

RRE1: an *Arabidopsis* E3 Ligase Involved in Plant Defense

Adam Perricone

Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487, USA

Introduction

Innate immunity is an ancient and conserved form of defense shared by plants, insects, and vertebrates. These defense responses in plants can be activated upon recognition of an array of general elicitors. Essential to this defense pathway is the role of the ubiquitin/26s proteasome pathway [4]. In this pathway, proteins to be degraded are tagged with ubiquitin, a 76-amino acid polypeptide; these tagged proteins are identified by the 26s proteasome, which subsequently catabolizes them. The mechanism by which ubiquitin is shuttled to the target protein to be degraded is facilitated by three classes of proteins (E1, E2, and E3); this mechanism is referred to as the ubiquitin conjugation cascade [5]. The focus of this paper is on *RRE1*, a gene that encodes an E3 protein, and is significantly up-regulated in response to the elicitor chitin. The protein structure of the *RRE1* gene-product is characteristic of E3 ligases. Recently, it has been shown that a RING-type E3 ubiquitin ligase plays a key role in regulating salicylic acid production [8]. Salicylic acid is one of several key signaling molecules that mediate defense responses in plants.

RRE1 Protein Structure

RRE stands for Rapid Response to Elicitor, underscoring the importance of the *RRE1* gene-product in plant innate immunity. RRE1 is a 378-amino acid protein containing the E3-characteristic RING (Really Interesting New Gene) zinc-finger domain [1, 7]. The RING zinc-finger domain serves as a binding site for ubiquitin during the ubiquitin conjugation cascade and facilitates the binding of ubiquitin to the target protein to be catabolized [5].

Zinc-finger domains are named due to their ability to bind one or more zinc atoms and for the multiple finger-like protrusions that extend from the protein and interact with the target molecule. Zinc-fingers are versatile protein motifs capable of binding DNA, RNA, protein, and lipid substrates. This versatility is achieved by the particular amino acid sequence of an individual finger, the sequence of the linker region

between fingers, and the particular higher-order structures of a particular finger. Zinc-fingers are usually grouped together, facilitating the binding of specific substrates [3]. This specificity is an important characteristic of E3 proteins, as their primary function is to identify the correct target molecule to be ubiquitinated and subsequently degraded during a defense response [5]. In addition to a RING zinc-finger domain, the *rre1* gene-product contains a PEST (Pro-Glu-Ser-Thr) domain that likely facilitates the rapid degradation of RRE1 after it has carried out its E3 function. This is an important quality of the protein as defense responses are energetically taxing and can be detrimental to the plant over long periods of time [1].

The Ubiquitin Conjugation Cascade

As an E3 protein, RRE1 plays a key role in the ubiquitin/26s proteasome pathway. The pathway begins with ubiquitin, a highly conserved protein in both plants and animals. Ubiquitin contains many intramolecular hydrogen bonds that give the molecule great stability. At the C-terminus is a flexible extension protruding from the bulk of the protein. This extension terminates with an essential glycine residue whose role is to facilitate binding of ubiquitin to its downstream partners. The first of these partners in the ubiquitin conjugation cascade is the E1 protein, a ubiquitin-activating enzyme. There are only two E1 isoforms found in the *Arabidopsis* genome. The role of E1 enzymes is to catalyze the formation of a thiol-ester linkage between the terminal ubiquitin glycine and an E1 cysteine residue. Once activated, ubiquitin is transferred to the E2 protein, a ubiquitin-conjugating enzyme. This protein contains a 150-amino acid core, at the center of which lies the active site. It is at this active site that the activated ubiquitin is bound via transesterification. Once bound, the E2 enzyme facilitates the transport of ubiquitin to the final protein in the cascade: the E3 ubiquitin-protein ligase. E3 ligases are responsible for identifying the correct protein to be ubiquitinated and as such, they are the most numerous and diverse components

of the cascade. The E3 ligase will facilitate the binding of the terminal glycine of ubiquitin to a lysine residue of the target protein to be degraded, forming the end product of the cascade: the ubiquitin-protein complex. This complex is subsequently recognized by the 26s proteasome, an ATP-dependent proteolytic complex. Within the proteasome is a large chamber that contains the protease active sites that will subsequently catabolize the ubiquitin-protein complex [5].

The Ubiquitin/26s Proteasome Pathway and Plant Defense

The ubiquitin/26s proteasome is a pathway that is paramount to plant growth, development, and health [5]. However, this pathway also plays an essential role in plant innate immunity. Goritschniger *et al.* (2006) demonstrated this in several experiments utilizing the *Arabidopsis* gain-of-function mutant *snc1*. This mutant shows a constitutive defense response in the plant resulting in decreased susceptibility to plant pathogens. Utilizing this mutant, downstream signaling molecules have been identified by screening for mutations that alter this constitutive defense response phenotype. One such mutant that was found to significantly restore susceptibility carried a mutation within one of the two E1 ubiquitin-activating enzymes. This mutant was named *modifier of snc1 5 (mos5)*. Furthermore, levels of salicylic acid, an important plant hormone involved in defense responses, in *mos5* plants were reduced 12-fold compared to the levels in *snc1* plants. Mutations in E1 ubiquitin-activating enzymes may inhibit downstream ubiquitin-conjugating reactions and the subsequent failure of ubiquitin to be ligated to the protein to be catabolized by the proteasome. This may lead to enhanced susceptibility to disease in *mos5* mutants [4]. The fact that a mutation within one of the E1 enzymes was capable of altering the disease-resistant phenotype of *snc1* plants strongly suggests that the ubiquitin conjugation cascade plays an essential role in plant defense.

E3 Ligases May Play an Important Role in Regulating Plant Defense Responses

Specific defense responses are regulated by key plant hormones such as salicylic acid (SA) and jasmonic acid (JA). These plant hormones accumulate

in response to invasion by plant pathogens, resulting in the activation of key defense genes [6]. The role of SA, in particular, is vital to plant defense as plants unable to produce SA are extremely susceptible to infection by a variety of pathogens. SA is synthesized in response to bacterial and fungal plant pathogens and upon recognition of general elicitors or pathogen-associated molecular patterns (PAMPs). The most important SA biosynthetic pathway involves the enzyme Isochorismate Synthase1 (ICS1); a mutation in the *ICS1* gene (also referred to as the *sid2* mutation) results in a significant reduction of SA, underlying the importance of this pathway. However, *sid2* plants still produce a significant level of SA, albeit substantially reduced, indicating that SA is capable of being synthesized by alternative pathways. Yaeno and Iba (2008) isolated, through mutant screens, an *Arabidopsis* plant line that accumulated excessive levels of SA upon treatment with benzoic acid (BA), a putative precursor of SA. This mutant was named *benzoic acid hypersensitive1-Dominant (bah1-D)* [8].

To further classify the function of BAH1, *bah1-D/sid2* double mutants were analyzed. These double mutants were found to have levels of SA that were slightly higher than *sid2* mutants, but were still significantly lower than *bah1-D* mutants. Therefore, because *sid2* mutants lack a functional *ICS1* gene and the SA level of the double mutant was near that of the *sid2* mutant, it can be concluded that the overproduction of SA in *bah1-D* mutants is a result of an over-stimulation of the ICS1 pathway [8].

The *bah1-D* mutant phenotype was created via a T-DNA insertion and sequences flanking the insertion site were identified by plasmid rescue. The insertion sites were found to be located within the 5' untranslated region of the *Arabidopsis* gene At1g02860. Unexpectedly, the *bah1-D* phenotype was not caused by the overexpression of the At1g02860 gene; rather the suppression of this gene was responsible for the *bah1-D* phenotype. In order to confirm that the suppression of At1g02860 was responsible for the *bah1-D* phenotype, Yaeno and Iba constructed RNAi plant lines in which At1g02860 was silenced. These RNAi plant lines had similar phenotypes to the *bah1-D* mutant plants confirming the fact that the suppression of At1g02860 was responsible for the *bah1-D* phenotype. Since the *bah1-D* mutation results in an overproduction of SA, it

can be inferred that the At1g02860 gene product plays a role in negative feedback regulation of SA accumulation during a defense response [8].

Analysis of the BAH1 protein indicates that the protein contains a RING-finger domain, a protein motif characteristic of E3 ligases. Although E3 ligase activity has not yet been directly confirmed, analysis of a related mutant line *nla* (nitrogen limitation adaptation) indicates that BAH1 may function as an E3 ligase. The evidence for this conclusion is that the *nla* phenotype is created by a mutation in the same gene as *bah1-D*, and this mutation results in the loss of the RING domain, inhibiting the E3 ligase ability of the NLA protein [8].

The accumulation of SA triggers the activation of a number of plant defense-related genes. One of the most important defense responses signaled by SA is known as Systemic Acquired Resistance (SAR). SAR is activated in response to pathogen attack, and it is capable of stimulating long-term resistance in a plant to an array of pathogenic microorganisms. SA activates a large set of genes, including those that encode the pathogenesis-related (PR) proteins. These proteins are able to inhibit colonization by harmful microorganisms leading to a state of resistance. SA is capable of activating the positive regulator protein NPR1 that is subsequently translocated to the nucleus where it interacts with various transcription factors responsible for activating genes necessary for SAR [2, 6]. The main source of SA during SAR is from the ICS1 biosynthetic pathway; the same pathway that is negatively regulated by BAH1, a protein that appears to function as an E3 ligase [2, 8].

Conclusion

There is substantial evidence demonstrating the importance of RRE1 in plant innate immunity. RRE1 is an E3 ubiquitin ligase containing a PEST domain and the E3-characteristic RING zinc-finger domain [1, 7]. The ubiquitin/26s proteasome pathway has been experimentally demonstrated to play an important role in plant defense [4]. As an E3 ligase, RRE1 plays an important role in the ubiquitin conjugation cascade: to identify the correct protein to be ubiquitinated and to facilitate the binding of ubiquitin to that protein [5]. Furthermore, *RRE1* has been experimentally shown to be up-regulated in *Arabidopsis* in response to the fungal elicitor chitin

[1]. The fact that an E3 ligase has been shown to regulate SA accumulation only bolsters the evidence in favor of the importance of RRE1 in plant innate immunity [8].

Although RRE1 and BAH1/NLA both function as E3 ubiquitin ligases, it is unlikely that the RRE1 protein functions in a similar manner to BAH1. While the overexpression of *RRE1* in plants results in a more disease-resistant phenotype, the overexpression of the At1g02860 gene would result in a more disease-susceptible phenotype since the BAH1/NLA protein negatively regulates SA accumulation. Nonetheless, the fact that both genes have E3 ubiquitin-ligase activity underlines the importance of this class of proteins in plant immune responses.

Unfortunately, generating plants that are more resistant to infection is not as simple as overexpressing *RRE1* or knocking out At1g02860. Plant defense responses have high energy requirements and can have negative effects on plant growth and reproduction if they are sustained over long periods of time. Yaeno and Iba (2008) demonstrated this fact by noting that the high levels of SA in the *bah1-D* mutant resulted in the inhibition of plant growth and the necrosis of plant tissue due to senescence. Therefore, one cannot simply create plant lines that are constantly undergoing a defense response, as these plants would not reproduce or grow well. Rather, one must be able to *modulate* the defense response. Creating plant lines that have modulated defense responses is a significant task for modern plant researchers. In order to accomplish this goal it is important to have a precise understanding of plant innate immunity and the pathways involved in order to generate appropriate plant lines. Therefore it is important to understand the action of RRE1 and other E3 ligases if useful plant lines more resistant to disease are to be created.

References

- [1] Berrocal-Lobo M, Ramonell K, Sánchez C, Stone S, Callis J, Molina A, and Somerville S. RRE1: an Ubiquitin Ligase Implicated in Plant Defense.
- [2] Durrant WE, Dong X. (2004). Systemic acquired resistance. Annual Review of Phytopathology, 42:185-209.

- [3] European Bioinformatics Institute (EBI) InterPro Database. 2008.
<http://www.ebi.ac.uk/interpro/IEntry?ac=IPR001841>
- [4] Goritschnig S, Zhang Y, and Li X. (2006). The ubiquitin pathway is required for Innate immunity in Arabidopsis. *The Plant Journal*, 49:540-51.
- [5] Smalle J and Vierstra RD. (2004). The Ubiquitin 26s Proteasome Proteolytic Pathway. *Annual Reviews of Plant Biology*, 55:555-90.
- [6] Spoel SH, Koornneef A, Claessens SMC, Korzelius JP, Van Pelt JA, Mueller MJ, Buchala AJ, Métraux JP, Brown R, Kazan K, Van Loon LC, Dong X, and Pieterse CMJ. (2003). NPR1 Modulates Cross-Talk between Salicylate- and Jasmonate-Dependent Defense Pathways through a Novel Function in the Cytosol. *The Plant Cell*, 15:760-770.
- [7] Universal Protein Resource. 2008. (July 22, 2008);
<http://www.uniprot.org/uniprot/O64763>.
- [8] Yaeno T, and Iba K. (2008). BAH1/NLA, a RING-Type Ubiquitin E3 Ligase, Regulates the Accumulation of Salicylic Acid and Immune Responses to *Pseudomonas syringae* DC3000. *Plant Physiology*, 148:1032-1041.

Adam Perricone is a senior majoring in Biology from New Orleans, LA. He studies the interactions between plants and pathogens in Dr. Katrina Ramonell's lab and has been awarded The American Society of Plant Biologists Research Fellowship. He is also the recipient of UA's National Hispanic Scholarship.