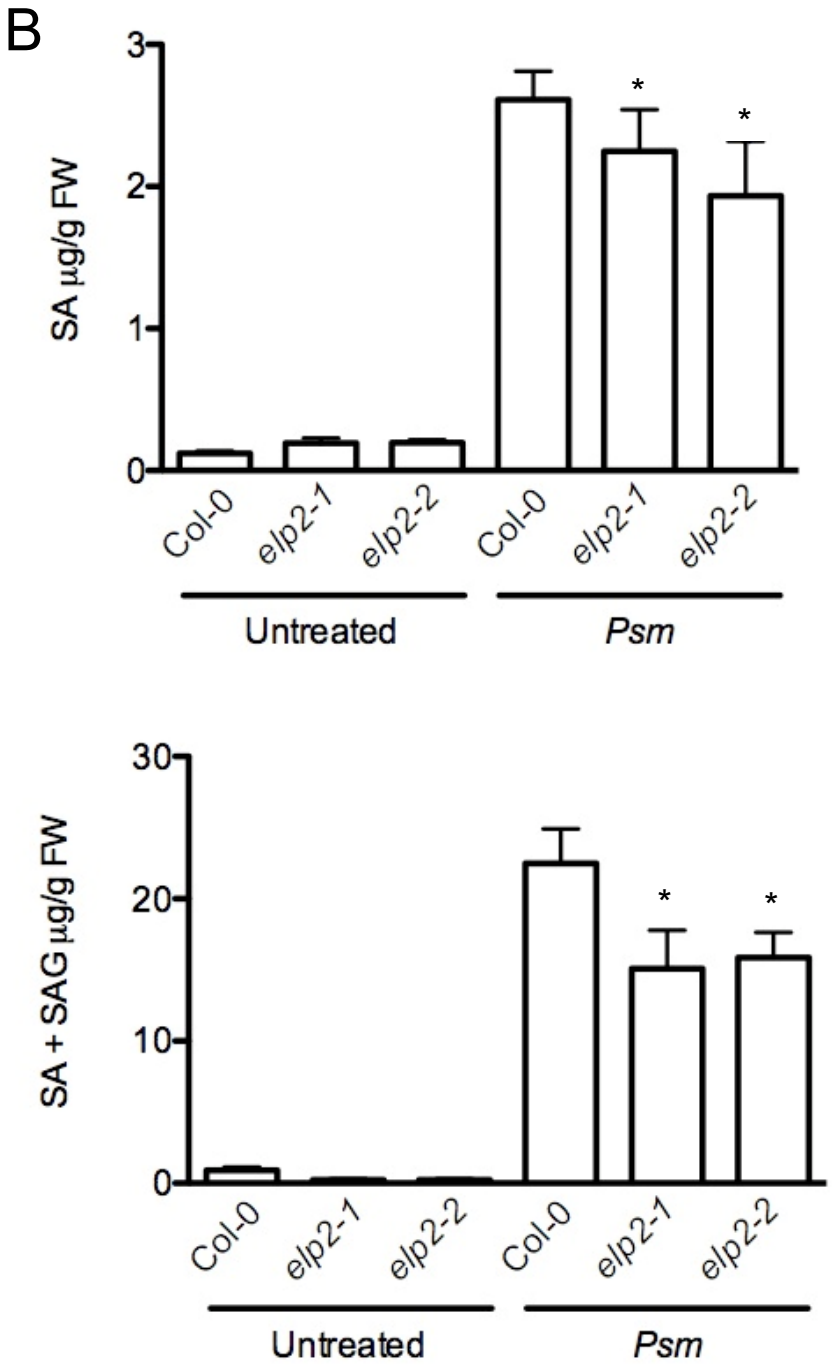
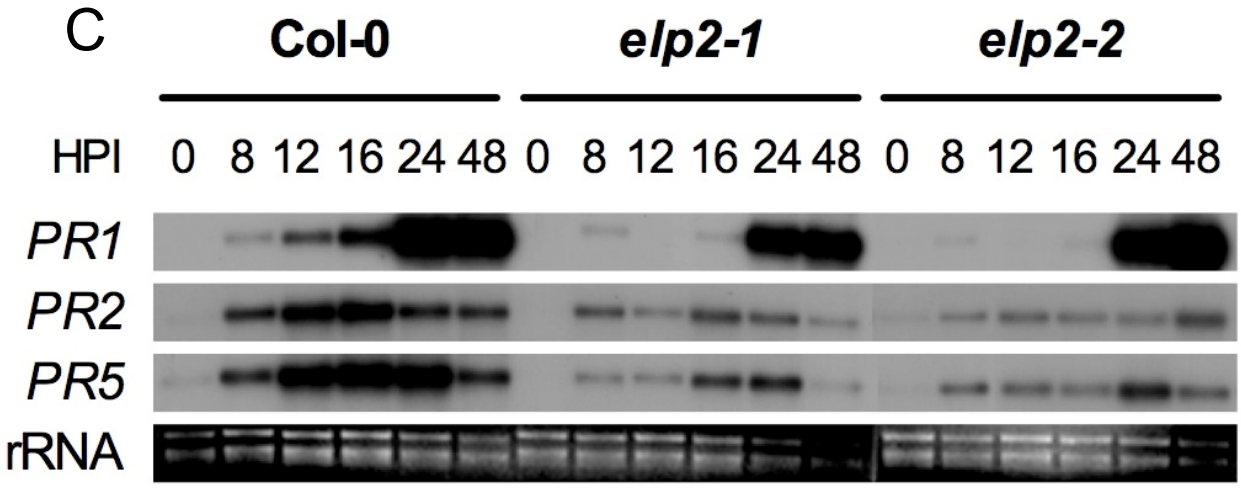


Figure 3-5. Continued



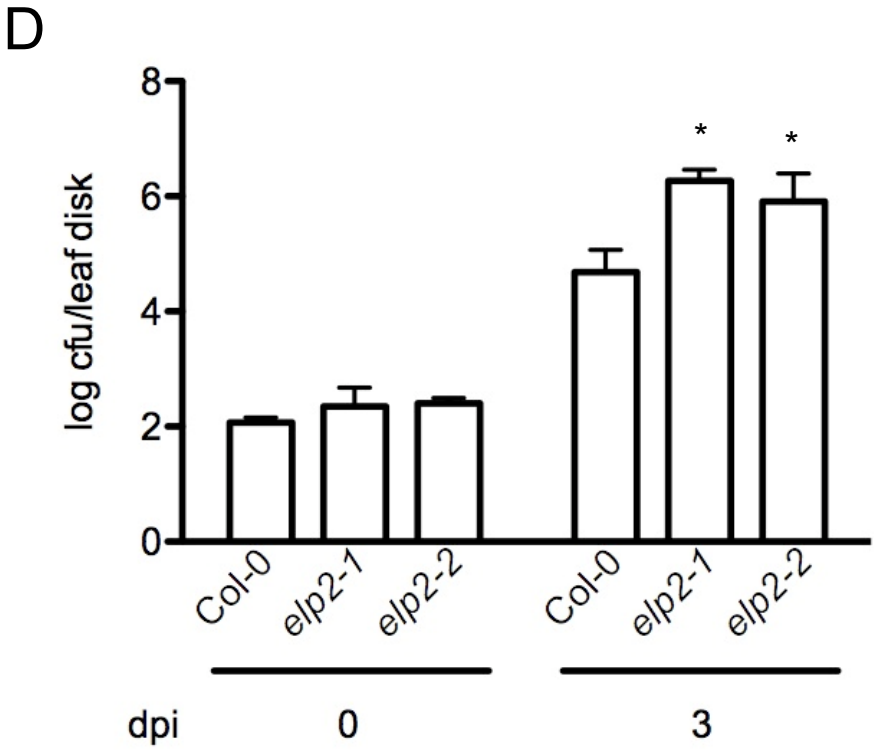


Figure 3-5. Continued

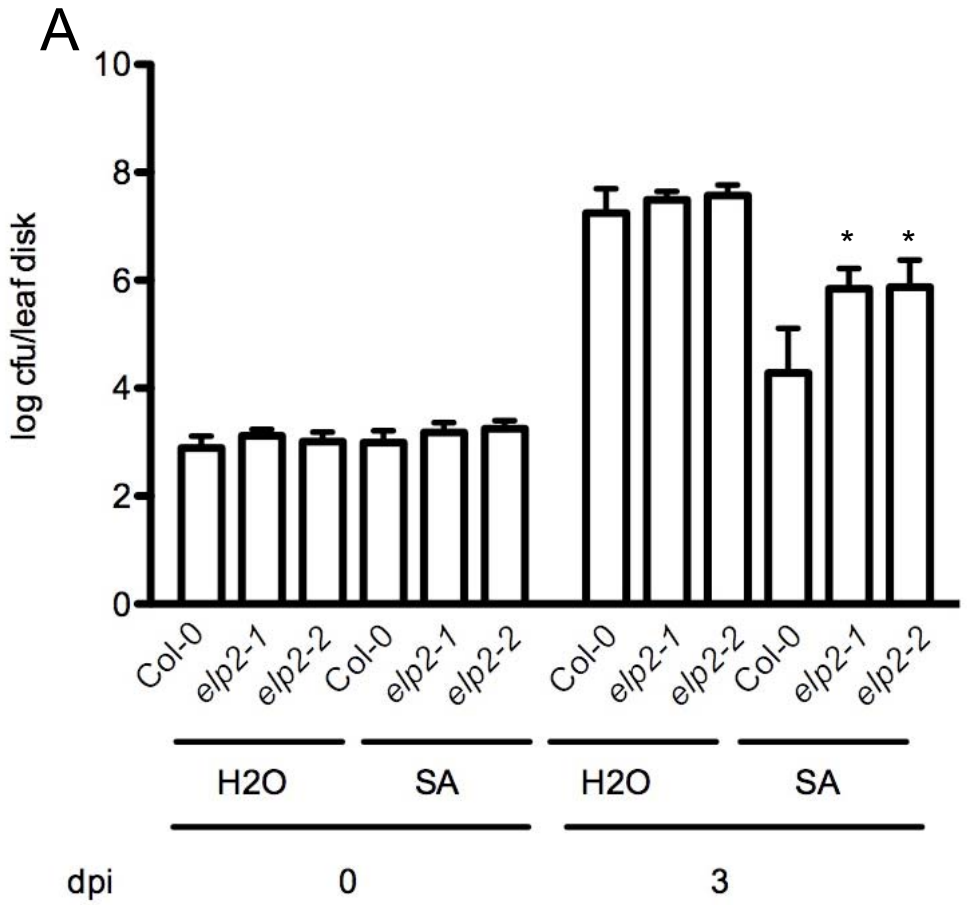


Figure 3-6. *ELP2* is essential for full-scale SA-induced resistance. (A) SA-induced resistance of *elp2* plants. Plants were treated with H_2O or 1 mM SA 24 hr prior to *Psm* infection (OD600 NM = 0.001), and bacterial numbers determined at 0 and 3 dpi. Values represent the mean of 4-10 samples (± SD). (B) SA-induced *PR* expression in *elp2* plants. *PR* expression in *Psm*-infected *elp2* plants. Transcript levels were determined by RNA blot analysis. The 25s rRNA band in the ethidium bromide-stained gel was photographed as a loading control before transferring to a nitrocellulose membrane. Blots were sequentially probed for the indicated genes.

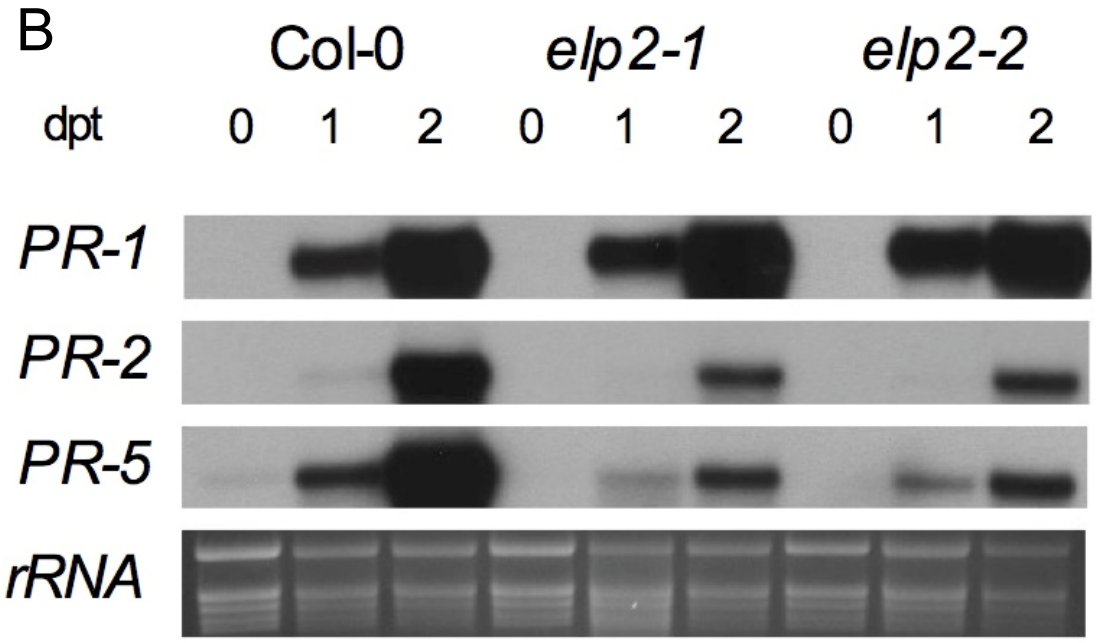


Figure 3-6. Continued

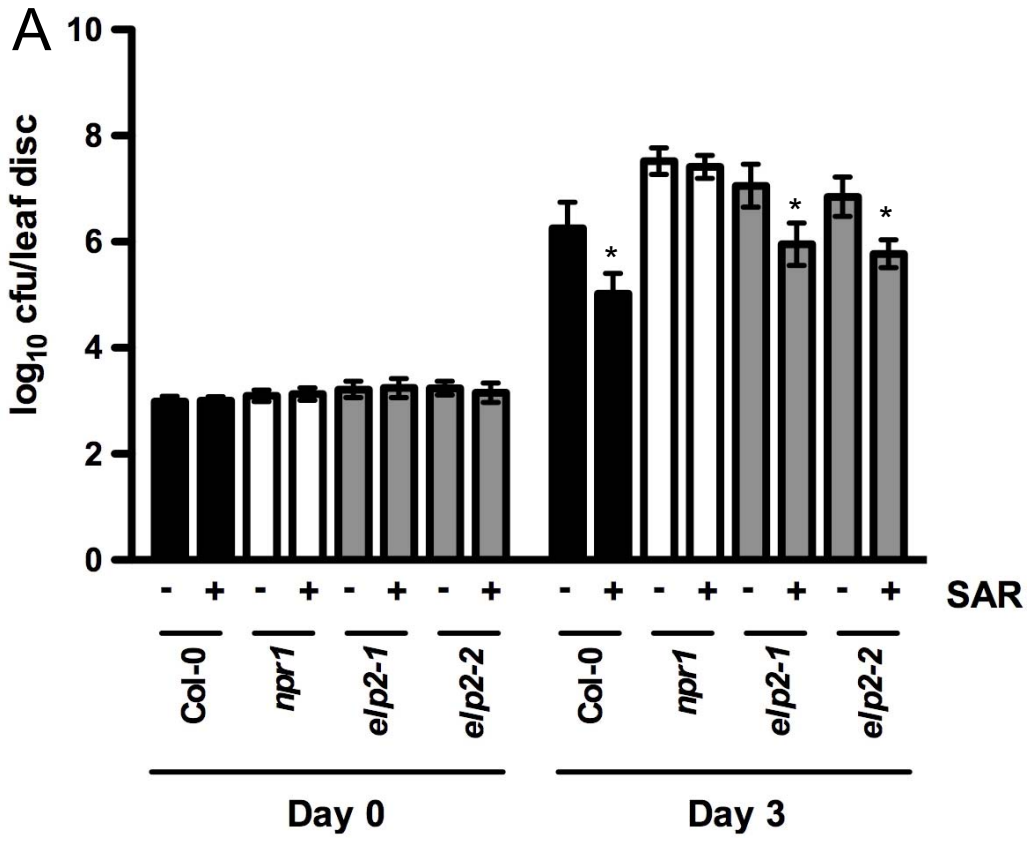


Figure 3-7. Systemic acquired resistance in *elp2* plants. (A) Three lower leaves of 4-week-old plants were pressure-infiltrated with a bacterial suspension of *Psm* (OD600 NM = 0.001). Two days later, two upper leaves were inoculated with the same dose of *Psm* and bacterial growth was determined. Values represent mean with standard deviation. (B) Expression of SAR-inducible genes was determined by qRT-PCR following infection two days after infection. Values represent the mean of three independent samples with standard deviation.

B

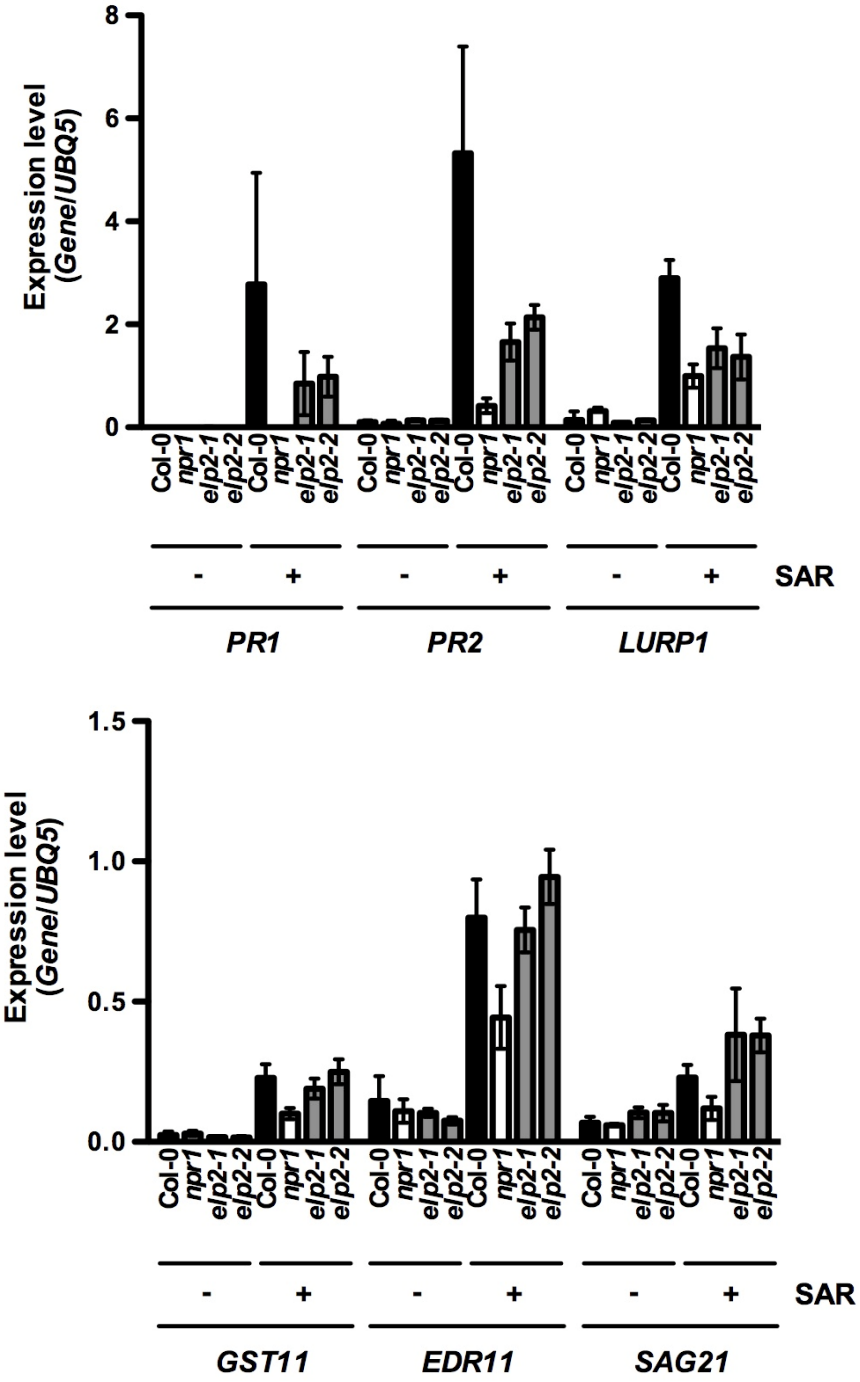


Figure 7. Continued

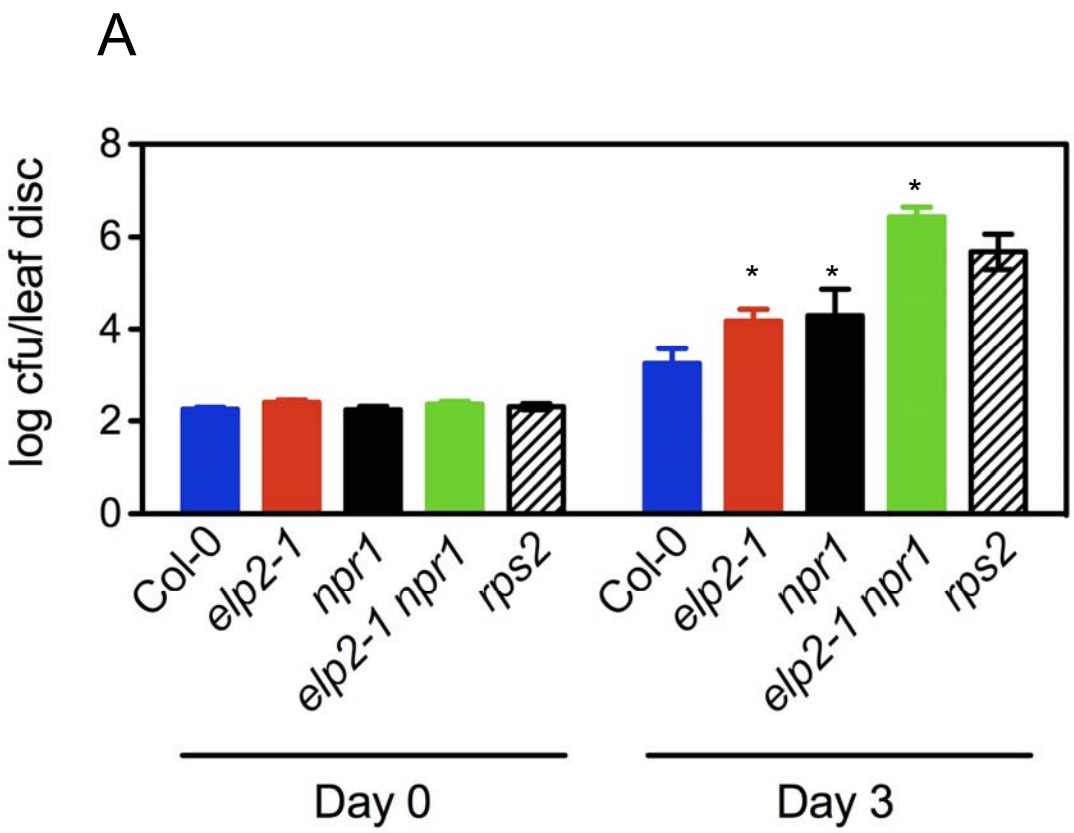


Figure 3-8. Effector-triggered resistance in *elp2* plants. (A) Plants were inoculated with *Pst* DC3000 *avrRpt2* (OD600 NM = 0.0001) and bacterial growth was determined after three days. (B) Expression of early-response genes defense genes was determined by qRT-PCR following infection with *Pst* DC3000 *avrRpt2* (OD600 NM = 0.001). Gene expression was normalized to *UBQ5* expression. Values represent the mean of three independent samples with standard deviation.

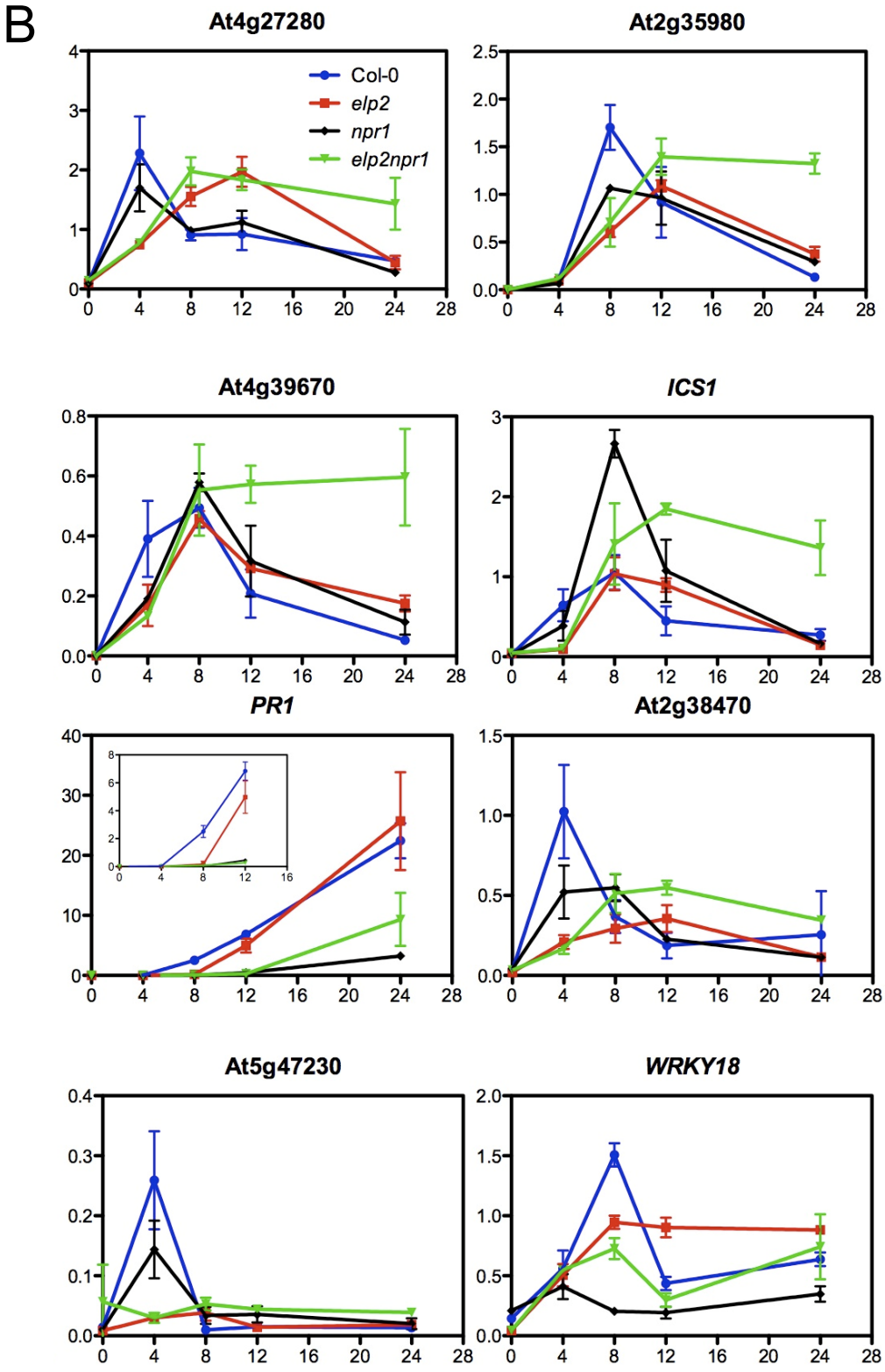


Figure 3-8. Continued

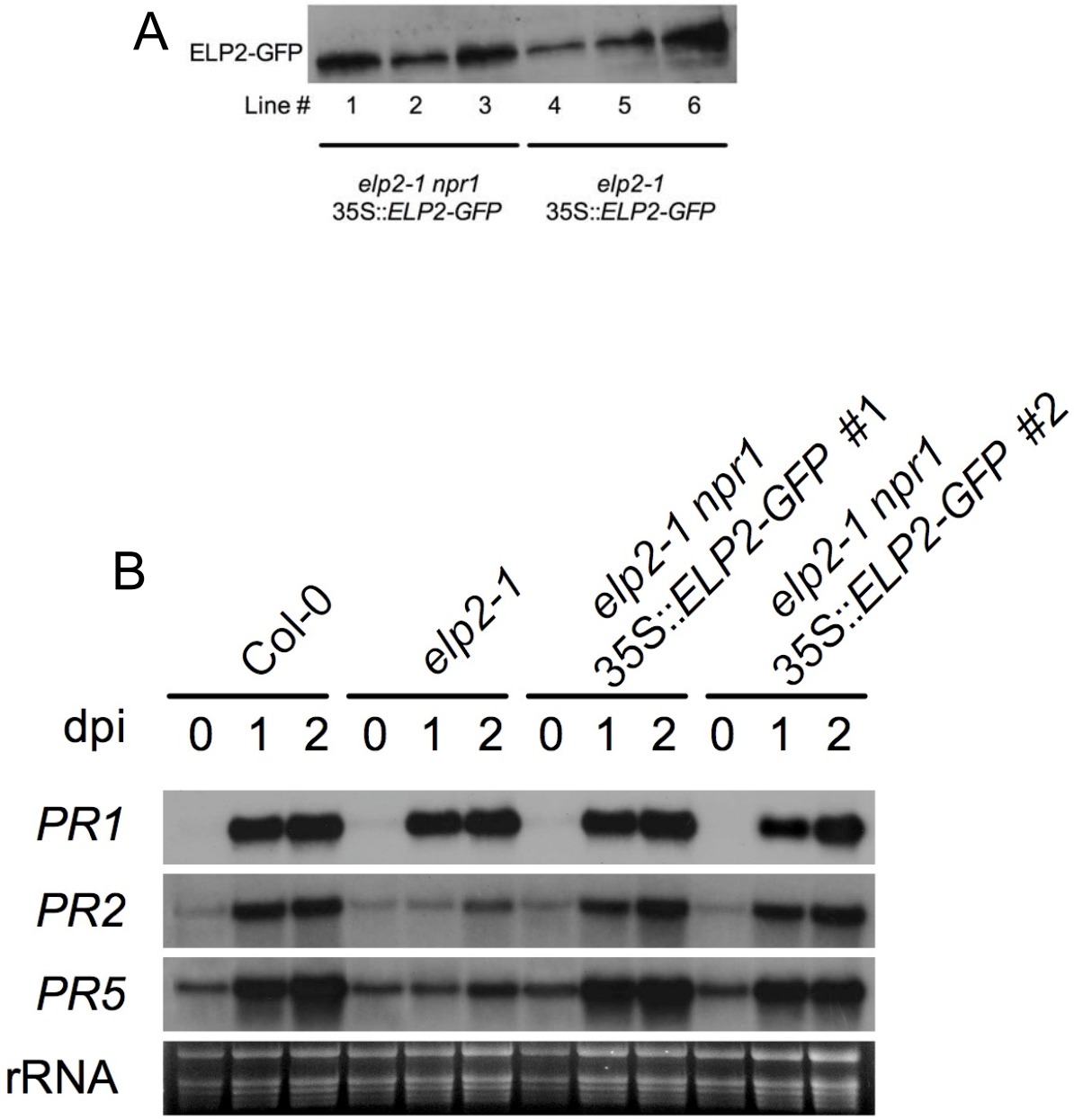


Figure 3-9. ELP2-GFP is a functional protein in planta. (A) ELP2-GFP expression in *elp2* and *elp2 npr1* lines. (B) Complementation of SA-induced PR expression in *elp2*. (C) Complementation of *Psm* resistance in *elp2*. (D) Complementation of SA tolerance in *elp2 npr1*. (E) Complementation of free and total SA hyper-accumulation in *elp2 npr1*. (F) Complementation of ICS1 overexpression in *elp2 npr1*. (G) Complementation of morphology in *elp2* and *elp2 npr1*. (G) Protein expression of ELP2-GFP in *elp2* and *elp2 npr1* plants. Total protein was analyzed by western blot as described in Methods.

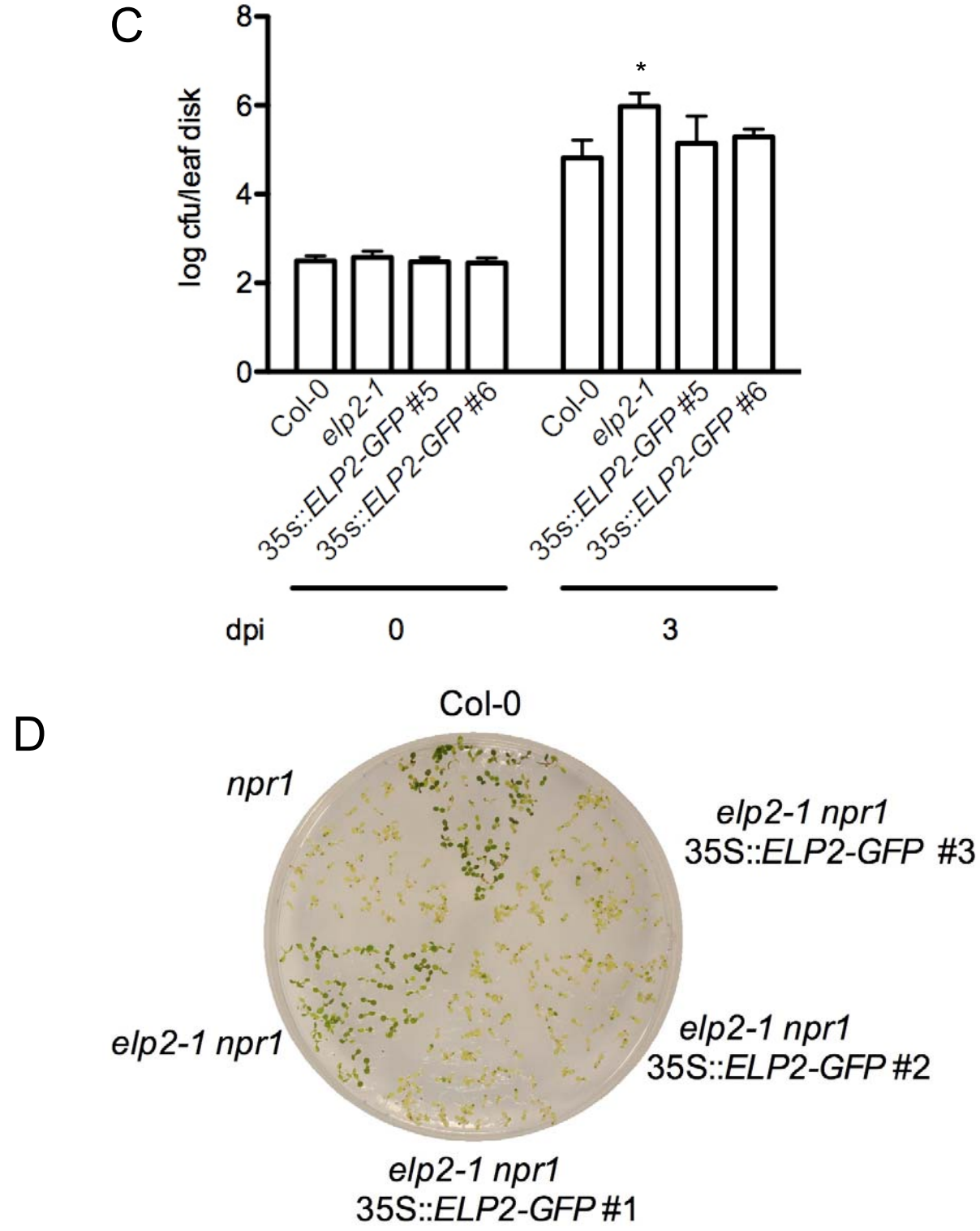


Figure 3-9. Continued

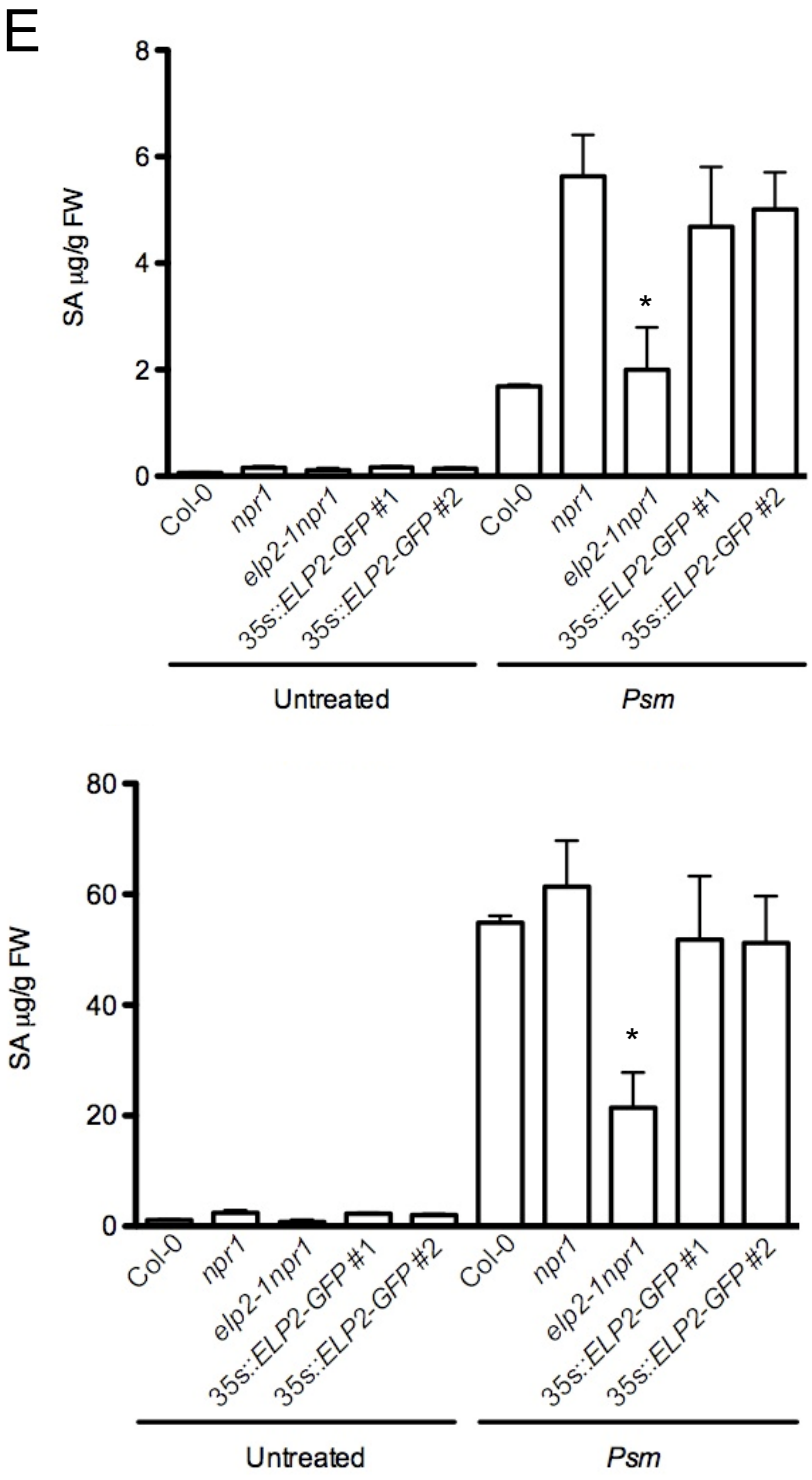
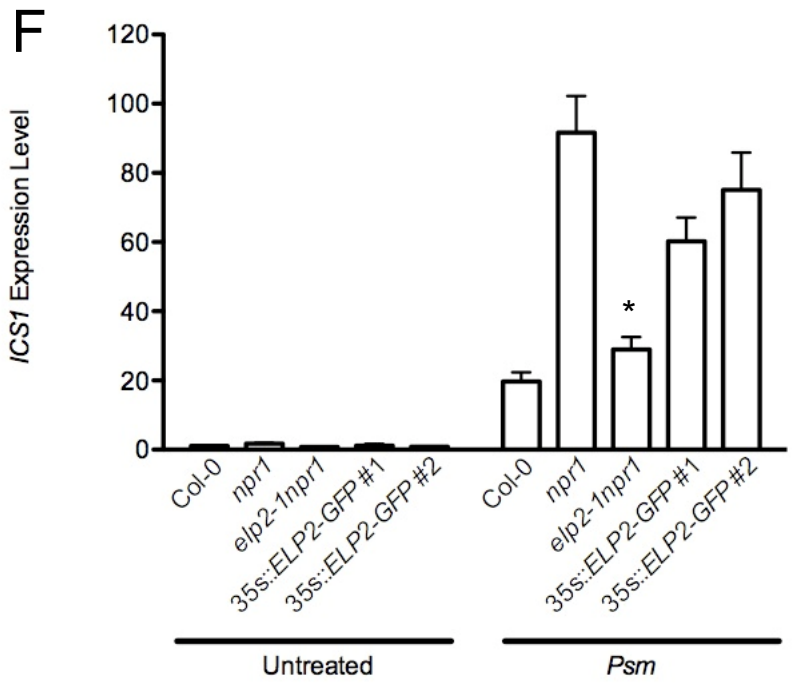


Figure 3-9. Continued



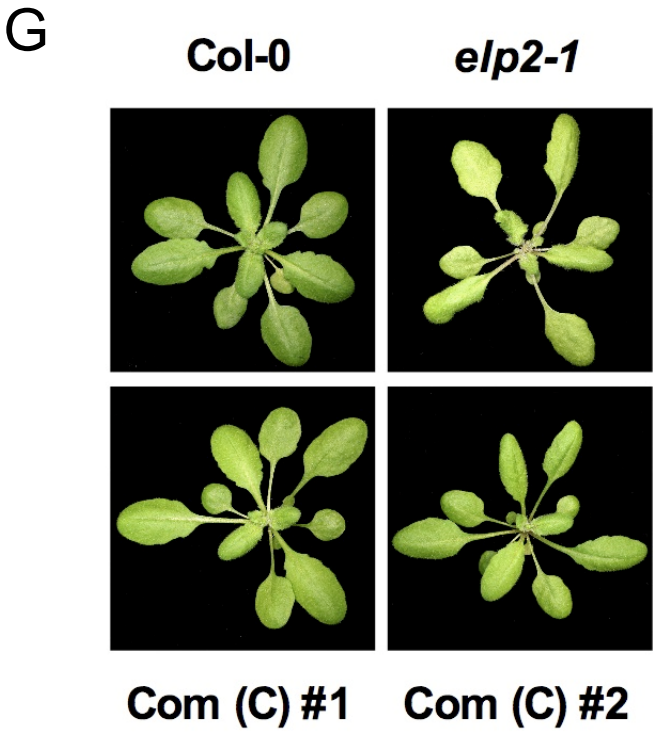


Figure 3-9. Continued

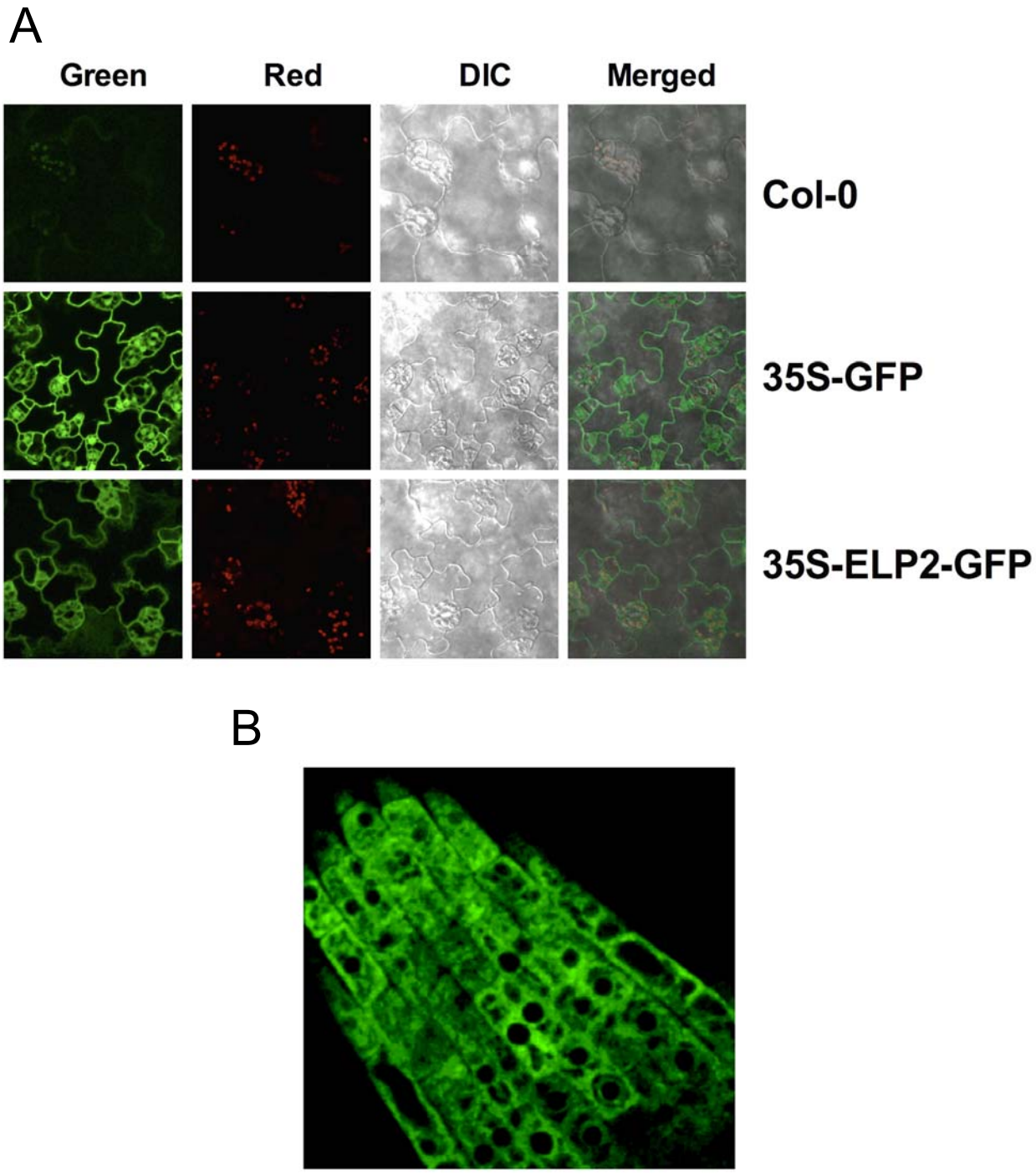


Figure 3-10. Subcellular localization of the ELP2 protein. (A) All three rows of images show, from left to right, GFP fluorescence, autofluorescence from chloroplasts, DIC images, and the overlay of all three channels. (B) GFP fluorescence from 5-day old root tips.

CHAPTER 4
ELP3 IS A POSITIVE REGULATOR OF PLANT IMMUNITY

Isolation and Genetic Analysis of *snt3*

Another mutation that restored SA tolerance in *npr1* was *snt3* (Figure 4-1a). To determine the effect of *snt3* on the enhanced pathogen susceptibility phenotype of *npr1*, we infected *snt3 npr1* plants with *Psm* and examined pathogen growth. *Psm* growth was ~3-fold greater in *snt3 npr1* compared to *npr1* (Figure 3-1b). These data suggest *snt3* confers SA tolerance to *npr1*, and functions in plant immunity at least partially independently of NPR1. *snt3 npr1* plants also exhibited wide leaves and a lighter green coloration (Figure 3-1c).

To determine the heritability of *snt3*, *snt3 npr1* was backcrossed to *npr1*, and progeny were observed. F_1 progeny resembled *npr1*, suggesting *snt3* is recessive or haplo-insufficient. Out of 72 F_2 plants, 22 or roughly one quarter ($P > 0.1$, Figure 4-2a) resembled *snt3 npr1*, further suggesting a recessive or haplo-insufficient mutation. To determine the co-segregation of the SA tolerant and *snt3* morphology, progeny from F_2 plants with either *npr1* or *snt3* morphology were examined. Progeny from *snt3*-like parents were nearly all SA-tolerant, while only a fraction of the progeny from *npr1*-like plants were SA tolerant (Figure 4-2b), suggesting SA-tolerance and *snt3* morphology co-segregate in *snt3* and are caused by the same mutation or two closely-linked mutations.

Map-Based Cloning of *snt3*

For rough mapping, 100 plants homozygous for *snt3* were identified on the basis of morphology. The *snt3* mutation was linked to the markers CIW9 and CIW10 on the

lower arm of chromosome 5. Further three-point mapping of *snt3* was carried out using various CAPS and SSLP markers (Table A-2), and the mapping interval was narrowed to the interval between markers at the loci At5g50180 and At5g50360 (Figure 4-3a). One gene within this interval was *ELP3* (At1g50320), which encodes the catalytic third subunit of the HAT Elongator complex. Since *snt1/elp2* phenotypically resembled *snt2*, and since ELP2 and ELP3 function in the same protein complex, *SNT3* may be *ELP3*. The *ELP3* coding region was therefore amplified from *snt3 npr1* and sequenced. A deletion of a cytosine was detected in the first exon of *ELP3*, resulting in frameshift and likely resulting in a non-functional protein (Figure 4-3c). This polymorphism was confirmed using a derived CAPS marker in the *snt3* mutant (Figure 4-3b) and in 100 homozygous *snt3* plants in the mapping population (not shown).

To determine if a loss of ELP3 function caused the *snt3* phenotypes, we examined a second allele of *ELP3*, *elo3-1,* which was previously generated in the Ler background. *elo3-1 npr1-L* plants were more SA tolerant than *npr1-L* and morphologically resembled *snt3* (not shown). Taken together, this data suggests the loss of *ELP3* function is responsible for the phenotypes seen in *snt3 npr1*, and that *SNT3* is *ELP3*.

Characterization of *snt3* Single Mutants

To determine the function of *ELP3* in pathogen resistance, growth of *Psm* was measured in the *elp3-1* and *elp3-2* single mutants. The *elp3-2* mutant is another *elp3* allele generated in the Wassilewskija ecotype. Both of these mutants were significantly more susceptible than their respective wild types (Figures 4-3a and 4-4b), suggesting *ELP3* plays an essential role in plant immune responses.

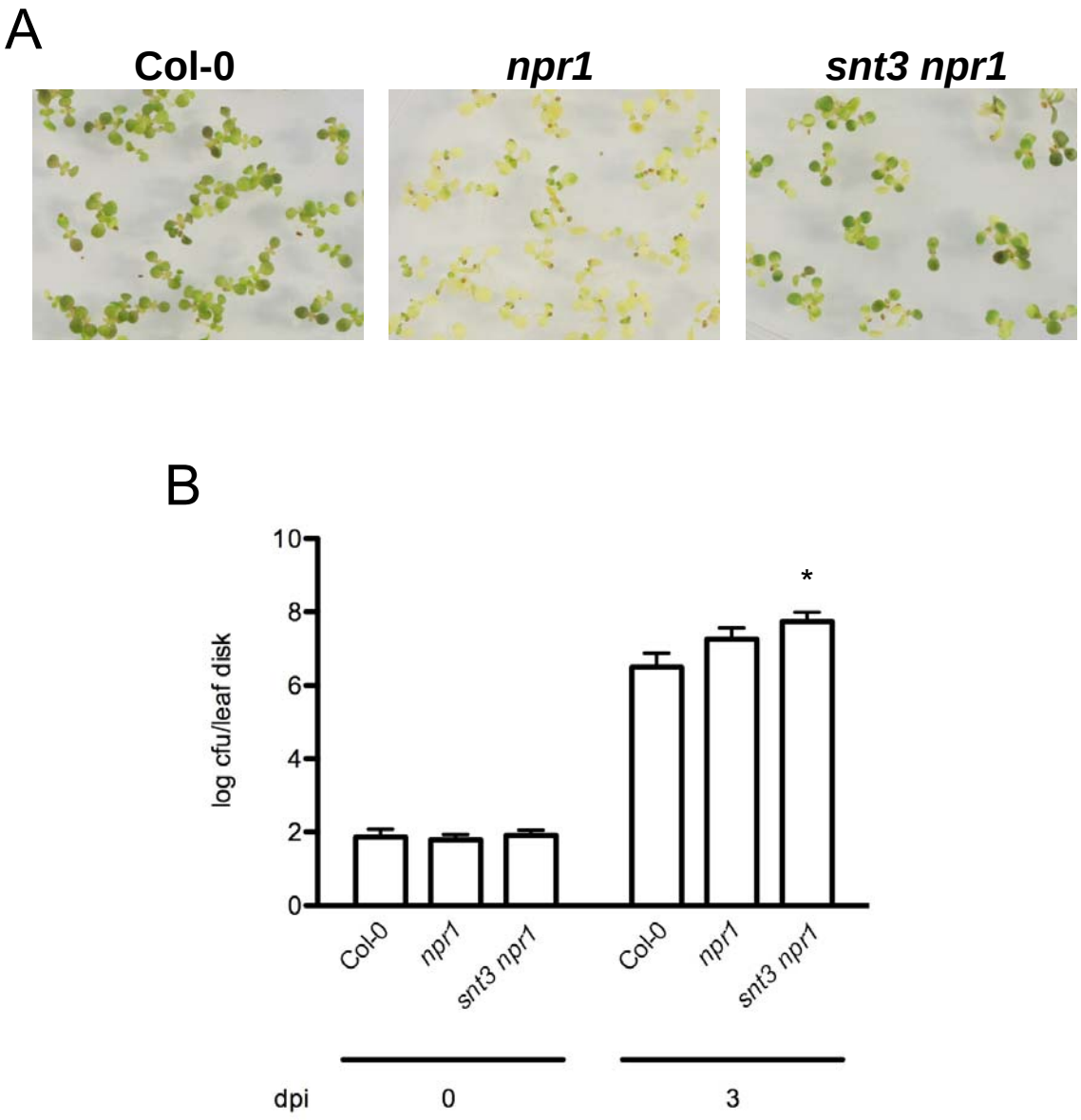


Figure 4-1. *snt3* suppresses SA toxicity and over-accumulation in *npr1*. (A) SA-tolerance of *snt3 npr1*. Plants were grown on $^{1}/_{2}$ MS medium containing 0.26 mM SA, and photographed 11 days after germination. (B) Morphology of the *snt3 npr1* mutants. Plants were grown under long-day conditions at 25°C and photographed 28 days after germination. (C) Growth of *Psm* in *snt3 npr1* plants. Leaves of 4-week-old plants were pressure-infiltrated with a bacterial suspension of OD600 NM = 0.0001 and bacterial numbers determined at 0 and 3 days after inoculation (dpi). Values represent the mean of 4-10 samples (± SD).

C

Figure 4-1. Continued

A

F2 Morphology	SA-tolerant	SA toxic
snt3	92.2 ± 9.4%	7.7 ± 9.5%
npr1	16.31 ± 10.74	83.6 ± 10.7

B

Generation	Wide leaves	Normal leaves
F1 snt3♀ x npr1-3♂	0	10
F1 snt3♂ x npr1-3♀	0	10
F2 snt3 x npr1-3	22	50

Figure 4-2. Genetic analysis of the *snt3* mutation. (A) Phenotypes of the progeny from backcrossed *snt3 npr1* plants. (B) Phenotypes of F_3 progeny from F_2 plants scored in (A) with either *snt3* or *npr1* (wild type) morphology.

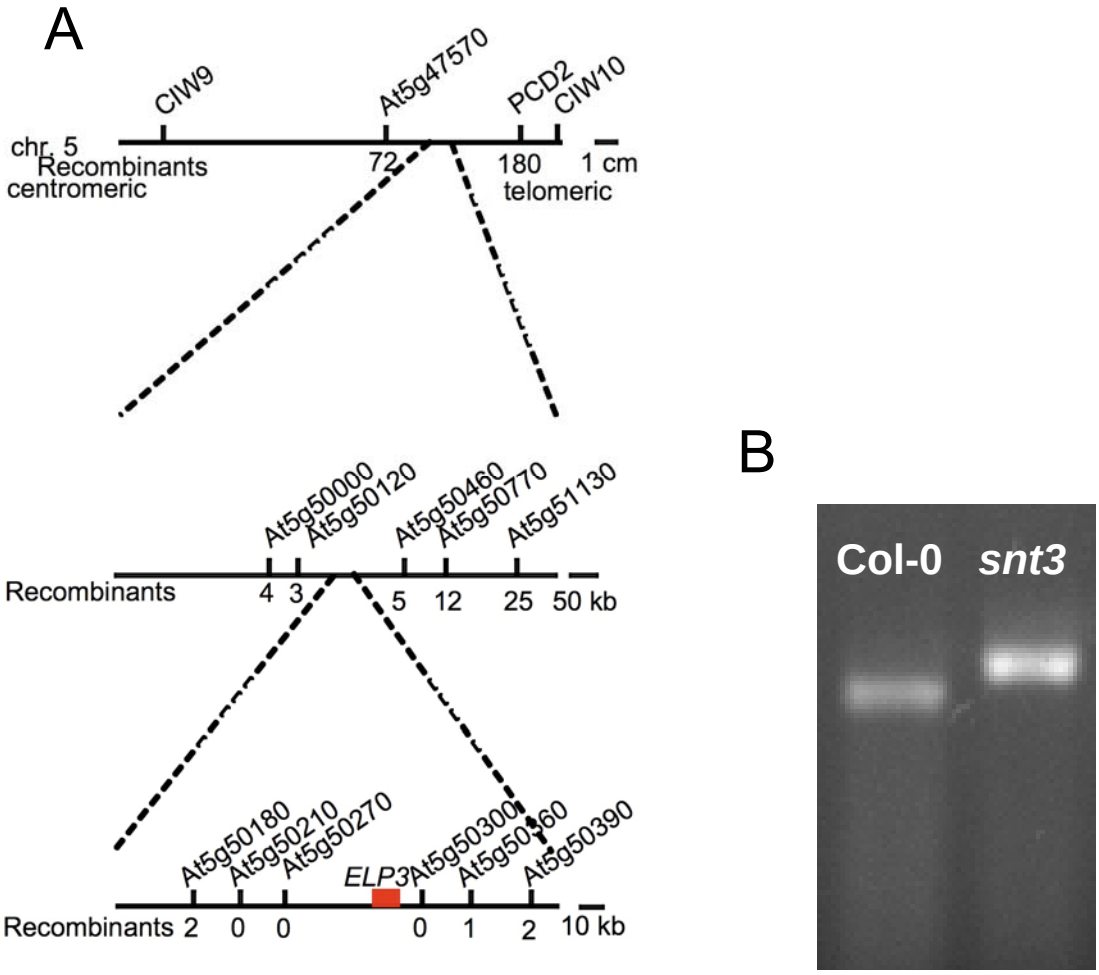


Figure 4-3. Identification of the *snt3* mutation. (A) Map-based cloning of *snt3*. 100 F_2 progeny homozygous for *snt3* were used to determine the approximate position of the *snt3* mutation using bulked segregant analysis. *snt3* was closely linked to the markers CIW9 and CIW10. Out of a total mapping population of 1352 plants homozygous for *snt3*, 72 were heterozygous or Ler at the marker At5g47570, and 180 were heterozygous or Ler at the marker PCD2. The recombinants found by these two markers were mostly mutually exclusive. Markers At5g50000, At5g50120, and At5g50180 had 4, 3, and 2 heterozygotes respectively. Markers At5g50360, At5g50390, At5g50460, At5g50770, and At5g51130 had 1, 2, 5, 12, and 25 heterozygotes respectively. No crossover was observed between these two groups of markers. (B) DNA polymorphism between *snt3* and wild type plants. Non-complementary primers were used to introduce a new MwoI site unique to wild type. The DNA fragments flanking the MwoI site were amplified from the wild type and *snt3* plants, digested with MwoI, and separated on an agarose gel. (C) Structure of the *ELP3* gene (At5g50320), the *snt3*, *elo3*, and *elp3-2* mutations. Boxes denote the coding sequence, and lines between denote introns.

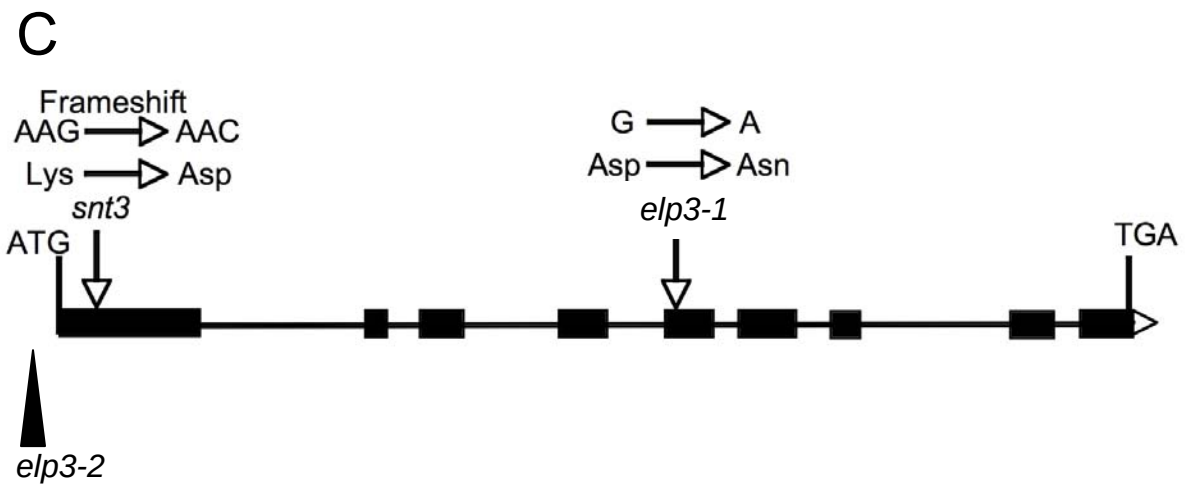


Figure 4-3. Continued

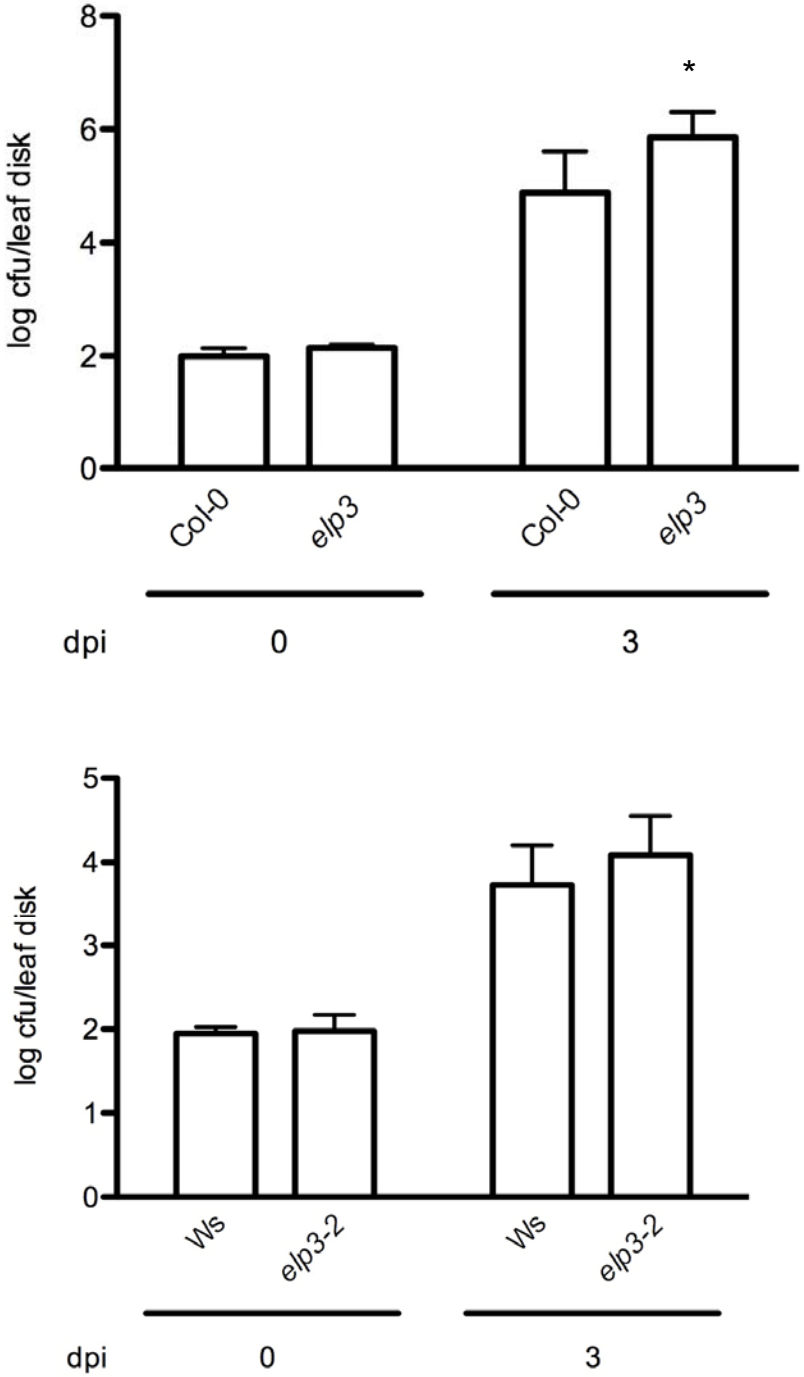


Figure 4-4. Growth of *Psm* in *elp3-1* and *elo3-2* single mutants. (A) *Psm* growth in *elp3*. (B) *Psm* growth in elo-2. Leaves of 4-week-old plants were pressure-infiltrated with a bacterial suspension of OD600 NM = 0.0001 and bacterial numbers determined at 0 and 3 days after inoculation (dpi). Values represent the mean of 4-10 samples (± SD).

CHAPTER 5
THE ROLE OF ANAC1 IN PLANT IMMUNITY

Isolation and Genetic Analysis of *snt2*

A third mutation that restored SA tolerance in *npr1* was *snt2* (Figure 5-1a). *snt2 npr1* plants also displayed reduced size and yellowing around the shoot apical meristem (Figure 5-1b). To determine if *snt2* suppresses the enhanced pathogen susceptibility phenotype of *npr1*, we infected *snt2 npr1* plants with *Psm* and examined pathogen growth. *Psm* growth was reduced by ~8-fold in *snt2 npr1* compared to *npr1* (Figure 5-1c). These results suggest *snt2* confers pathogen resistance and SA tolerance to *npr1*. *npr1* lacks SAR as well as local resistance. We tested the SAR response in *snt2 npr1*, but observed no significant decrease in pathogen growth compared to *snt2 npr1* plants that received a mock primary infection. The same was true for *npr1*, whereas SAR was induced in the wild type. Therefore *snt2* does not restore SAR to *npr1*. To determine the heritability of the yellowing phenotype we carried out genetic analysis by backcrossing *snt2 npr1* to its parent, *npr1*, and observing the progeny. F_1 progeny from reciprocal crosses resembled *npr1*, suggesting *snt2* is recessive or haplo-insufficient. Out of 192 F_2 plants, 64 resembled *snt2 npr1* (Figure 5-1a), further suggesting an *snt2* is possibly a recessive or haplo-insufficient allele ($P < 0.1$). To determine the co-segregation of the SA tolerant and yellowing phenotypes of *snt2*, we examined progeny from F_2 plants with either *npr1* or *snt2* morphology. The *snt2* progeny were nearly all SA-tolerant, while only a fraction of the progeny from *npr1*-like plants were SA tolerant (Figure 5-1b), suggesting SA-tolerance and yellowing co-segregate and are caused by the same mutation or two closely-linked mutations.

Map-Based Cloning of *snt2*

To map the *snt2* locus, *snt2 npr1* was crossed to *npr1-L* to generate an F_2 segregating population. For rough mapping, 198 plants homozygous for *snt2* were identified on the basis of morphology. The *snt2* mutation was linked to the marker F12M12 on chromosome 1. Further three-point mapping of *snt2* was carried out using the original 198 plants using various CAPS markers (Table A-2), and the mapping interval was narrowed to the telomeric side of At1g01050 (Figure 5-2a). The progeny of the plants heterozygous at this marker were all SA-tolerant, confirming *snt2* homozygosity (not shown). The genes in this region were then amplified from *snt2 npr1* and sequenced. A transition point mutation was found in the first intron of At1g01010 (Figure 5-2c). This gene encodes a putative NAC (no apical meristem domain containing) transcription factor (ANAC1). This polymorphism was confirmed using a derived CAPS marker (Figure 3-2b) in the *snt2* mutant and in 100 homozygous *snt2* plants in the mapping population (not shown). Interestingly, intron 1 lacks a consensus branch-point sequence (Figure 5-3d). The *snt2* mutation introduces such a sequence thirty-six bases upstream of the 3' splice site.

To test the notion that *snt2* phenotypes result from a loss of *ANAC1* function, two lines containing T-DNA insertions in *ANAC1* were isolated and crossed into an *npr1* background. The double mutants did not exhibit SA tolerance, nor did they have any detectable morphological phenotype (not shown), though *ANAC1* mRNA levels were undetectable (Figure 3-3e). This data suggests that *snt2* may be a gain-of-function mutation.

Since *snt2* is located in an intron, it may affect splicing of *ANAC1*. To test this, *ANAC1* cDNA from *snt2* was amplified by RT-PCR. The single amplicon obtained was the same size as that from wild type. The cDNA was then sequenced and compared to the wild type cDNA sequence. The two sequences were 100% identical (not shown). This data suggests *snt2* does not affect the structure of the *ANAC1* transcript. *ANAC1* mRNA levels in *snt2* were then tested using semi-quantitative RT-PCR. In *snt2, ANAC1* transcript levels were higher than in *npr1*, suggesting *snt2* may somehow increase the expression of this gene. This overexpression might be responsible for *snt2* phenotypes. If *snt2* overexpression were responsible for pathogen resistance in *snt2*, then this gene would be expected to be induced by pathogen infection. Indeed, *ANAC1* expression is increased during pathogen infection (Figure 5-3e).

Characterization of *ANAC1* single mutants

To determine the function of *ANAC1* in pathogen resistance, we infected both *anac1* T-DNA insertion mutants and the *snt2* single mutant with *Psm* and measured pathogen growth. The *snt2* single mutant, but neither knockout mutant, was significantly more resistant than wild type. This suggests that the *snt2* mutation confers pathogen resistance, and acts independently of NPR1. The lack of detectable phenotypes in the knockout mutants suggests *ANAC1* is not essential for resistance to *Psm*, but that overexpression of *ANAC1* may be sufficient to confer resistance.

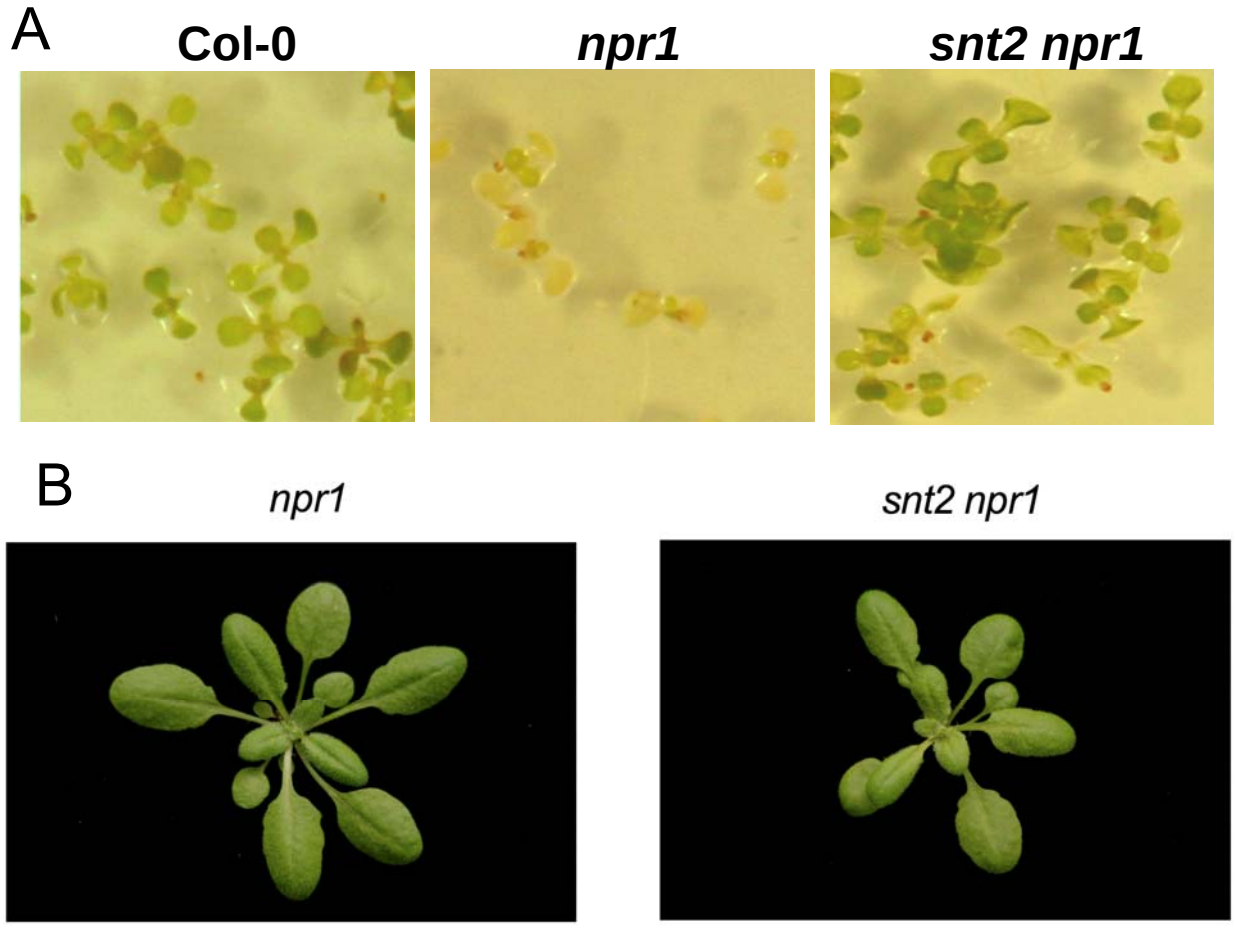


Figure 5-1. The *snt2* mutation suppresses SA toxicity and over-accumulation in *npr1*. (A) SA-tolerance of *snt2 npr1*. Plants were grown on $^{1}/_{2}$ MS medium containing 0.26 mM SA, and photographed 10 days after germination. (B) Morphology of the *snt2 npr1* mutants. Plants were grown under long-day conditions at 25°C and photographed 28 days after germination. (C) Growth of *Psm* in *snt2 npr1* plants. Leaves of 4-week-old plants were pressure-infiltrated with a bacterial suspension of OD600 NM = 0.0001 and bacterial numbers determined at 0 and 3 days after inoculation (dpi). Values represent the mean of 4-10 samples (± SD). (D) Systemic acquired resistance of *snt2 npr1* plants. Three lower leaves of 4-week-old plants were pressure-infiltrated with a bacterial suspension of OD600 NM = 0.002. Two days later, two upper leaves were inoculated (OD600 NM = 0.001) and bacterial growth was determined as in (C).

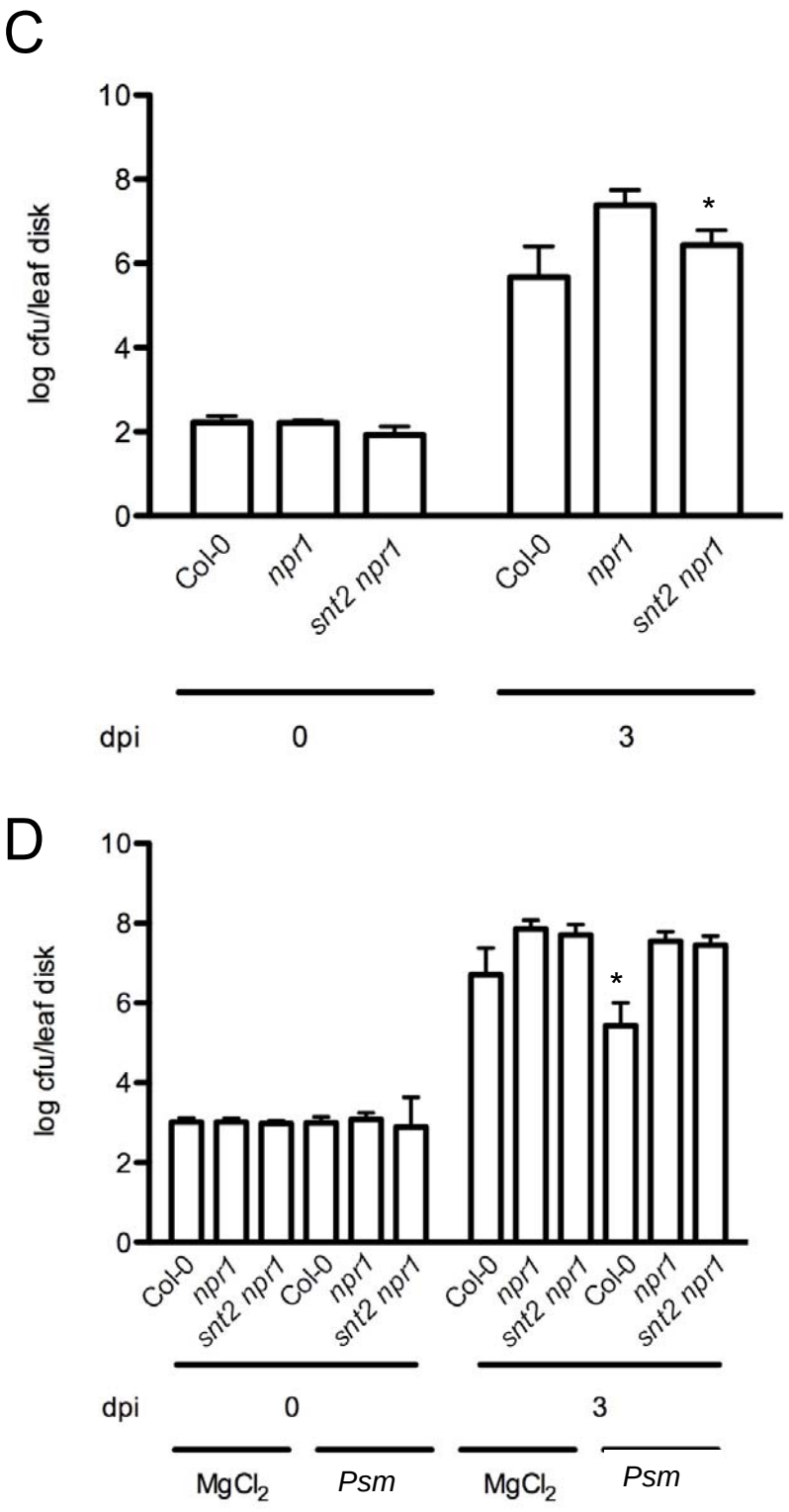


Figure 5-1. Continued

A

Generation	Yellowing	Normal
F_1 *snt2*♀ x *npr1-3*♂	0	10
F_1 snt2♂ x *npr1-3*♀	0	10
F_2 *snt2* x *npr1-3*	64	134

B

F2 Morphology	SA-tolerant	SA toxic
snt2	96.6 ± 4.8%	3.8 ± 5.7%
npr1	16.31 ± 10.74	83.6 ± 10.7

Figure 5-2. Genetic analysis of the *snt2* mutation. (A) Phenotypes of the progeny from backcrossed *snt2 npr1* plants. (B) Phenotypes of F_3 progeny from F_2 plants scored in (A) with either *snt2* or *npr1* morphology.

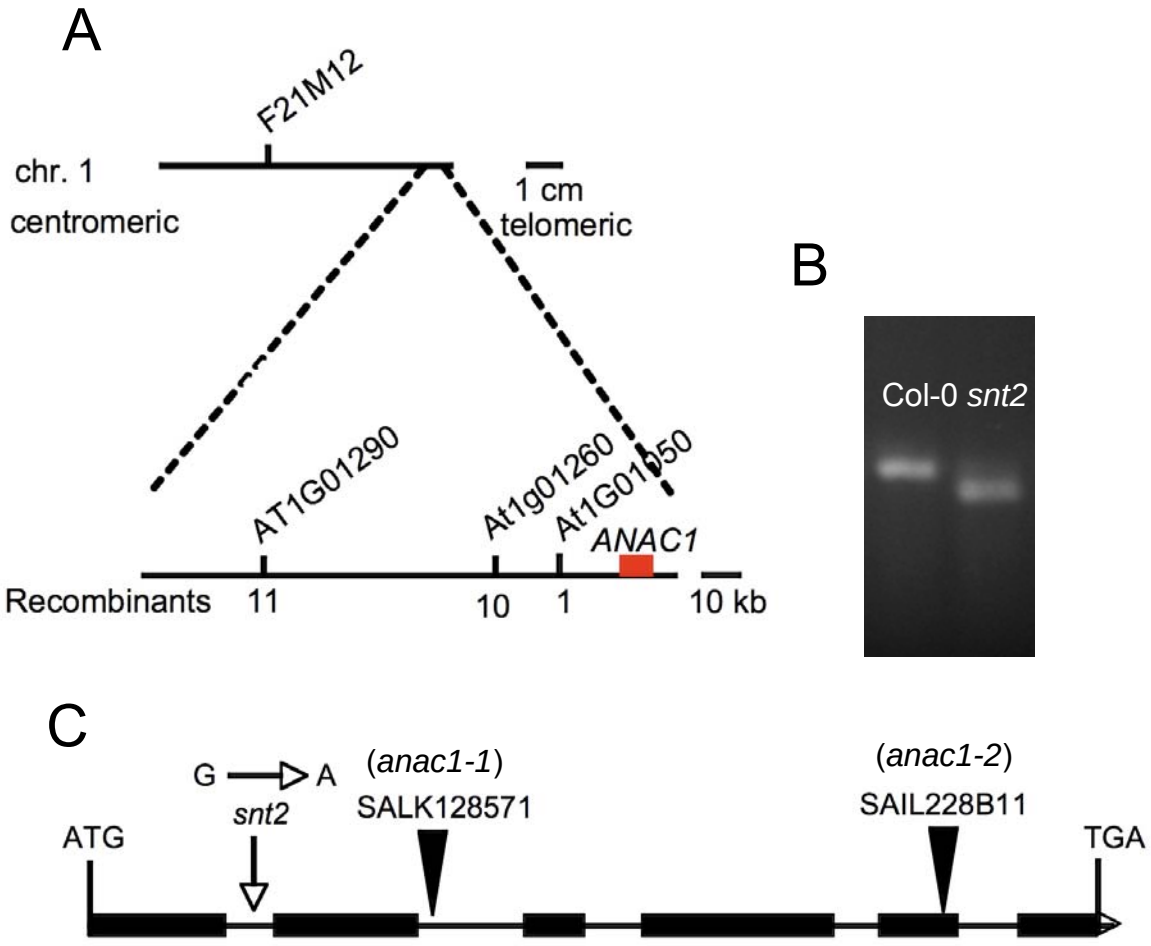
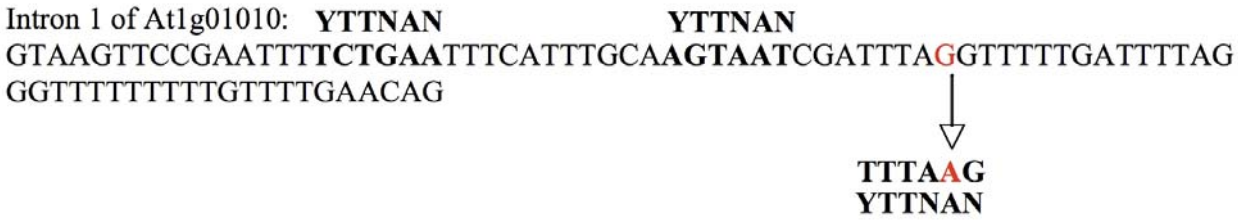


Figure 5-3. Identification of the *snt2* mutation. (A) Map-based cloning of *snt2*. 198 F_2 progeny homozygous for *snt2* were used to determine the approximate position of the *snt2* mutation using bulked segregant analysis. *snt2* was closely linked to the marker F12M12. Out of a mapping population of the same 198 plants homozygous for *snt2*, 11 were heterozygous at At1g01290, 10 were heterozygous at At1g01260, and one was heterozygous at At1g01050. Therefore *snt2* is on the telomeric side of these markers. (B) DNA polymorphism between *snt2* and wild type plants. Non-complementary primers were used to introduce a new DdeI site unique to wild type. The DNA fragments flanking the DdeI site were amplified from the wild type and *snt2* plants, digested with DdeI, and separated on an agarose gel. (C) Structure of the *ANAC1* gene (At1g01010), the *snt2* mutation, and the insertion sites of two T-DNA insertion mutants. Boxes denote the coding sequence, and lines between denote introns. (D) Possible branch-points in intron 1 of At1g01010. Bases in bold indicate possible branch points, with the branch-point consensus sequence for Arabidopsis shown above the sequence. The *snt2* mutation is shown in red. (E) Expression of At1g01010. Gene expression was analyzed by semi-quantitative RT-PCR as described in Methods.

D



E

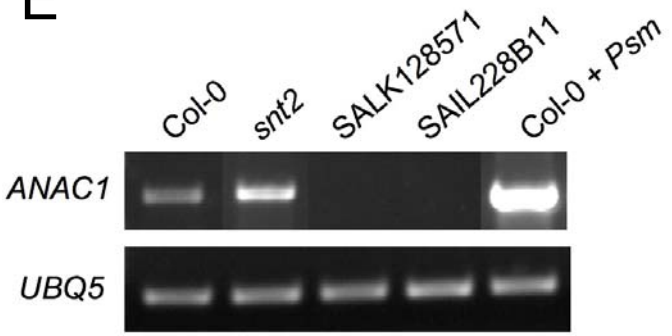


Figure 5-3. Continued

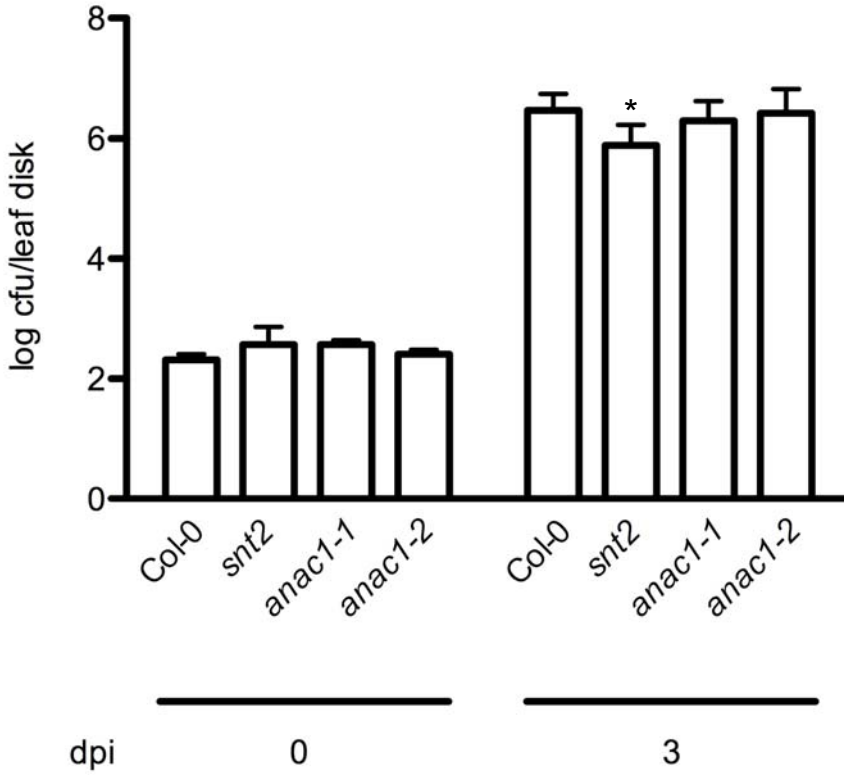


Figure 5-4. Growth of *Psm* in *snt2*, *anac1-1*, and *anac1-2* single mutants. Leaves of 4-week-old plants were pressure-infiltrated with a bacterial suspension of OD600 NM = 0.0001 and bacterial numbers determined at 0 and 3 days post inoculation (dpi). Values represent the mean of 4-10 samples (± SD).

CHAPTER 6
DISCUSSION

The isolation of two Elongator mutants with deficient immune responses from the same genetic screen strongly supports the notion that this protein complex plays an important role in plant immunity. Although efforts within the last decade have revealed diverse functions for Elongator, how Elongator accomplishes these functions is only beginning to be understood.

Functions of Elongator

Elongator and Transcription

Elongator was first identified as an interactor of hyperphosphorylated (elongating) RNA Polymerase II (RNAPII) in yeast, and subsequently co-purified with RNAPII in mammalian cells. Elongator is comprised of six subunits. Holo-Elongator is a relatively unstable six-subunit complex composed of two subcomplexes: core-Elongator, comprised of Elp1, Elp2, and Elp3 (Wittschieben et al., 1999) (Otero et al., 1999) (Fellows et al., 2000), and a smaller three-subunit module comprised of Elp4, Elp5, and Elp6 (Li et al., 2001b). All six subunits are highly conserved in eukaryotic organisms. The first and second subunits, ELP1 and ELP2, are WD40 proteins that act as scaffolds for complex assembly (Smith et al., 1999). ELP1 also contains a functional nuclear localization sequence (Fichtner et al., 2003). ELP3 is a histone acetyltransferase (HAT), and has been shown to acetylate histone 3 and possibly histone 4 (Winkler et al., 2002). Other than their requirement for ELP3 HAT activity, the functions of ELP4-6 are unknown. Multiply acetylated H3 and H4 are decreased in yeast Elongator mutants (Winkler et al., 2002). Additionally, disruption of subunits in both Elongator and SAGA

(another HAT) results in severe growth defects, while disruption of Elongator and H3 or H4 N-terminal tails results in synthetic lethality (Wittschieben et al., 2000). These results suggest Elongator's HAT activity is important for its function.

ELP3 also contains a putative radical S-adenosyl methionine (SAM)-binding domain, suggesting Elongator may also contain methyltransferase activity (Chinenov, 2002). In support of this notion, disruption of ELP1, ELP4, ELP3, and the ELP3 SAM-binding domain but not its HAT domain, prevented DNA demethylation of paternal DNA in mouse zygotes (Okada et al., 2009). However, DNA methyltransferase or histone methyltransferase activity of ELP3 has not yet been demonstrated.

Elongator preferentially acetylates K14 on histone 3 (Winkler et al., 2002). This modification is generally associated with actively transcribed chromatin (Lusser, 2002) (Fransz and de Jong, 2002). Not surprisingly, Elongator has been shown to function in gene expression. Disruption of Elongator affected the activation of many genes in yeast, while only a few constitutively expressed genes were affected (Krogan and Greenblatt, 2001). Removal of any of the six Elongator subunits or the ELP3 HAT domain results in slow adaptation to new growth conditions and environmental stresses. The induction of genes required for growth in the new conditions was also delayed (Otero et al., 1999). These studies suggest Elongator functions in transcription activation. However, chromatin immunoprecipitation experiments failed to detect an interaction between Elongator and its target genes in yeast (Winkler et al., 2002). In contrast, Elongator was detected at several target and non-target genes in human cell lines. Additionally, cells lacking Elongator displayed defects in neuronal migration, and several genes important

for cell migration were under-expressed and their chromatin hypoacetylated only in the coding region (Close et al., 2006). In yeast *elp* mutants, total histone acetylation was decreased, yet no clear clustering of histone acetylation was observed in the promoter or coding regions (Winkler et al., 2002). Though these studies examined different histone modifications, organisms, and genes, further experiments are needed to test the notion that Elongator facilitates gene induction by histone modification.

Elongator genes have also been identified in Arabidopsis, and disruption of all Elongator genes tested resulted in nearly identical developmental defects such as elongated leaves and reduced fertility (Nelissen et al., 2005). These results suggest that removal of any Elongator subunit abrogates the function of the entire complex, though an exception to this is discussed below.

Elongator and tRNA Modification

Although Elongator certainly plays a distinct role in transcription, *elp* mutants also display translational defects. Yeast *elp* mutants lack 5-methoxycarbonylmethyl (mcm^5) and 5-carbamoylmethyl (ncm^5) groups on uridines at the wobble position in 11 tRNA species, one of which was found to co-immunoprecipitate with Elongator (Huang et al., 2005). Overexpression of two of these tRNAs lacking the mcm^5 side chain was sufficient to rescue the *elp* phenotypes, but not mcm5 and ncm^5 synthesis (Esberg et al., 2006). Elongator also seems to interact with naked RNA (Otero et al., 1999). This data suggests Elongator may directly modify tRNAs, and defects in tRNA modification and translation may explain the phenotypes of *elp* mutants. Interestingly, an *elp3* mutant has been isolated that is zymocin-insensitive (presumably lacks mcm^5 and ncm^5), yet does not display the other *elp3* phenotypes (Jablonowski et al., 2001). This data calls into

question the assertion that all the phenotypes of Elongator mutants are caused by tRNA modification defects.

These studies raise an important question: Are the *elp* phenotypes due to defects in tRNA or chromatin modification? In other words, does a defect in translation then affect transcription, or vice versa? For example, in mouse zygotes, delayed translation of DNA demethylases resulting from decreased mcm^5 and ncm^5 levels in tRNA might result in delayed paternal DNA demethylation. Alternatively, Elongator might directly demethylate the paternal genome or acetylate chromatin in genes important in this process, initiating a somatic cell transcriptional program.

Elongator in Familial Dysautnomia

In humans, a mutation resulting in a truncated ELP1 protein results in familial dysautonomia (FD), an autosomal recessive disease, resulting in abnormally low numbers of neurons in the autonomic and sensory nervous systems. In FD cells, Elongator is not present at genes involved in neuron migration (Close et al., 2006). Underexpression of these genes in FD neurons was thought to contribute to lowered neuron numbers. Additionally, lowered α-tubulin acetylation, which is involved in cell migration, was observed in cells lacking Elongator subunits. Expression of a nonacetylatable α-tubulin mutant led to comparable defects in cortical neurons. ELP3 associates with and promotes acetylation of α-tubulin, suggesting α-tubulin may be a target of Elongator, and that α-tubulin acetylation is essential for normal neuron migration (Creppe et al., 2009). In a subsequent study, the molecular basis of Polymicrogyria (abnormal development of the cerebral cortex) was shown to be a

mutation in an α-tubulin variant of unknown function that is not susceptible to the acetylation that regulates microtubule function during cortical neuron migration (Abdollahi et al., 2009). These studies suggest α-tubulin hypoacetylation due to Elongator disruption is the cause of FD and possibly other neural disorders. In light of these results, the possibility that hypoacetylation of α-tubulin may underlie some or all of the Elongator mutant phenotypes in yeast and plants must be considered.

ELP3 is Essential for Plant Immunity

The isolation of two mutants with deficient immune responses that lack different subunits of Elongator strongly implicates this complex in plant immunity. A discussion of the *elp3* mutants is presented here, while a more detailed discussion of Elongator's function in plant immunity is presented below in the context of *elp2* characterization. The *elp3* mutation was found to be a deletion of a cytosine and a subsequent frameshift, likely resulting in a non-functional protein. This was unexpected, as EMS mutagenesis normally causes transition mutations, and in rare cases (~1%), transversions (Kim et al., 2006). This deletion might have been due to a spontaneous mutation. Alternatively, another transition mutation may have occurred somewhere in the vicinity of the *elp3* mutation, and the *elp3* mutation was introduced during error-prone DNA repair. In a large-scale EMS mutagenesis reverse-genetic screen, Greene et al. reported several non-transition mutants, some of which may have resulted from error-prone repair (Greene et al., 2003). Regardless of how the *elp3* mutation was generated, the data shows that disruption of *ELP3* results in SA tolerance, susceptibility to *Psm*, and abnormal leaf development.

The wide leaf phenotype is somewhat surprising, given that the *elp2* mutants and the *elo3* mutant all exhibit a narrow leaf phenotype. The reason for this is not known. It is possible that a second-site mutation that is closely linked to *ELP3* causes this phenotype. Obtaining an independent mutant allele in Columbia background might address this question. However, mutants containing a T-DNA insertion in *ELP3* in this ecotype were unavailable. Another possibility is that loss of *ELP3* function confers different phenotypes to different ecotypes of Arabidopsis.

With the exception of leaf size, the *elo3* mutant closely resembled the *elp3* mutant, suggesting these mutants are allelic. The mapping of these mutations to the same gene confirms this notion. The phenotypes of these alleles also suggest that Elongator functions in plant immunity in at least two Arabidopsis ecotypes. Although *elo3* is more susceptible than wild type Ws, the difference between *elo3* and Ws is less than the difference between *elp3* and Col-0. This may be because Ws supports less *Psm* growth than Col-0 (Figure 4-3).

The Role of Elongator in Plant Immunity

The transcription coactivator NPR1 is a master regulator of plant immune responses. Mutations in the *NPR1* gene block SA- or INA-induced defense gene expression and disease resistance and completely compromise SAR (Cao et al., 1994). To identify additional regulators of SAR, two genetic screens for suppressors of *npr1* have been performed (Bowling et al., 1997) (Clarke et al., 1998) (Shah et al., 1999) (Li et al., 1999; Shah et al., 2001) (Li et al., 2001a). The suppressors identified in these screens either restored inducible defense gene expression and pathogen resistance or displayed constitutive defense responses. In this study, a genetic screen was performed

which selected for suppressors of *npr1* based on the SA-nontolerant phenotype of *npr1* on MS medium containing high concentrations of SA. Isolation and characterization of the *npr1*-suppressor mutant *elp2* revealed that the Elongator subunit 2 (ELP2) functions upstream and downstream of SA in plant immunity, and is an accelerator of plant immune responses, which primarily regulates the NPR1-independent defense pathway.

Mutations in the *ELP2* gene partially restored SA tolerance in *npr1* (Figure 3-1a and 3-3d), suggesting that the wild-type ELP2 protein may play a role in producing SA cytotoxicity in *npr1*. ELP2 may negatively regulate the expression of antioxidant genes that help scavenge reactive oxygen species (ROS) generated by high levels of SA, thus attenuating SA-caused oxidative damage to plant cells (Rao et al., 1997). Previous work has reported that *elp* mutant plants (including *elp1*, *elp2*, *elp4*, and *elp6*) are more resistant to oxidative stress caused by methyl viologen and cesium chloride under light, which is correlated with increased expression of antioxidant genes such as *CAT3* (encoding catalase 3) (Chen et al., 2006) (Zhou et al., 2009). The elevated antioxidant capacity in *elp2* plants may alleviate oxidative damage caused by SA, thus rendering *npr1* plants more SA tolerant. This conclusion is in agreement with the result that SA cytotoxicity is partially caused by SA-induced oxidative stress (Rao et al., 1997).

The *Atelp2* mutations did not restore pathogen resistance in *npr1*. Instead, these mutations further compromised basal resistance of *npr1* to the virulent pathogen *Psm* (Figure 3-1c and 3-3f), suggesting that ELP2 is essential for NPR1-independent immunity. Indeed, compared to *npr1*, *Psm*-induced expression of *PR1*, *PR2*, and *PR5* is further delayed or reduced in *elp2 npr1* plants (Figure 3-3B). *elp2* exhibited reduced

pathogen-induced *ICS1* expression (Figure 3-5a) and SA biosynthesis (Figures 3-5b), indicating that ELP2 promotes immunity upstream of SA. Consistently, *elp2* mutations also suppressed pathogen-induced hyperaccumulation of *ICS1* transcripts and SA in *npr1* (Figures 3-4a, 3-1b, and 3-2e). Furthermore, *elp2* mutations partially blocked SA-induced defense gene expression and pathogen resistance (Figure 3-6), suggesting that ELP2 also functions downstream of SA. The function of ELP2, both upstream and downstream of SA, may explain the delayed or reduced expression of *PR1*, *PR2*, and *PR5* (Figures 3-1c and 3-4b) and enhanced disease susceptibility (Figures 3-1c and 3-5d) of *elp2* and *elp2 npr1* plants.

Surprisingly, SAR is nearly intact in *elp2* plants. When SAR-inducible gene expression was monitored, all six genes tested were induced and three of them were induced to wild-type levels (Figure 3-7b). The SAR-induced inhibition of pathogen growth was similar in wild type and *elp2* plants (Figure 3-7a), indicating that SAR induction can compensate for the immune defects in *elp2*. These results, together with the delayed and reduced basal defense in *elp2*, indicate that ELP2 may function as an accelerator of defense responses in Arabidopsis. This conclusion is in agreement with the results from yeast Elongator mutants (*elp*Δ) (Otero et al., 1999). Yeast *elp*Δ cells exhibit a delay in growth recovery when introduced to new growth conditions. However, once adapted to the new conditions, they grow with a doubling time comparable to wild type. The slow adaptation phenotype is most likely caused by delayed expression of genes induced by and required for growth under new conditions. For instance, when yeast *elp*Δ cells were transferred from glucose to galactose, the transcript level of the

galactose-inducible *GAL1-10* gene was ~8-fold lower in *elp*Δ cells after 30 minutes but reached wild-type levels after 2-4 hours. Similarly, expression of the low-phosphate inducible *PHO5* gene and the low-inositol inducible *INO1* gene was significantly delayed when *elp*Δ cells were transferred to low phosphate and low inositol media, respectively. These results suggest that Elongator is required for rapid transcriptional reprogramming in response to environmental changes.

The role of AtELP2 as an accelerator in immune responses was further substantiated by the results from characterization of defense gene expression in *elp2* plants during ETI. Following infection by the ETI-inducing pathogen *Pst* DC3000/*avrRpt2*, activation of defense genes was delayed in *elp2* plants, but expression of some genes eventually reached wild-type levels (Figure 3-8b). Among the genes tested, ELP2 is required for induction of At5g47230, suggesting the existence of ELP2-dependent genes. In contrast, gene activation in *npr1* was mostly not delayed, but expression failed to reach wild-type levels. Interestingly, the delayed gene activation in *elp2* plants or decreased gene expression in *npr1* plants only had a moderate effect on RPS2-mediated resistance (Figure 3-8b). However, RPS2-mediate resistance was completely abolished in the *elp2 npr1* double mutant plants, indicating that ELP2 and NPR1 function primarily independently of each other in ETI.

Characterization of the *elp2*, *npr1*, and *elp2 npr1* mutant plants in this study provided new insight into the function of NPR1 and its relationship with ELP2 in plant immune responses. Both ELP2 and NPR1 are required for basal immunity and ETI, but only NPR1 is required for SAR. Mutations in either *ELP2* or *NPR1* significantly block the

slow and weak basal immunity, but only moderately affect the fast and strong ETI. The result that removal of both ELP2 and NPR1 completely abolishes the RPS2-mediated resistance demonstrates that both ELP2 and NPR1 are major positive regulators of ETI. ELP2 and NPR1 appear to act at different signaling nodes in immune responses. ELP2 is mainly essential for the timely activation of defense genes, whereas NPR1 mostly controls the scale of gene expression, though these two functions are not mutually exclusive. How much overlap exists between the functions of ELP2 and NPR1 remains to be determined. Interestingly, expression of several defense genes in *elp2 npr1* plants remained high long after repression had occurred in wild type, *elp2*, and *npr1* plants, suggesting that ELP2 and NPR1 may function redundantly to repress the expression of these genes at later time points. These functions may help switch off immune responses once pathogen challenge subsides, and the underlying regulatory mechanism merits further investigation.

How does Elongator promote pathogen resistance? Elongator most likely facilitates RNAPII transcription through acetylation of histones. The yeast Elongator subunit 3, ELP3, was shown to have histone acetyltransferase (HAT) activity (Winkler et al., 2002). In mammals, several genes involved in cell motility, neurulation and vascular development were found to be direct targets for acetylation by the Elongator complex during transcription elongation (Close et al., 2006). In Arabidopsis, ELP3 was colocalized with euchromatin and the phosphorylated form of RNAPII, and reduced expression of two auxin-related genes was correlated with reduced histone H3 lysine 14 acetylation at the coding region of these genes (Nelissen et al., 2009). Although ELP3's

HAT activity has not been tested, it is possible that the Arabidopsis Elongator complex directly promotes the induction of defense genes through its interaction with the hyperphosphorylated form of RNAP II and subsequent acetylation of histones of these genes. This and other possibilities of the underlying mechanism for Elongator's immune function are discussed below, as well as future studies to address these possibilities.

How Does Elongator Regulate Pathogen Resistance?

Transcription and Histone Modification

Given Elongator's multifunctional nature, this protein complex might positively regulate plant immunity through several different, but not mutually exclusive, mechanisms. Elongator might directly promote the induction of defense genes through its interaction with RNAP and subsequent acetylation of histones. These modifications would convert chromatin to the "open" state, facilitating more efficient transcription. To test this hypothesis, defense gene chromatin will be immunoprecipitated with H3K9 and H3K14 antibodies to determine the effect of Elongator disruption on pathogen-induced H3 acetylation. Chromatin immunoprecipitation (ChIP) experiments will also test the possibility that Elongator acts directly on defense genes by probing Interactions between Elongator and defense gene chromatin before and during pathogen infection.

tRNA Modification and Translation

Since defects in translation may be responsible for most *elp* phenotypes in yeast, these defects might also explain the immune deficiencies of *elp* plants. It will be interesting to see if plant *elp* mutants also lack mcm^5 and ncm^5 tRNA modifications, as these modifications are conserved in eukaryotes (de Crecy-Legard et al., 1990). If *elp* plants lack these modifications, the decreased transcription of defense genes in these

mutants might be due reduced synthesis of proteins that regulate these genes. Future work will attempt to determine the effect of Elongator disruption on tRNA modification in plants.

Localization

NPR1 prevents SA toxicity and regulates defense genes through its co-activator activity in the nucleus (Zhang et al., 2009). Since Elongator interacts genetically with NPR1, Elongator may also carry out its immune function in the nucleus. Although nuclear florescence in 35S::ELP2-GFP plants could not be detected (Figure 3-10), nuclear localization of a fraction of Elongator seems likely. Previous studies established that yeast and human cells posses both cytosolic and nuclear Elongator (Rahl et al., 2005) (Fichtner et al., 2003) (Creppe et al., 2009), and that this dual localization is essential for Elongator function. The NLS sequence on the C-terminus of ELP1 is conserved (Rahl et al., 2005); suggesting plant Elongator can enter the nucleus. Failure to detect nuclear ELP2-GFP by fluorescence microscopy may be due to low levels of nuclear Elongator, or masking of the GFP fluorophore by other proteins in the vicinity. Introduction of ELP2-GFP driven by its native promoter to *elp2* mutants resulted in partial complementation of morphological phenotypes in three lines. However, none of these lines had detectable ELP2-GFP protein levels (not shown). It is possible that fusion of GFP to ELP2 impedes its function, possibly by steric hindrance, and that overexpression of ELP2-GFP might alleviate these effects. Even when driven by the strong 35S promoter, no single-insertion transgenic lines were isolated that completely complemented the plant defense phenotypes of *elp2* (Figure 9). In light of these results, other approaches may be needed to determine the subcellular localization of ELP2.

Efforts to detect nuclear ELP2-GFP via western blot from nuclear extracts are underway. In future work, the immune response of plants lacking the NLS of ELP1 will be tested to determine if nuclear localization is essential for Elongator's immune function.

Dissecting Elongator Function

To determine Elongator function in plant immunity, transcriptional and translational defects need to be uncoupled *in vivo*, and the immune responses of the resulting mutants characterized. Transcriptional and translation inhibitors are unsuitable for this purpose because of their global and non-specific effects. Removal of the HAT domain and SAM-binding domain of ELP3, and the nuclear localization sequence of ELP1 may uncouple these defects, and will clarify the function of each motif.

Possible Functions of ELP4-ELP6 in Plant Immunity

In yeast, disruption of any Elongator subunit results in similar phenotypes. The only evidence for divergent functions between Core-Elongator and Holo-Elongator comes from studies with Arabidopsis. Only mutations in core Elongator subunits caused stomatal closure to be hypersensitive to ABA (Zhou et al., 2009). This is surprising, given that all six subunits are essential for histone acetylation, and suggests HAT activity may not be essential for Elongator's function in ABA signaling. To determine if the non-core subunits ELP4-ELP6 are essential for plant immunity, *elp1, elp4* and *elp6* mutants have been isolated, and *elo1*, *elo2*, and *elo3* plants were obtained and crossed into an *npr1* background. All double mutants were SA tolerant and both single and double mutants displayed *elp2*-like morphology (not shown). Preliminary results show

that these mutants are also susceptible to *Psm* infection, suggesting Elongator's immune function may require both core and non-core subunits.

Does Elongator Regulate Crosstalk Between Hormone Signaling Pathways?

Plant pathogens are known to use stomata for entry into the leaf during pathogenesis. Pathogen-induced stomata closure requires ABA and SA signal transduction, and is antagonized by the virulence factor coronatine, which is a jasmonate-mimic that antagonizes SA signaling (Melotto et al., 2008). Elongator antagonizes ABA-mediated signaling (Zhou et al., 2009) and promotes SA signaling (this study), suggesting Elongator might modulate crosstalk between these two signaling networks. In this study, *Psm* was infiltrated directly into the apoplast, bypassing the leaf infiltration step in pathogenesis. During an infection where the pathogen must infiltrate an intact leaf, Elongator may act as both a negative (through antagonizing stomatal closure) and a positive (through its activation of defense genes) regulator of plant immunity. Elongator might also function in stomatal closure via its role in SA signaling. In plant immunity, virulent *P. syringae* induces ABA biosynthesis. ABA levels are correlated with pathogen susceptibility, and ABA represses SA accumulation and defense gene expression (Spoel and Dong, 2008). This suggests ABA might also promote virulence after infiltration occurs. However, in some cases ABA may function to promote resistance (Ton et al., 2009). Further experiments are needed to determine if elongation functions in plant immunity through its role in ABA signaling. Regardless of how the Elongator complex regulates defense gene transcription, this study clearly demonstrates that AtELP2 is an essential component of plant immunity, which functions

together with the transcription coactivator NPR1, to orchestrate plant immune responses.

The Role of ANAC1 in Plant Immunity

In addition to Elongator, this study has implicated ANAC1 in SA tolerance and plant immunity. This genetic screen aimed to isolate mutants that restored SA tolerance to *npr1*. One such mutant was *snt2*. It was possible that *snt2* might also affect plant immunity. The *snt2* mutation partially restored pathogen resistance to *npr1*, and also conferred greater resistance to wild type plants. The *snt2* mutation was mapped to *ANAC1*, and found to be a gain-of-function mutation, as knockout alleles of *ANAC1* did not confer *snt2* phenotypes and *ANAC1* expression was increased in *snt2*. Wild type and *npr1* plants containing the *35S:ANAC1-GFP* transgene were obtained to attempt to recapture the *snt2* phenotype and show that *SNT2* is *ANAC1*. Unfortunately, none of the transgenic lines expressed detectable amounts of ANAC1-GFP (not shown), nor did they display *snt2* phenotypes. Therefore we could not confirm the identity of *SNT2*. Perhaps high expression levels of *ANAC1* are lethal during embryogenesis, as *ANAC1* expression is lowest during this stage of plant development (AK, 2006). Alternatively, the GFP fused to ANAC1 may somehow interfere with its function. However this seems unlikely, as other ANAC-GFP fusions proteins have been shown to be function normally in vivo (Bu et al., 2008). Transgenic overexpression of *ANAC1* without GFP driven by a weaker or the *ANAC1* native promoter might allow the isolation o *ANAC1*-overepressing plants.

Surprisingly, the *snt2* mutation was found in intron 1 of *ANAC1*. The fact that a single cDNA amplicon was obtained which was identical to the wild type cDNA suggests

this gene is spliced correctly in *snt2*. *ANAC1* expression was also increased in *snt2*. The possibility that alteration of an intron cis-element in *snt2* caused *ANAC1* overexpression was then examined. Three cis-elements are essential for intron splicing in plants. The first and second elements are located at the 5' and 3' splice junctions, respectively, and consist of several semi-conserved bases that span the intron/exon borders. The third element is the less conserved branchpoint sequence, with a consensus sequence of YTTNAN or, more stringently, CTRAY). The Adenine is the absolutely conserved and essential branchpoint and functions in formation of the lariat intermediate. The branchpoint consensus is usually located -19 to -50 bases upstream of the 3' splice junction (Schuler, 2008). One possible explanation for *ANAC1* overexpression in *snt2* is that this mutation increases splicing efficiency by introducing a novel branchpoint, which in turn increases steady-state levels of mature *ANAC1* mRNA and ANAC1 protein. Studies with two Arabidopsis mutants support the plausibility of this model.

The *det3-1* mutation destroys a consensus branch-point sequence that is 32 base pairs upstream of the 3' splice junction, and reduces expression of *DET3* by half. The *DET3* cDNA is unchanged, and overexpression of *DET3* is sufficient for complementation of *det3-1* (Schumacher et al., 1999). This suggests branch-point sequences may be essential for optimum gene expression. Another mutation, *ap3-1*, is a loss-of-function allele of the *APATELA* gene, which is involved in flower development. The mutant phenotype results from the skipping of exon 5 during splicing. An intragenic suppressor mutation, *ap3-11*, partially restores normal splicing and gene function. This

mutation is located 32 nucleotides upstream from the 3' splice junction in intron 4, and creates a novel putative branch point through a G to A transition. The authors of this study propose this novel branchpoint may cause exon 5 to be more frequently recognized by the splicing machinery (Yi and Jack, 1998).

The similarities between *ap3-11* and *snt2* are striking. Both are G to A transitions, both are found in the interval where branchpoints are commonly found, both introduce putative novel branchpoints, and both result in an increase in correctly spliced transcript levels. Therefore *snt2*, like *ap3-11*, may increase transcript levels by increasing exon recognition by the splicing machinery.

ANAC transcription factors play diverse roles in Arabidopsis. The ANAC family is specific to plants and consists of 105 members in Arabidopsis. These transcription factors posses a conserved N-terminal DNA-binding NAC domain and a variable C-terminal transactivation domain (Ooka et al., 2003).

Several ANAC factors have been implicated in pathogen resistance. ANAC55 and ANAC92 are essential for age-related resistance to *Pst* and *H. parasitica*, and are induced by pathogen infection (Carviel et al., 2009). JA treatment induces ANAC55 and ANAC19. This induction is dependant on COI1 and AtMYC2, which are central mediators of the JA response. Removal of both *ANAC55* and *ANAC19*, but not one or the other alone, resulted in reduced JA-dependant defense gene expression, although this double mutant exhibited enhanced resistance to the necrotrophic pathogen *B. cinerea*. Conversely, plants overexpressing each of these genes showed enhanced JA-induced defense gene expression (Bu et al., 2008).

Overexpression of *ANAC1* may increase pathogen resistance, but apparently *ANAC1* is not essential for immunity (Figure 5-4). Perhaps *ANAC1* functions redundantly with another *ANAC*, as is the case with *ANAC19* and *ANAC55*. A BLASTP search against the C-terminal transactivation domain of ANAC1 suggests ANAC68 is the only close homolog in Arabidopsis and may functionally overlap with ANAC1. However, according to microarray data (Genevestigator), *ANAC68* is not induced by pathogen treatment. Analysis of an *anac68 anac1* double mutant may address this question of redundancy.

In another study, the NAC factor ATAF2 was found to positively regulate plant Tobacco Mosaic Virus (TMV) resistance in Tobacco, and was induced during infection and SA treatment. Interaction between ATAF and the TMV replicase may act to suppress the defense response.

The data presented here adds *ANAC1* to the list of NACs implicated in pathogen resistance. The following arguments suggest *ANAC1* may play a role in plant immunity: 1) Plants overexpressing *ANAC1* display increased pathogen resistance 2) *ANAC1* expression is induced upon pathogen infection 3) *ANAC1* interacts genetically with NPR1, a central regulator of plant immune responses. However, these facts are insufficient to assign an immune function to *ANAC1*, and attempts to recapture the *snt2* phenotype by *ANAC1* overexpression were unsuccessful, as discussed above. Although an increase in *ANAC1* expression was observed in *snt2*, the possibility that a fraction of *ANAC1* transcripts were alternately spliced cannot be ruled out. Cloning and sequencing a large number of ANAC1 cDNAs might address this possibility. In future

work, SA accumulation and ICS1 expression in untreated and infected *snt2* and *snt2 npr1* plants will be determined. The yellowing near the shoot apical meristem in *snt2* is reminiscent of other Arabidopsis mutants that constitutively accumulate SA and are resistant to biotrophic pathogens (Clarke et al., 2000) (Zhang et al., 2003a). These mutants require *ICS1* for their pathogen resistance phenotype. *snt2 sid2-1* and *snt2 npr1 sid2-1* mutants will be tested for SA accumulation, ICS1 expression, and pathogen resistance to determine if *snt2* phenotypes also require ICS1-dependent SA accumulation.

APPENDIX
SUPPLEMENTAL FIGURES AND TABLES

Ler *npr1-L*

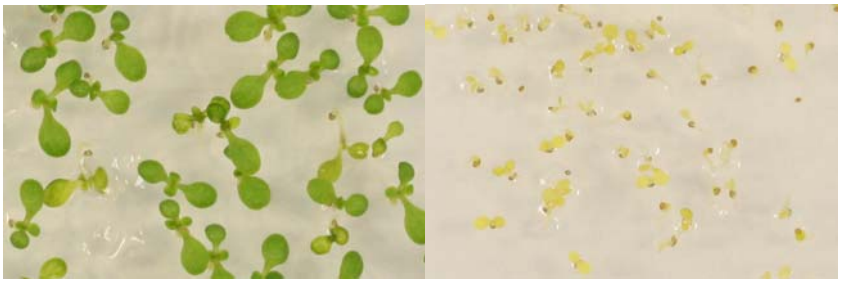


Figure A-1. Loss of SA tolerance in *npr1-L*. The experiment was done as for Figure 3-1a, except that a concentration of 0.18 mM SA was used.

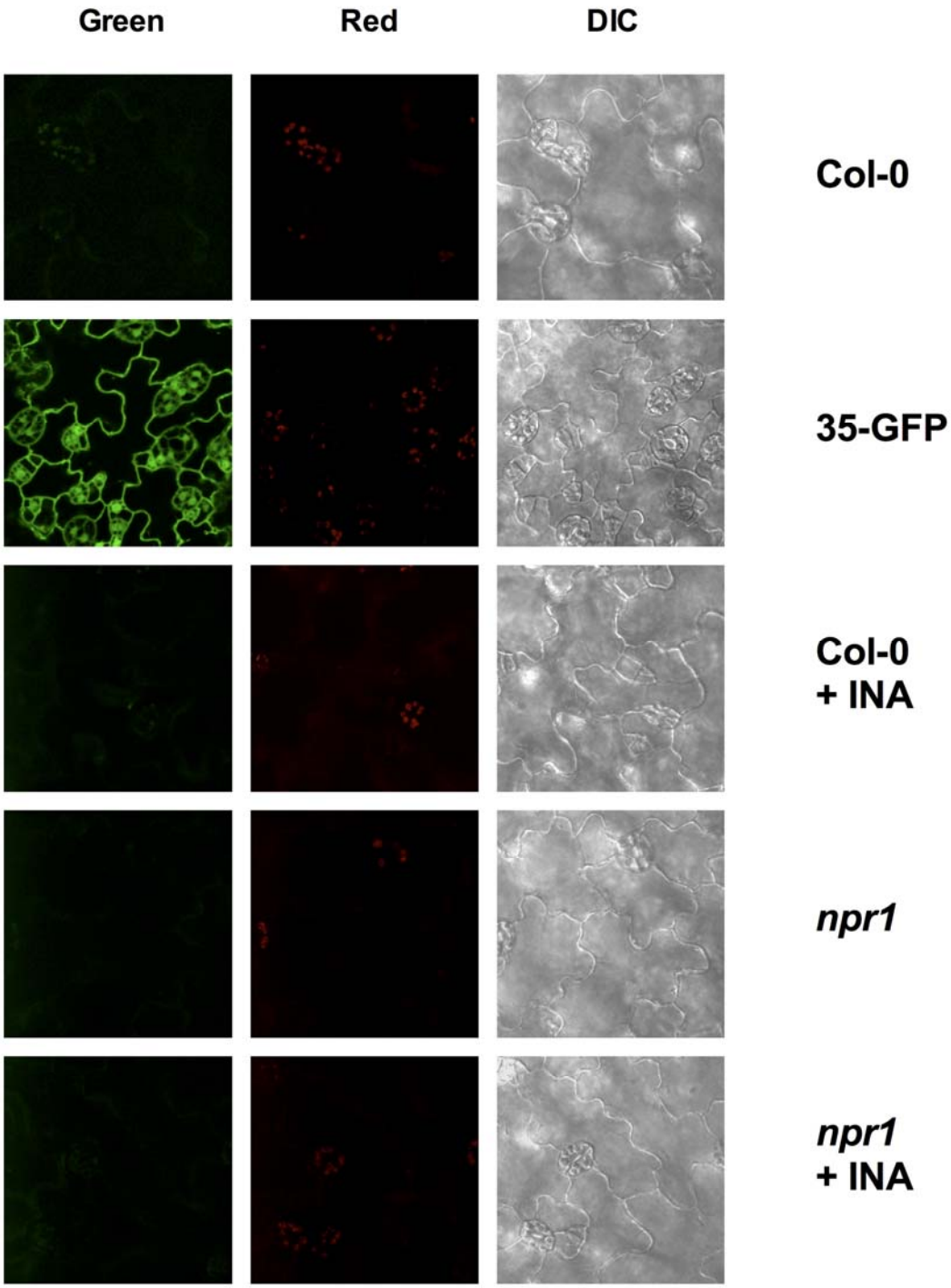


Figure A-2. ELP-GFP fluorescence in *npr1* and INA-treated seedlings. The experiment was done as for Figure 3-10.

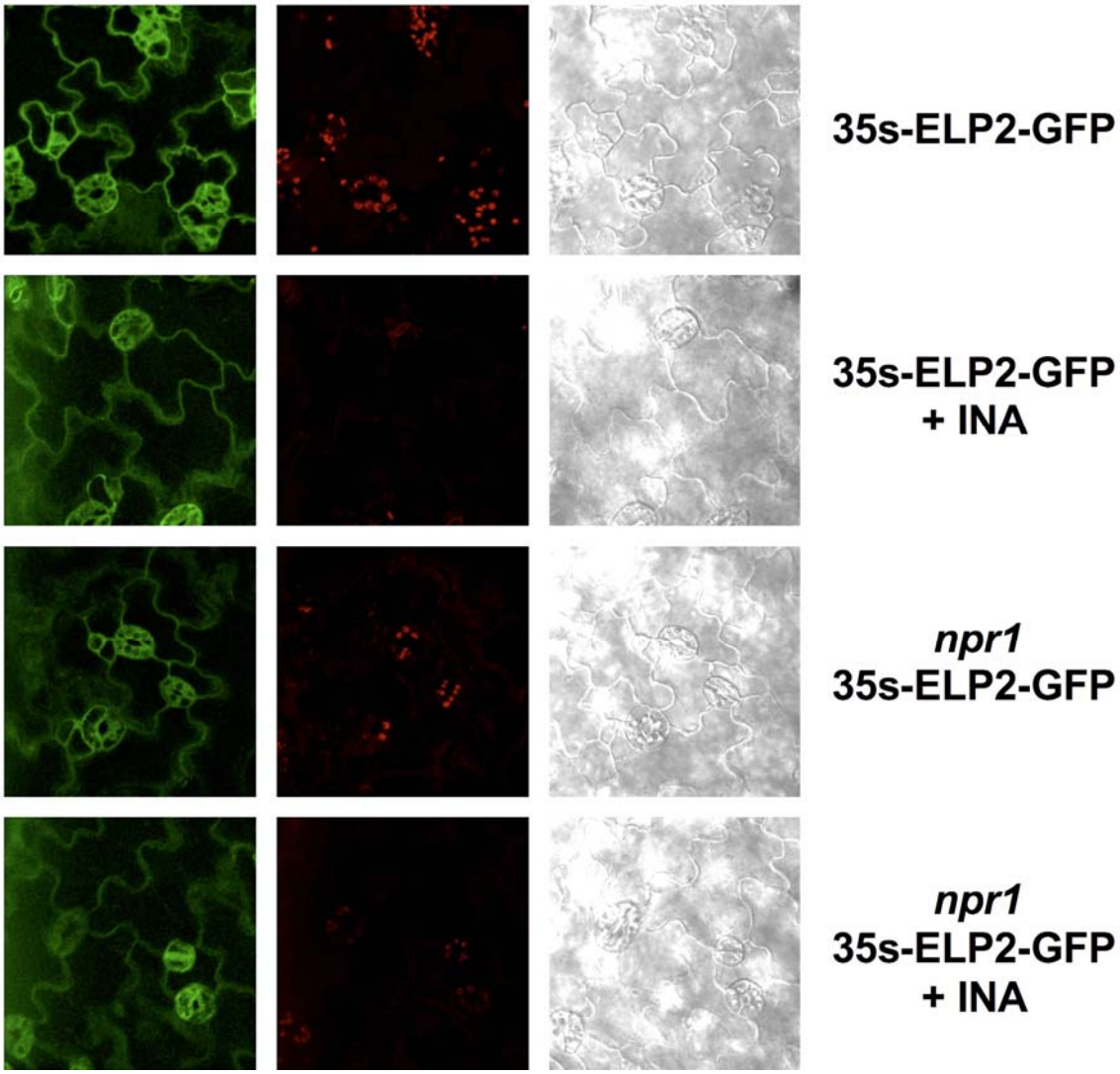


Figure A-2. Continued

Rough Map-Based Cloning Markers					
Chromosome (cM)	Marker	Forward Primer	Reverse Primer	Col (bp)	Ler (bp)
I					
(10)	F21M12	GGCTTTCTCGAAATCTGTCC	TTACTTTTTGCCTCTTGTCATTG	200	160
(39)	ciw12	AGGTTTTATTGCTTTTCACA	CTTTCAAAAGCACATCACA	128	115
(72)	ciw1	ACATTTTCTCAATCCTTACTC	GAGAGCTTCTTTATTTGTGAT	159	135
(81)	nga280	CTGATCTCACGGACAATAGTGC	GGCTCCATAAAAAGTGCACC	105	85
(113)	nga111	CTCCAGTTGGAAGCTAAAGGG	TGTTTTTTAGGACAAATGGCG	128	162
II					
(11)	ciw2	CCCAAAAGTTAATTATACTGT	CCGGGTTAATAATAAATGT	105	90
(30)	ciw3	GAAACTCAATGAAATCCACTT	TGAACTTGTTGTGAGCTTTGA	230	200
(50)	nga1126	CGCTACGCTTTTCGGTAAAG	GCACAGTCCAAGTCACAACC	191	199
(73)	nga168	TCGTCTACTGCACTGCCG	GAGGACATGTATAGGAGCCTCG	151	135
III					
(20)	nga162	CATGCAATTTGCATCTGAGG	CTCTGTCACTCTTTTCCTCTGG	107	89
(43)	ciw11	CCCCGAGTTGAGGTATT	GAAGAAATTCCTAAAGCATTC	179	230
(70)	ciw4	GTTCATTAAACTTGCGTGTGT	TACGGTCAGATTGAGTGATTC	190	215
(86)	nga6	TGGATTTCTTCCTCTCTTCAC	ATGGAGAAGCTTACACTGATC	143	123
IV					
(10)	ciw5	GGTTAAAAATTAGGGTTACGA	AGATTTACGTGGAAGCAAT	164	144
(47)	ciw6	CTCGTAGTGCACTTTCATCA	CACATGGTTAGGGAAACAATA	162	148
(65)	ciw7	AATTTGGAGATTAGCTGGAAT	CCATGTTGATGATAAGCACAA	130	123
(104)	nga1107	GCGAAAAAACAAAAAAATCCA	CGACGAATCGACAGAATTAGG	150	140
V					
(10)	CTR1	CCACTTGTTTCTCTCTCTAG	TATCAACAGAAACGCACCGAG	159	143
(42)	ciw8	TAGTGAAACCTTTCTCAGAT	TTATGTTTTCTTCAATCAGTT	100	135
(71)	PHYC	CTCAGAGAATTCCCAGAAAAATCT	AAACTCGAGAGTTTTGTCTAGATC	207	222
(88)	ciw9	CAGACGTATCAAATGACAAATG	GACTACTGCTCAAACTATTCGG	165	145
(115)	ciw10	CCACATTTTCCTTCTTTCATA	CAACATTTAGCAAATCAACTT	140	130

Table A-1. Primers used for rough mapping.

Fine Mapping Markers					
Marker	Forward Primer	Reverse Primer	Restriction Enzyme	Col (bp)	Ler (bp)
elp2-1					
At1g49580	TGTACCCACCAACTGTCAAC	ATCACAGTAATGCCATCGAAG	MspI	270, 217	487
At1g49470 CTD1)	CTTTGTGCCTGGTCTCTCAC	GGTCCACTATCATTAGTGCG	BamHI	566, 454	1020
At1g49550 (CTD3)	GGCAGCTTTGTATCTCATATG	AGTGACCAAGTCCAAGAGTG	AciI	477, 274	751
At1g49610	TGCTTGACAGAGGAATTGATG	TATGAACCCAATACGAAGGAG	AccI	368, 234	602
At1g49580 (CTD2)	TGTACCCACCAACTGTCAAC	ATCACAGTAATGCCATCGAAG	MspI	270, 217	487
BEDSSLP1F	TTGGACAAAACTTGCTGCAC	AAAATTTCCCTTAAGAAACATGC	none	155	125
GAPB	GGCACTATGTTCAGTGCTG	TCTGATCAGTTGCAGCTATG	BfaI	1211	850, 361
elp3-1					
At5g47570	CCACTCAACCATGCCAATGC	CGTCGGCAAACACATCGTCC	ApoI	250	211, 39
At5g50270	CGGTTTGTTGACAGATCTTTG	CATATCCTGCATACAAGACAG	BstUI	758	521, 237
At5g50120	TTACCAAAGATTTCACTGCTCC	CATCCTACTCATCATCCCATC	Bbs1	500	248, 252
At5g50180	GAACCATGTGATATGTTTCACC	GCAAGATTACTTCAAACCTTGC	BtsCI	604	372, 232
At5g50210	TTGTTGGGTGGAGAGAGATC	CCAGCTTGATCTAGGATGGC	RsaI	793	463, 329
At5g50270	CGGTTTGTTGACAGATCTTTG	CATATCCTGCATACAAGACAG	BstUI	758	521, 237
At5g50300	TAGAAACAATGCATGCACAAGC	AGTGGTGTGTGAGTGTGTACCG	BsrI	228	197, 31
At5g50360	ACCACCAGATCCGTTCTTCG	CACATCCAAAGGAGATTCGTG	EcoRV	782	540, 242
At5g50390	CCACAAGAACACTTCCCTTC	GGCAGATATACCAAAAAGTGG	Tsp509I	318	216, 102
At5g50460	CCATTATTGCTCGTTAGTTAC	TGGGATGCAATATTTTGGCC	BslI	279	211, 68
At5g50770	CTAAGAGCCTCCATCATTGG	GCAAGTTCATGACAAAGGAC	DpnII	586	483, 103
AT5G51130	GCCTTACAGGAGGTTTCTAG	GGTCGTAAAACAATCGTTCG	ApoI	477	288, 189
PDC2	CAGTGGATCACTCCCAAGACGCCTC	GCACTCAACTTATATATATTTCAG	BamHI	425	360, 65
anac1					
AT1G01440	CCCAAAGCTATACACGTCAG	GAGAATATACCACGGAGAG	Taq I	267	236, 31
AT1G01290	GGGTTCTGTTCTTGATCTCTTG	CGTAAGTCTACACGAACATGC	MnlI	820	428, 392
AT1G01260	CGACAAAACAGGGAGCCGATAC	GCACAGTTCTCTTCTTCTGAGCC	BstF5I	897	517, 380
AT1G01050	GCACAGGTTCCTACACAAAG	CAAGGTTAGATGAGAACAAAG	HinfI	296	217, 79

Table A-2. Primers used for fine mapping.

Mutant Genotyping Markers			Restriction enzyme	Col-0 (bp)	Mutant (bp)
Primer	Forward Primer	Reverse Primer			
SK_004690	ATGATGCCATTAGGGTGTGG	CACTATCATCACTACTACGAG			
SK_011529	CTGCGAGAGTAAGACAAATGC	TTACCTTATCTCCAGGAGCTG			
SK_084199	TGCACTGCATCGGTGGTATG	GAGATTTGAGGCGCCAGAG			
SK_100099	GCAGAGACATGTCATCCACC	GGATGGTTTGGTAGTGGTG			
SK_028216	CAGTGTCTTACTGTGTTACAG	CAGAGTTATTGGCAGTGGTAC			
SK_003541	CAGCAAATTCTGCTCAAGCTC	CCTTGTGTATGTTGAGGAATC			
SK_079193	CTGAGTTCCGTGTGTGATTAC	GGATGCCACACAACCTTGG			
SK_143430	TGGATACTGTTATTGCAGCC	GTTGGAGAAGCCCTTAGGTG			
SK_128571	CCTCTGTAAAATTTCCGGAGG	GGAACTTGCGCTGTAAGTTCCG			
SK_128569	CCTCTGTAAAATTTCCGGAGG	GGAACTTGCGCTGTAAGTTCCG			
Sail_228_B11	GGAGATGAACAACAAGACACC	CAGCAAATCATGGCCAGCAG			
elp2-2	CATCCCAGACATTCACAACTC	CGTTACACTGCTTCCAAACG			
elp2-3	CATCCCAGACATTCACAACTC	CGTTACACTGCTTCCAAACG			
elp2-4	CTCACGGATAGCAAGTACTC	CGTTACACTGCTTCCAAACG			
LBa1	TGGTTCACGTAGTGGGCCATCG				
LB3	TAGCATCTGAATTTCATAACCAA-TCTCGATACAC				
*anac1*dCAPS	CTGAATTTCATTTGCAAGTAATCGACTTA	CCTTGACCTCAACAGATTCTCC	Dde1	176, 25	201
*elp2-1*dCAPS	TGTTAGTAGCAGCAAAGAGTCCGAGAATT	GACCTGATCTTGGCTCACGGATAGC	BslI	187, 26	213
*elp3-1d*CAPS-F	CCTTTACCTGGCCGAGGTTG	CATTTTATGTCTTTTCTGTTGCCTC	MwoI	192	225
npr1-3	GGCCGACTATGTGTAGAAATACTAGCG	TGAGACGGTCAGGCTCGAGG	HhaI		
sid2-1	AAGCTTGCAAGAGTGCAA	TTTTAGCTGTCCTGCCAAT		~200	~180, 20
snc1-1	TGATCGTGCAAAGTCCAAGG	GTGAGATTGAGGTACTCGAG	XbaI	~400	~800

Table A-3. Primers used for mutant genotyping.

Gene Expression Analysis Primers		
RNA Blot Probe Primers		
Primer Set	Forward Primer	Reverse Primer
PR1	CTCATACACTCTGGTGGG	TTGGCACATCCGAGTC
PR2	CAAATCGGAGTATGCTACGG	CATCTCTGTAGCTCTGAACG
PR5	AGATGTGTAACCGGAGACTG	CTCGTTTCGTCGTCATAAGC
Quantitative PCR primers		
Gene	Forward Primer	Reverse Primer
ICS1	GAATTTGCAGTCGGGATCAG	AATTAATCGCCTGTAGAGATGTTG
UBQ5	TCTCCGTGGTGGTGCTAAG	GAACCTTTCCAGATCCATCG
ANAC1	GAATCGACAGAGCAGGACAA	CTACGACCTCTTACCAGAACATCAG
At4g27280F	ACGGTGCGTTGAATCAGATG	TCCCAAATCTCTCCAGTGTC
At5g47230F	CGTTTCCGTTTGTAACGTCG	TCCACGTCAGCATACACATC
At2g38470	GAATCGTAGTGCAGACAACG	TCCACATGTTTCCTCACTGG
At2g35980	GATCAAGTTCAGGCTTAGGG	AGAAGTCGAAGTCGCACTTG
At4g39670	TTACACGGAAGTGTGTGCAC	CTTCCATGTACCTCCTCATG
GST11	CGAGCTCAAAGATGGTGAAC	AGGGAGACAAGTTGGTTTCC
EDR11	AGCCTTTCATCCTTCGCAAC	ATGTCCTTGCCAGTTGAGAG
SAG21	GGCTCGTTCTATCTCTAACG	TTCTTCATCACAGCCGAAGC
LURP1	TGGCTAACAACGTAGAGGAG	CAACAGTGACGGAGAAATGG
WRKY18	TTAGATGCTCGTTTGCACCG	CCAAAGTCACTGTGCTTGAC
PR1	CTCATACACTCTGGTGGG	ATTGCACGTGTTCGCAGC
PR2	ATCAAGGAGCTTAGCCTCAC	TGTAAAGAGCCACAACGTCC

Table A-4. Primers used in analysis of gene

REFERENCES

Abdollahi, M.R., Morrison, E., Sirey, T., Molnar, Z., Hayward, B.E., Carr, I.M., Springell, K., Woods, G., Ahmed, M., Hattingh, L., Corry, P., Pilz, D.T., Stoodley, N., Crow, Y., Taylor, G.R., Bonthron, D.T., and Sheridan, E. (2009). Mutation of the variant a-tubulin TUBA8 results in polymicrogyria with optic nerve hypoplasia. The American Journal of Human Genetics 85, 737–744.

Abramoff, M.D., Magelhaes, P.J., and Ram, S.J. (2004). Image processing with ImageJ. Biophotonics International 11, 36-42.

AK, G. (2006). Genevestigator. Facilitating web-based gene-expression analysis. Plant Physiology 141, 1164-1166.

Alam, K., and Rolfe, J. (2006). Economics of plant disease outbreaks. Agenda 13, 161-174.

Alvarez-Venegas, R., Abdallat, A.A., Guo, M., Alfano, J.R., and Avramova, Z. (2007). Epigenetic control of a transcription factor at the cross section of two antagonistic pathways. Epigenetics 2, 106-117.

Asai, T., Tena, G., Plotnikova, J., Willmann, M.R., Chiu, W.L., Gomez-Gomez, L., Boller, T., Ausubel, F.M., and Sheen, J. (2002). MAP kinase signalling cascade in Arabidopsis innate immunity. Nature. 415, 977-983.

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

Belkhadir, Y., Subramaniam, R., and Dangl, J.L. (2004). Plant disease resistance protein signaling: NBS-LRR proteins and their partners. Curr Opin Plant Biol 7, 391-399.

Bergelson, J., Kreitman, M., Stahl, E.A., and Tian, D. (2001). Evolutionary dynamics of plant R-genes. Science 292, 2281-2285.

Blanco, F., Salinas, P., Cecchini, N.M., Jordana, X., Hummelen, P.V., Alvarez, M.E., and Holuigue, L. (2009). Early genomic responses to salicylic acid in Arabidopsis. Plant Mol Biol. 70, 79-102.

Bowling, S.A., Clarke, J.D., Liu, Y., Klessig, D.F., and Dong, X. (1997). The *cpr5* mutant of Arabidopsis expresses both NPR1-dependent and NPR1-independent resistance. Plant Cell 9, 1573-1584.

Boyes, D.C., Nam, J., and Dangl, J.L. (1998). The *Arabidopsis thaliana RPM1* disease resistance gene product is a peripheral plasma membrane protein that is degraded coincident with the hypersensitive response. Proc. Natl. Acad. Sci., USA 95, 15849-15854.

Broglie, R., Broglie, K., Chet, I., Roby, D., and Holliday, M. (1991). Chitinase expression in transgenic plants: a molecular approach to fungal disease resistance. UCLA Symposia on Molecular and Cellular Biology. Journal of Cellular Biochemistry, 9.

Bu, Q., Jiang, H., Li, C.-B., Zhai, Q., Zhang, J., Wu, X., Sun, J., Xie, Q., and Li, C. (2008). Role of the Arabidopsis thaliana NAC transcription factors ANAC019 and ANAC055 in regulating jasmonic acid-signaled defense responses. Cell Research 18, 756-767.

Buell, R., Joardar, V., Lindeberg, M., Selengut, J., Paulsen, I.T., Gwinn, M.L., Dodson, R.J., Deboy, R.T., Durkin, A.S., Kolonay, J.F., Madupu, R., Daugherty, S., Brinkac, L., Beanan, M.J., Haft, D.H., Nelson, W.C., Davidsen, T., Zafar, N., Zhou, L., Liu, J., Yuan, Q., Khouri, H., Fedorova, N., Tran, B., Russell, D., Berry, K., Utterback, T., Aken, S.E.V., Feldblyum, T.V., D'Ascenzo, M., Deng, W.-L., Ramos, A.R., Alfano, J.R., Cartinhour□, S., Chatterjee, A.K., Delaney, T.P., Lazarowitz, S.G., Martin, G.B., Schneider□, D.J., Tang, X., Bender, C.L., White, O., Fraser, C.M., and Collmer, A. (2003). The complete genome sequence of the Arabidopsis and tomato pathogen Pseudomonas syringae pv. tomato DC3000. Proc. Natl. Acad. Sci. 100, 10181-10186

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 Molecular Biology 61, 665-674.

Cao, H., Bowling, S.A., Gordon, S., and Dong, X. (1994). Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. The Plant Cell 6, 1583-1592.

Cao, H., Glazebrook, J., Clark, J.D., Volko, S., and Dong, X. (1997). The Arabidopsis *NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell 88, 57-63.

Carviel, J.L., Al-Daoud, F., Neumann, M., Mohammad, A., Provart, N.J., Moeder, W., Yoshioka, K., and Cameron, R.K. (2009). Forward and reverse genetics to identify genes involved in the age-related resistance response in Arabidopsis thaliana. Molecular Plant Pathology 10, 621-634.

Chaturvedi, R., Krothapalli, K., Makandar, R., Nandi, A., Sparks, A.A., Roth, M.R., Welti, R., and Shah, J. (2008). Plastid x3-fatty acid desaturase-dependent accumulation of a systemic acquired resistance inducing activity in petiole exudates of Arabidopsis thaliana is independent of jasmonic acid. Plant J. 54, 106-117.

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 Advance Online Publication.

Chen, Z., Kloek, A.P., Boch, J., Katagiri, F., and Kunkel, B.N. (2000). The *Pseudomonas syringae avrRpt2* gene product promotes pathogen virulence from inside plant cells. Molecular Plant-Microbe Interactions 13, 1312-1321.

Chen, Z., Zhang, H., Jablonowski, D., Zhou, X., Ren, X., Hong, X., Schaffrath, R., Zhu, J.-K., and Gong, Z. (2006). Mutations in ABO1/ELO2, a subunit of Holo-Elongator, increase abscisic acid sensitivity and drought tolerance in Arabidopsis thaliana. Molecular and Cellular Biology 26, 6902–6912

Chinenov, Y. (2002). A second catalytic domain in the Elp3 histone acetyltransferases:a candidate for histone demethylase activity? Trends in Biochemical Sciences 27, 115-117.

Church, G.M., and Gilbert, W. (1984). Genomic sequencing. Proc. Natl. Acad. Sci. USA 81, 1991-1995.

Clarke, J.D., Liu, Y., Klessig, D.F., and Dong, X. (1998). Uncoupling PR gene expression from NPR1 and bacterial resistance: Characterization of the dominant Arabidopsis *cpr6-1* mutant. The Plant Cell 10, 557-569.

Clarke, J.D., Volko, S.M., Ledford, H., Ausubel, F.M., and Dong, X. (2000). Roles of salicylic acid, jasmonic acid, and ethylene in *cpr*-induced resistance in Arabidopsis. Plant Cell 12, 2175-2190.

Close, P., Hawkes, N., Cornez, I., Creppe, C., Lambert, C.A., Rogister, B., Siebenlist, U., Merville, M.-P., Slaugenhaupt, S.A., Bours, V., Svejstrup, J.Q., and Chariot, A. (2006). Transcription impairment and cell migration defects in Elongator-depleted cells: Implication for familial dysautonomia. Molecular Cell 22, 521–531.

Clough, S.J., and Bent, A.F. (1998). Floral Dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidosis thaliana*. Plant J. 16, 735-743.

Creppe, C., Malinouskaya, L., Volvert, M.-L., Gillard, M., Close, P., Malaise, O., Laguesse, S., Cornez, I., Rahmouni, S., Ormenese, S., Belachew, S., Malgrange, B., Chapelle, J.-P., Siebenlist, U., Moonen, G., Chariot, A., and Nguyen, L. (2009). Elongator controls the migration and differentiation of cortical neurons through acetylation of a-tubulin. Cell 136, 551-564.

Daniels, M. (1982). Editorial: Possible adverse effects of antibiotic therapy in plants. Reviews of Infectous Diseases 4, 167-170.

Davis, K.R., Lyon, G.D., Darvill, A.G., and Albersheim, P. (1984). Endopolygalacturonic acid lyase from *Erwinia carotovora* elicits phytoalexin accumulation by releasing plant cell wall fragments. Plant Physiol. 74, 52-60.

de Crecy-Legard, V., Glaser, P., Lejeune, P., Sismeiro, O., Barber, C.E., Daniels, M.J., and Danchin, A. (1990). A *Xanthomonas campestris* pv. *campestris* protein similar to catabolite activation factor is involved in regulation of phytopathogenicity. J. Bacteriol. 172, 5877-5883.

Delaney, T.P., Friedrich, L., and Ryals, J.A. (1995). *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. Proc. Natl. Acad. Sci. USA 92, 6602-6606.

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.

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. The Plant Cell 15, 2181-2191.

Donofrio, N.M., and Delaney, T.P. (2001). Abnormal callose response phenotype and hypersusceptibility to Peronospoara parasitica in defence-compromised arabidopsis nim1-1 and salicylate hydroxylase-expressing plants. Mol Plant Microbe Interact 14, 439-450.

Durrant, W.E., and Dong, X. (2004). Systemic acquired resistance. Annu Rev Phytopathol 42, 185-209.

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. 104, 4223– 4227.

Edreva, A. (2005). Pathogenesis-related proteins: Research in the last 15 years. Gen. Appli. Plant Physiology 31, 105-124.

Esberg, A., Huang, B., Johansson, M.J.O., and Bystro, A.S. (2006). Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. Molecular Cell 24, 139–148.

Falk, A., Feys, B.J., Frost, L.N., Jones, J.D.G., Daniels, M.J., and Parker, J.E. (1999). *EDS1*, an essential component of *R* gene-mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. Proc. Natl. Acad. Sci. 96, 3292-3297.

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.

Felix, G., Duran, J.D., Volko, S., and Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J. 18, 265-276.

Fellows, J., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J.Q. (2000). The Elp2 subunit of Elongator and elongating RNA Polymerase II Holoenzyme is a WD40 repeat protein. Journal of Biological Chemistry 275, 12896 – 12899.

Feys, B.J., Moisan, L.J., Newman, M.-A., and Parker, J.E. (2001). Direct interaction between the *Arabidopsis* disease resistance signaling proteins, EDS1 and PAD4. EMBO Journal 20, 5400-5411.

Fichtner, L., Jablonowski, D., Schierhorn, A., Kitamoto, H.K., Stark, M.J.R., and Schaffrath, R. (2003). Elongator's toxin-target (TOT) function is nuclear localization sequence dependent and suppressed by post-translational modification. Molecular Microbiology 49, 1297–1307

Forouhar, F., Yang, Y., Kumar, D., Chen, Y., Fridman, E., Park, S.W., Chiang, Y., Acton, T.B., Montelione, G.T., Pichersky, E., Klessig, D.F., and Tong, L. (2005). Structural and biochemical studies identify tobacco SABP2 as a methyl salicylate esterase and implicate it in plant innate immunity. Proc. Natl. Acad. Sci. 102, 1773-1778.

Fransz, P.F., and de Jong, J.H. (2002). Chromatin dynamics in plants. Current Opinion in Plant Biology 5, 560-567.

Friedrich, L., Lawton, K., Reuss, W., Masner, P., Specker, N., Gut Rella, M., Meier, B., Dincher, S., Staub, T., Uknes, S., Métraux, J.-P., Kessman, H., and Ryals, J. (1996). A benzothiadiazole induces systemic acquired resistance in tobacco. Plant J. 10, 61-70.

Fry, W. (1982). Principles of plant disease management.

Funke, T., Han, H., Healy-Fried, M.L., Fischer, M., and Schönbrunn, E. (2006). Molecular basis for the herbicide resistance of roundup ready crops. Proc. Natl. Acad. Sci. 103, 13010-13015

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., and Ryals, J. (1993). Requirement of salicylic acid for the induction of systemic acquired resistance. Science 261, 754-756.

Glawischnig, E. (2007). Camalexin. Phytochemistry 68, 401-406.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43, 205-227.

Glazebrook, J., Chen, W., Estes, B., Chang, H.-S., Nawrath, C., Métraux, J.-P., Zhu, T., and Katagiri, F. (2003). Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. Plant J. 34, 217-228.

Gomez-Gomez, L., Felix, G., and Boller, T. (1999). A single locus determines the sensitivity to bacterial flagellin in *Arabidopsis thaliana*. Plant J. 18, 277-284.

Goodman, R.N., and Novacky, A.J. (1994). The hypersensitive response in plants to pathogens. (St. Paul: APS Press).

Gopalan, S., Bauer, D.W., Alfano, J.R., Loniello, A.O., He, S.Y., and Collmer, A. (1996). Expression of the *Pseudomonas syringae* avirulence protein AvrB in plant cells alleviates its dependence on the hypersensitive response and pathogenicity (Hrp) secretion system in eliciting genotype-specific hypersensitive cell death. Plant Cell 8, 1095-1105.

Gráda, C.Ó. (2006). Ireland's Great Famine:. University College Dublin Press.

Greene, E.A., Codomoa, C.A., Taylora, N.E., Henikoff, J.G., Till, B.J., Reynolds, S.H., Enns, L.C., Burtner, C., Johnson, J.E., Odden, A.R., Comai, L., and Henikoff, S. (2003). Spectrum of Chemically Induced Mutations From a Large-Scale Reverse-Genetic Screen in Arabidopsis Genetics 164, 731-730.

Gundlach, H., Muller, M.J., Kutchan, T.M., and Zenk, M.H. (1992). Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc. Nat'l Acad. Sci. 89, 2389-2393.

Hahn, M.G., Bucheli, P., Cervone, F., Doares, S.H., and O'Neill, R.A. (1989). Roles of cell wall constituents in plant-pathogen interactions. In Plant-Microbe Interactions: Molecular and Genetic Perspectives, T. Kosuge and E.W. Nester, eds (New York: Macmilllan), pp. 343-379.

Hammond-Kosack, K.E., Tang, S., Harrison, K., and Jones, J.D.G. (1998). The tomato *Cf-9* disease resistance gene functions in tobacco and potato to confer responsiveness ot the fungal avrulence gene product Avr9. Plant Cell 10, 1251-1266.

Hayat, S., and Ahmed, A. (2007). Salicylic acid a plant hormone. (Springer).

Heidel, A.J., Clarke, J.D., Antonovics, J., and Dong, X. (2004). Fitness costs of mutations affecting the systemic acquired resistance pathway in Arabidopsis thaliana. Genetics 168, 2197-2206.

Heil, M. (2002). Ecological costs of induced resistance. Current Opinion in Plant Biology 5, 345-350.

Heil, M., Hilpert, A., Kaiser, W., and Linsenmair, K.E. (2000). Reduced growth and seed set following chemical induction of pathogen defence: does systemic acquired resistance (SAR) incur allocation costs? Journal of Ecology 88, 645-654.

Hildman, T., Ebneth, M., Pena-Cortes, H., Sanchez-Serrano, J.J., Willmitzer, L., and Prat, S. (1992). General roles of abscisic and jasmonic acids in gene activation as a result of mechanical wounding. Plant Cell 4, 1157-1170.

Holliday, M.J., Keen, N.T., and Long, M. (1981). Cell death patterns and accumulation of fluorescent material in the hypersensitive response of soybean leaves to *Pseudomonas syringae pv. glycinea*. Physiolog. Plant Pathol. 18, 279-287.

Huang, B., Johansson, M.J.O., and Bystrom, A.S. (2005). An early step in wobble uridine tRNA modification requires the Elongator complex. RNA 11, 424-436.

Jablonowski, D., Frohloff, F., Fichtner, L., Stark, M., and Schaffrath, R. (2001). Kluyveromyces lactis zymocin mode of action is linked to RNA polymerase II function via Elongator. Mol Microbiol. 42, 1095-1105.

Jacobs, W.P. (1952). The role of auxin in differentiation of xylem around a wound. Amer. J. Bot. 39, 301-309.

Jander, G., Norris, S.R., Rounsley, S.D., Bush, D.F., Levin, I.M., and Last, R.L. (2002). Arabidopsis map-based cloning in the post-genome era. Plant Physiol 129, 440-450.

Jensen, P.J., Hangarter, R.P., and Estelle, M. (1998). Auxin transport is required for hypocotyl elongation in light-grown but not dark-grown Arabidopsis. Plant Physiol 116, 455-462.

Jirage, D., Tootle, T.L., Reuber, T.L., Frost, L.N., Feys, B.J., Parker, J.E., Ausubel, F.M., and Glazebrook, J. (1999). *Arabidopsis thaliana PAD4* encodes a lipase-like gene that is important for salicylic acid signaling. Proc. Natl. Acad. Sci. 96, 13583-13588.

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.

Jones, D.A., and Takemoto, D. (2004). Plant innate immunity - direct and indirect recognition of general and specific pathogen-associated molecules. Curr Opin Immunol 16, 48-62.

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

Kachroo, P., Shanklin, J., Shah, J., Whittle, E.J., and Klessig, D.F. (2001). A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proc. Natl. Acad. Sci. 98, 9448-9453.

Katagiri, F., Thilmony, R., and He, S.Y. (2002). The Arabidopsis Book: The Arabidopsis thaliana-Pseudomonas syringae interaction.

Kauss, H., Theisinger-Hinkel, E., Mindermann, R., and Conrath, U. (1992). Dichloroisonicotinic and salicylic acid, inducers of systemic acquired resistance, enhance fungal elicitor responses in parsley cells. Plant J. 2, 655-660.

Keen, N.T., and Bruegger, B. (1977). Phytoalexins and chemicals that elicit their production in plants. ACE Symp. Ser. 62, 1-26.

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.

Kim, Y.S., Schumaker, K.S., and Zhu, J.K. (2006). EMS mutagenesis of Arabidopsis.

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.

Kirik, V., Bouyer, D., Schobinger, U., Bechtold, N., Herzog, M., Bonneville, J.M., and Hulskamp, M. (2001). CPR5 is involved in cell proliferation and cell death control and encodes a novel transmembrane protein. Curr Biol 11, 1891-1895.

Koo, Y.J., Kim, M.A., Kim, E.H., Song, J.T., Jung, C., Moon, J.-K., Kim, J.-H., Seo, H.S., Song, S.I., Kim, J.-K., Lee, J.S., Cheong, J.-J., and Choi, Y.D. (2007). Overexpression of salicylic acid carboxyl methyltransferase reduces salicylic acid-mediated pathogen resistance in Arabidopsis thaliana. Plant Mol Biol. 64, 1-15.

Koornneef, A., Rindermann, K., Gatz, C., and Pieterse, C.M. (2008). Histone modifications do not play a major role in salicylate-mediated suppression of jasmonate-induced PDF1.2 gene expression. Commun Integr Biol. 1, 143-145.

Kreig, N.R., and Holt, J.G. (1984). Bergey's Manual of Systematic Biology.

Krogan, N.J., and Greenblatt, J.F. (2001). Characterization of a six-subunit Holo-Elongator complex required for the regulated expression of a group of genes in Saccharomyces cerevisiae. Molecular and Cellular Biology 21, 8203–8212.

Kunkel, B.N., and Brooks, D.M. (2002). Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol. 5, 325-331.

Kunkel, B.N., Bent, A.F., Dahlbeck, D., Innes, R.W., and Staskawicz, B.J. (1993). *RPS2*, an *Arabidopsis* disease resistance locus specifying recognition of *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. Plant Cell 5, 865-875.

Kunze, G., Chinchilla, D., Caniard, A., Jones, J.D.G., Boller, T., and Felix, G. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell 125, 749-760.

Lawton, K., Weymann, K., Friedrich, L., Vernooij, B., Uknes, S., and Ryals, J. (1995). Systemic acquired resistance in *Arabidopsis* requires salicylic acid but not ethylene. Molecular Plant Microbe Interactions 8, 863-870.

Leathers, H.D., and Foster, P. (2004). The World Food Problem. (Boulder, CO: Lynne Rienner Pub).

Lee, H.-I., León, J., and Raskin, I. (1995). Biosynthesis and metabolism of salicylic acid. Proceedings of the National Academy of Sciences, U.S.A. 92, 4076-4079.

Lee, J., Nam, J., Park, H.C., Na, G., Miura, K., Jin, J.B., Yoo, C.Y., Baek, D., Kim, D.H., Jeong, J.C., Kim, D., Lee, S.Y., Salt, D.E., Mengiste, T., Gong, Q., Ma, S., Bohnert, H.J., Kwak, S.S., Bressan, R.A., Hasegawa, P.M., and Yun, D.J. (2007). Salicylic acid-mediated innate immunity in Arabidopsis is regulated by SIZ1 SUMO E3 ligase. Plant J 49, 79-90.

Li, X., Clarke, J.D., Zhang, Y., and Dong, X. (2001a). Activation of an EDS1-mediated *R*-gene pathway in the *snc1* mutant leads to constitutive, NPR1-independent pathogen resistance. Molecular Plant-Microbe Interactions 14, 1131-1139.

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.

Li, Y., Takagi, Y., Jiang, Y., Tokunaga, M., Erdjument-Bromage, H., Tempst, P., and Kornberg, R.D. (2001b). A multiprotein complex that interacts with RNA Polymerase II Elongator. Journal of Biological Chemistry 276, 29628 –29631.

Loidl, P. (2004). A plant dialect of the histone language. Trends in Plant Science 9, 1360-1385.

Lukowitz, W., Gillmor, C.S., and Scheible, W.-R. (2000). Positional cloning in Arabidopsis. Why it feels good to have a genome initiative working for you. Plant Physiol. 123, 795–805.

Lund, S.T., Stall, R.E., and Klee, H.J. (1998). Ethylene regulates the susceptible response to pathogen infection in tomato. Plant Cell 10, 371-382.

Lusser, A. (2002). Acetylated, methylated, remodeled: chromatin states for gene regulation. Current Opinion in Plant Biology 5, 437-443.

Mackey, D., Holt, B.F., Wiig, A., and Dangl, J.L. (2002). RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell 108, 743-754.

Mackey, D., Belkhadir, Y., Alonso, J.M., Ecker, J.R., and Dangl, J.L. (2003). Arabidopsis RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. Cell 112, 379-389.

Malamy, J., Hennig, J., and Klessig, D.F. (1992). Temperature-dependent induction of salicylic acid and its conjugates during the resistance response to tobacco mosaic virus infection. Plant Cell 4, 359-366.

Maldonado, A.M., Doerner, P., Dixon, R.A., Lamb, C.J., and Cameron, R.K. (2002). A putative lipid transfer protein involved in systemic resistance signalling in *Arabidopsis*. Nature 419, 399-403.

Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K., Dangl, J., and Dietrich, R. (2000). The transcriptome of Arabidopsis thaliana during systemic acquired resistance. Nature Genetics 4, 403-410.

Martin, J. (1964). The role of the cuticle in the defense against plant disease. Ann. Rev. Phytopathol. 2, 81-100.

McGuinness, M., and Dowling, D. (2009). Plant-associated bacterial degradation of toxic organic compounds in soil. Int J Environ Res Public Health 6, 2226-2247.

Melotto, M., Underwood, W., and He, S.Y. (2008). Role of stomata in plant innate immunity and foliar bacterial diseases. Annu. Rev. Phytopathol.

46, 101-122.

Métraux, J.-P. (2002). Recent breakthroughs in the study of salicylic acid biosynthesis. Trends in Plant Science 7, 332-334.

Métraux, J.-P., Ahl-Goy, P., Staub, T., Speich, J., Steinemann, A., Ryals, J., and Ward, E. (1991). Induced resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens. In Advances in Molecular Genetics of Plant-Microbe Interactions, H. Hennecke and D.P.S. Verma, eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 432-439.

Métraux, J.P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W., and Inverardi, B. (1990). Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science 250, 1004-1006.

Mishina, T., and Zeier, J. (2007). Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in Arabidopsis. Plant J. 50, 500-513.

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.

Nawrath, C., and Métraux, J.-P. (1999). Salicylic acid induction-deficient mutants of Arabidopsis express *PR-2* and *PR-5* and accumulate high levels of camalexin after pathogen inoculation. Plant Cell 11, 1393-1404.

Nelissen, H., Fleury, D., Bruno, L., Robles, P., Veylder, L.D., Traas, J., Micol, J.L., Montagu, M.V., Inze, D., and Lijsebettens, M.V. (2005). The elongata mutants identify a functional Elongator complex in plants with a role in cell proliferation during organ growth. Proc. Natl. Acad. Sci. 102, 7754 –7759.

Nelissen, H., Groeve, S.D., Fleury, D., Neyt, P., Bruno, L., Bitonti, M.B., Vandenbussched, F., Straetend, D.V.D., Yamaguchie, T., Tsukayae, H., Wittersg, E., Jaegera, G.D., Houbenh, A., and Lijsebettens, M.V. (2009). Plant Elongator regulates auxin-related genes during RNA polymerase II transcription elongation. Proc. Natl. Acad. Sci. Early edition.

Nicholson, R., and Hammerschmidt, R. (1992). Phenolic compounds and their role in disease resistance. Annu. Rev. Phytopathol. 30, 369-389.

Niggeweg, R., Thurow, C., Weigel, R., Pfitzner, U., and Gatz, C. (2000). Tobacco TGA factors differ with respect to interaction with NPR1, activation potential and DNA-binding properties. Plant Molecular Biology 42, 775-788.

Nimchuk, Z., Eulgem, T., Holt, B.F., 3rd, and Dangl, J.L. (2003). Recognition and response in the plant immune system. Annu Rev Genet 37, 579-609.

Nuhse, T.S., Peck, S.C., Hirt, H., and Boller, T. (2000). Microbial elicitors induce activation and dual phosphorylation of the Arabidopsis thaliana MAPK 6. J Biol Chem 275, 7521-7526.

Nurnberger, T., Brunner, F., Kemmerling, B., and Piater, L. (2004). Innate immunity in plants and animals: striking similarities and obvious differences. Immunol Rev 198, 249-266.

Okada, Y., Yamagata, K., Hong, K., Wakayam, T., and Zhang, Y. (2009). A role for the elongator complex in zygotic paternal genome demethylation. Nature 463, 554-558.

Ooka, H., Satoh, K., Doi, K.j., Nagata, T., Otomo, Y., Murakami, K., Matsubara, K., Osato, N., Kawai, J., Carninci, P., Hayashizaki, Y., Suzuki, K.j., Kojima, K., Takahara, Y., Yamamoto, K.j., and Kikuchi, S. (2003). Comprehensive analysis of NAC family genes in Oryza sativa and Arabidopsis thaliana. DNA Research 10, 239–247.

Otero, G., Fellows, J., Li, Y., Bizemont, T.d., Dirac, A.M.G., Gustafsson, C.M., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J.Q. (1999). Elongator, a multisubunit component of a novel RNA Polymerase II Holoenzyme for transcriptional elongation. Molecular Cell 3, 109–118.

Parnell, S., Gottwald, T., van den Bosch, F., and Gilligan, C. (2009). Optimal strategies for the eradication of asiatic citrus canker in heterogeneous host landscapes. Phytopathology 99, 1370-1376.

Phipps, T. (1989). Externalities and the returns to agricultural research: discussion. American Journal of Agricultural Economics 71, 466-467.

Pontier, D., Miao, Z.-H., and Lam, E. (2001). Trans-dominant suppression of plant TGA factors reveals their negative and positive roles in plant defense responses. Plant J. 27, 529-538.

Rahl, P.B., Chen, C.Z., and Collins, R.N. (2005). Elp1p, the Yeast homolog of the FD disease syndrome protein, negatively regulates exocytosis independently of transcriptional elongation Molecular Cell 17.

Rao, M.V., Paliyath, G., Ormrod, D.P., Murr, M.P., and Watkins, C.B. (1997). Influence of salicylic acid on H2O2 production, oxidative stress, and H2O2-metabolizing enzymes. Salicylic acid-mediated oxidative damage requires H2O2. Plant Physiol. 115, 137-149.

Raskin, I., Turner, I.M., and Melander, W.R. (1989). Regulation of heat production in the inflorescences of Arum lily by endogenous salicylic acid. Proc. Natl. Acad. Sci. USA 86, 2214-2218.

Ritter, C., and Dangl, J.L. (1995). The *avrRpm1* gene of *Pseudomonas syringae* pv. *maculicola* is required for virulence on *Arabidopsis*. Mol. Plant-Microbe Interact. 8, 444-453.

Roberts, J.A., Tucker, G.A., and Maunders, M.J. (1980). Ethylene and foliar senescence. In Senescence in Plants, K.V. Thimann, ed (Boca Raton, FL: CRC Press), pp. 267-275.

Rojo, E., Leon, J., and Sanchez-Serrano, J.J. (1999). Cross-talk between wound signalling pathways determines local versus systemic gene expression in Arabidopsis thaliana. Plant J 20, 135-142.

Romantschuk, M. (1992). Attachment of plant pathogenic bacteria to plant surfaces. Annu. Rev. Phytopathol. 30, 225-243.

Romantschuk, M., and Bamford, D.H. (1986). The causal agent of halo blight in bean, *Pseudomonas syringae* pv. *phaseolicola*, attaches to stomata via its pili. Microbial Pathogenesis 1, 139-148.

Ronald, P.C., Salmeron, J.M., Carland, F.M., and Staskawicz, B.J. (1992). The cloned avirulence gene *avrPto* induces disease resistance in tomato cultivars containing the *Pto* resistance gene. J. Bacteriol. 174, 1604-1611.

Ross, A.F. (1961). Systemic acquired resistance induced by localized virus infections in plants. Virology 14, 340-358.

Ryals, J., Weymann, K., Lawton, K., Friedrich, L., Ellis, D., Steiner, H.-Y., Johnson, J., Delaney, T.P., Jesse, T., Vos, P., and Uknes, S. (1997). The Arabidopsis *NIM1* protein shows homology to the mammalian transcription factor inhibitor IκB. Plant Cell 9, 425-439.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.-Y., and Hunt, M.D. (1996). Systemic acquired resistance. Plant Cell 8, 1809-1819.

Sanger, F., Nicklen, S., and Coulson, A.R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci., USA 74, 5463-5467.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C., and Manners, J.M. (2000). Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc. Natl. Acad. Sci. 97, 11655-11660.

Schuler, M.A. (2008). Splice site requirements and switches in plants. In Current Topics in Microbiology and Immunology (

Schumacher, K., Vafeados, D., McCarthy, M., Sze, H., Wilkins, T., and Chory, J. (1999). The Arabidopsis det3 mutant reveals a central role for the vacuolar H+-ATPase in plant growth and development. Genes and Development 13, 3259–3270.

Shah, J. (2003). The salicylic acid loop in plant defense. Current Opinion in Plant Biology 6, 365-371.

Shah, J., Tsui, F., and Klessig, D.F. (1997). Characterization of a salicylic acid-insensitive mutant (*sai1*) of *Arabidopsis thaliana* identified in a selective screen utilizing the SA-inducible expression of the *tms2* gene. Molecular Plant-Microbe Interactions 10, 69-78.

Shah, J., Kachroo, P., and Klessig, D.F. (1999). The Arabidopsis *ssi1* mutation restores pathogenesis-related gene expression in *npr1* plants and renders defensin gene expression salicylic acid dependent. Plant Cell 11, 191-206.

Shah, J., Kachroo, P., Nandi, A., and Klessig, D.F. (2001). A recessive mutation in the *Arabidopsis SSI2* gene confers SA- and *NPR1*-independent expression of *PR* genes and resistance against bacterial and oomycete pathogens. Plant Journal 25, 563-574.

Shirano, Y., Kachroo, P., Shah, J., and Klessig, D.F. (2002). A gain-of-function mutation in an Arabidopsis toll interleukin receptor–nucleotide binding site–leucine-rich repeat type R gene triggers defense responses and results in enhanced disease resistance. Plant Cell 14, 3149–3162.

Silva, H., Yoshioka, K., Dooner, H.K., and Klessig, D.F. (1999). Characterization of a new Arabidopsis mutant exhibiting enhanced disease resistance. Molecular Plant-Microbe Interactions 12, 1053-1063.

Skou, J.P. (1985). On the enhanced callose deposition in barley with *mlo* powdery resistance genes. Phytopath. Z. 112, 207-216.

Smith, D.A. (1982). Toxicity of phytoalexins. In Phytoalexins, J.A. Bailey and J.W. Mansfield, eds (New York: John Wiley and Sons), pp. 218-252.

Smith, T.F., Gaitatzes, C., Saxena, K., and Neer, E.J. (1999). The WD-40 repeat: a common architecture for diverse functions. TIBS 24, 181-184.

Song, J.T., Koob, Y.J., Seoc, H.S., Kime, M.C., Choib, Y.D., and Kimf, J.H. (2008). Overexpression of AtSGT1, an Arabidopsis salicylic acid glucosyltransferase, leads to increased susceptibility to Pseudomonas syringae. Phytochemistry 69, 1128-1134

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., 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., Koornneef, A., Claessens, S.M.C., Korzelius, J.P., Van Pelt, J.A., Mueller, M.J., Buchala, A.J., Metraux, J.-P., Brown, R., Kazan, K., Van Loon, L.C., Dong, X., and Pieterse, C.M.J. (2003). NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell 15, 760-770.

Stacey, M.G., Hicks, S.N., and Arnim, A.G.v. (1999). Discrete domains mediate the light-responsive nuclear and cytoplasmic localization of Arabidopsis COP1. Plant Cell 11, 349-364.

Staskawicz, B.J., Mudgett, M.B., Dangl, J.L., and Galan, J.E. (2001). Common and contrasting themes of plant and animal diseases. Science 292, 2285-2289.

Strawn, M., Marr, S., Inoue, K., Inada, N., Zubieta, C., and Wildermuth, M. (2006). Arabidopsis isochorismate synthase functional in pathogen-induced salicylate biosynthesis exhibits properties consistent with a role in diverse stress responses 282, 5919 –5933.

Tada, Y., Spoel, S.H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., Zuo, J., and Dong, X. (2008). Plant immunity requires conformational charges of NPR1 via S-nitrosylation and thioredoxins. Science 321, 952-955.

Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H.S., Han, B., Zhu, T., Zou, G., and Katagiri, F. (2003). Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen Pseudomonas syringae. Plant Cell 15, 317-330.

The Arabidopsis Genome Initiative. (2000). Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408, 796-815.

Thompson, J.N., and Burdon, J.J. (1992). Gene-for-gene coevolution between plants and parasites. Nature 360, 121-125.

Ton, J., Flors, V., and Mauch-Mani, B. (2009). The multifaceted role of ABA in disease resistance. Trends Plant Sci 14, 310-317.

Ton, J., Van Pelt, J.A., Van Loon, L.C., and Pieterse, C.M.J. (2002). Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in *Arabidopsis*. Molecular Plant-Microbe Interactions 15, 27-34.

Trethwewny, R. (2004). Metabolite Profiling as an Aid to Metabolic Engineering in Plants. Curr Opin Plant Biol. 7, 196-201.

Turner, N. (2009). From dwarves to giants? Plant height manipulation for biomass yield. Nat Chem Biol 5, 567-573.

van Dijk, K., Fouts, D.E., Rehm, A.H., Hill, A.R., Collmer, A., and Alfano, J.R. (1999). The Avr (effector) proteins HrmA (HopPsyA) and AvrPto are secreted in culture from *Pseudomonas syringae* pathovars via the Hrp (type III) protein secretion system in a temperature- and pH-sensitive manner. J. Bacteriol. 181, 4790-4797.

van Kan, A.L. (2006). Licensed to kill: the lifestyle of a necrotrophic plant pathogen. Trends in Plant Science 11, 1360-1385.

van Wees, S.C.M., de Swart, E.A.M., van Pelt, J.A., van Loon, L.C., and Pieterse, C.M.J. (2000). Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. 97, 8711-8716.

Verberne, M.C., Verpoorte, R., Bol, J.F., Mercado-Blanco, J., and Linthorst, H.J.M. (2000). Overproduction of salicylic acid in plants by bacterial transgenes enhances pathogen resistance. Nature Biotechnology 18, 779-783.

Vernooij, B., Friedrich, L., Morse, A., Reist, R., Kolditz-Jawhar, R., Ward, E., Uknes, S., Kessmann, H., and Ryals, J. (1994). Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. Plant Cell 6, 959-965.

Vidal, S., Eriksson, A.R.B., Montesano, M., Denecke, J., and Palva, E.T. (1998). Cell wall-degrading enzymes from *Erwinia carotovora* cooperate in the salicylic acid-independent induction of a plant defense response. Molec. Plant-Microbe Interact. 11, 23-32.

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., Weaver, N.D., Kesarwani, M., and Dong., X. (2005). Induction of protein secretory pathway is required for systemic acquired resistance. Science 308, 1036-1039.

Wei, W., Plovanich-Jones, A., Deng, W.-L., Jin, Q.-L., Collmer, A., Huang□, H.-C., and He, S.Y. (1999). The gene coding for the Hrp pilus structural protein is required for type III secretion of Hrp and Avr proteins in Pseudomonas syringae pv. tomato. Proc. Natl. Acad. Sci. 97, 2247-2252

Weigel, D., and Glazebrook, J. (2002). Arabidopsis: A Laboratory Manual. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Whenham, R.J., Fraser, R.S.S., and Snow, A. (1985). Tobacco mosaic virus-induced increase in abscisic acid concentration in tobacco leaves: intracellular location and relationship to symptom severity and to extent of virus multiplication. Physiological Plant Pathology 26, 379-387.

White, R.F. (1979). Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. Virology 99, 410-412.

Wiermer, M., Feys, B.J., and Parker, J.E. (2005). Plant immunity: the EDS1 regulatory node. Curr Opin Plant Biol 8, 383-389.

Wildermuth, M.C., Dewdney, J., Wu, G., and Ausubel, F.M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414, 562-565.

Winkler, G.S., Kristjuhan, A., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J.Q. (2002). Elongator is a histone H3 and H4 acetyltransferase important for normal histone acetylation levels in vivo. Proc. Natl. Acad. Sci. 99, 3517–3522.

Wittschieben, B.O., Otero, G., de Bizemont, T., Fellows, J., Erdjument-Bromage, H., Ohba, R., Li, Y., Allis, C.D., Tempst, P., and Svejstrup, J.Q. (1999). A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. Mol. Cell 4, 123–128.

Wittschieben, B.Ø., Fellows, J., Du, W., Stillman, D.J., and Svejstrup, J.Q. (2000). Overlapping roles for the histone acetyltransferase activities of SAGA and Elongator in vivo. EMBO J. 19, 3060–3068.

Xia, Y., Suzuki, H., Borevitz, J., Blount, J., Guo, Z., Patel, K., Dixon, R., and Lamb, C. (2004). An extracellular aspartic protease functions in Arabidopsis disease resistance signaling. EMBO J. 23, 980-988.

Yang, P., Chen, C., Wang, Z., Fan, B., and Chen, Z. (1999). A pathogen- and salicylic acid-induced WRKY DNA-binding activity recognizes the elicitor response element of the tobacco class I chitinase gene promoter. Plant J. 18, 141-149.

Yi, Y., and Jack, T. (1998). An intragenic suppressor of the Arabidopsis floral organ identity mutant apetala3-1 functions by suppressing defects in splicing. Plant Cell 10, 1465–1477.

Yu, G.L., Katagiri, F., and Ausubel, F.M. (1993). Arabidopsis mutations at the *RPS2* locus result in loss of resistance to *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. Molecular Plant-Microbe Interactions 6, 434-443.

Zhang, X., Chen, S., and Mou, Z. (2009). Nuclear localization of NPR1 is required for regulation of salicylate tolerance, isochorismate synthase 1 expression and salicylate accumulation in Arabidopsis. Journal of Plant Physiology 167, 144–148

Zhang, Y., and Li, X. (2005). A putative nucleoporin 96 is required for both basal defense and constitutive resistance responses mediated by suppressor of npr1-1, constitutive 1. Plant Cell 17, 1306–1316.

Zhang, Y., Goritschnig, S., Dong, X., and Li, X. (2003a). A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in *suppressor of npr1-1*, *constitutive 1*. Plant Cell 15, 2636-2646.

Zhang, Y., Tessaro, M.J., Lassner, M., and Li, X. (2003b). 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., 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. 96, 6523-6528.

Zhang, Y., Cheng, Y.T., Bi, D., Palma, K., and Li, X. (2005). MOS2, a protein containing G-patch and KOW motifs, is essential for innate immunity in Arabidopsis thaliana. Current Biology 15.

Zhou, N., Tootle, T.L., Tsui, F., Klessig, D.F., and Glazebrook, J. (1998). *PAD4* functions upstream from salicylic acid to control defense responses in Arabidopsis. Plant Cell 10, 1021-1030.

Zhou, X., Hua, D., Chen, Z., Zhou, Z., and Gong, Z. (2009). Elongator mediates ABA responses, oxidative stress resistance and anthocyanin biosynthesis in Arabidopsis. Plant J. Advanced Online Publication.

Zhu, Y., Chen, H., Fan, J., Wang, Y., Li, Y., Chen, J., Fan, J., Yang, S., Hu, L., Leung, H., Mew, T.W., Teng, P.S., Wang, Z., and Mundt, C.C. (2000). Genetic diversity and disease control in rice. Nature 406, 718-722.

BIOGRAPHICAL SKETCH

Christopher Thomas DeFraia was born in Kingston, New York, to parents Gary and Isabelle DeFraia. He has one younger brother, Daniel. In May 2005, he graduated with a Bachelor of Science degree in biotechnology from Rutgers University. In November of 2005, he joined the laboratory of Dr. Zhonglin Mou, where he studies plant immunity and is pursuing his Ph.D.