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Complex regulation of an *R* gene *SNC1* revealed by autoimmune mutants

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Plants have evolved resistance (R) proteins to detect pathogen effectors and trigger plant defense responses in the so named effector-triggered immunity (ETI). R proteins are under negative regulation in plants as upregulated activation of R protein is detrimental to plant growth. Autoimmune mutants have been instrumental in understanding the fine tuning of plant defense responses. Recently, a number of such mutants have been molecularly characterized, and some of them result from over-activation of SNC1, a TIR-NBS-LRR type of R protein. Studies of these mutants revealed a complex negative regulation of SNC1 activity from transcriptional to post-translational regulation. Here, we summarize studies on these SNC1-dependent autoimmune mutants and discuss the fine regulation of R proteins in plant immunity.

To ward off pathogen invasion, plants have evolved at least two layers of defense responses. The first layer is triggered through the perception of pathogen associated molecular patterns (PAMP) by pattern recognition receptors (PRR), namely 'PAMP triggered immunity' (PTI). The second layer of defense is triggered through the recognition of pathogen-secreted effectors by resistance (R) proteins, namely 'effector triggered immunity' (ETI).[1] Specific recognition of effectors by R proteins leads to a rapid programmed cell death called hypersensitive response (HR) which restricts the growth and spread of pathogens.[2] Although R activation is essential for effective plant defense, R protein activity needs to be fine controlled to avoid extensive cell death by R protein over-activation. R proteins are normally expressed at a low level and assume an inactive state in the absence of pathogen triggers. The mechanisms underlying the negative regulation of R proteins are far from well understood. Here we will review the study of autoimmune mutants that have revealed negative regulation at multiple levels on SNC1, a TIR-NBS-LRR R protein.

snc1-1 and Other Auto-immune Mutants

In the last decade, a number of mutants were isolated in Arabidopsis showing dwarf morphology, constitutive expression of pathogen-related (*PR*) genes, and enhanced disease resistance. As they display immune responses without pathogen triggers, these mutants are named as auto-immune mutants. Since many such mutants also display microscopic cell deaths at least under some environmental conditions, they are called lesion mimic mutants as well.[3,4] *snc1* (referred to as *snc1-1*), isolated as a suppressor of *npr1-1*[5], has a missense mutation in a TIR-NBS-LRR R protein SNC1, leading to constitutive activation of SNC1 and thus autoimmune responses.[5,6] The *snc1-1* mutant can be suppressed to different extent by a set of *modifer of snc1* (*mos*) mutants. Characterizations of these *MOS* genes indicate that SNC1-mediated resistance is regulated by many processes and pathways.[7] The *bon1/cpn1* mutants also exhibit dwarfism and auto-immune responses.[8,9] Interestingly, this autoimmune phenotype is present in the Col-0 accession but not the Ws accession, and the study of this natural modification of *bon1* reveals that the autoimmune response is dependent on the Col-0 haplotype-specific *SNC1*.[8,10] In the last few years, many more autoimmune mutants were found to be *SNC1* dependent, similarly to *bon1*. They include: *bap1*, *bir1*, *srfr1*, *cpr1*, and *mkp1* (**Table 1**). Therefore, *SNC1* seems to be under complex regulation by molecules with diverse functions and subcellular localizations (**Table 1**).

Regulation of SNC1 by BON1 Associated Proteins on the Plasma Membrane

BON1/CPN1 is a member of evolutionarily conserved copine proteins characterized by two C2 domains at the N-terminus and a VWA domain at the C-terminus.[8] Though the C2 domain could confer a calcium-dependent phospholipid binding to the membranes, the localization of BON1 to the plasma membrane is mediated by the N-terminal myristoylation of BON1.[11] Several BON1 interacting proteins have been isolated through the yeast two-hybrid screens. The first one identified is BAP1 (BON1-ASSOCIATED PROTEIN 1) which contains one C2 domain and a short C-terminal tail.[8,12] Similar to *bon1-1*, the loss-of-function mutant *bap1-1* shows auto-immune phenotype that is dependent on *SNC1*.[12] Overexpression of BAP1 partially suppressed the *bon1-1* phenotype, indicating that BON1 and BAP1 work together to negatively regulate SNC1.[12] The BAP1 protein is membrane associated, however its exact location has not been determined. The second BON1 interacting protein identified is BIR1 (BAK1-INTERACTING RECEPTOR-LIKE KINASE 1) which encodes a receptor like kinase interacting with BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE 1),[13] a co-receptor

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Table 1. SNC1 dependent auto-immune mutants

Gene name	Mutant name (in Col-0)	Severity of dwarfness	suppression by *snc1–11*	Subcellular localization	Biochemical function	References
BON1	*cpn1-1* *bon1-1*	++	Fully	Plasma membrane	Phospholipid-binding Copine protein	8–10
BAP1	*bap1-1*	+	Fully	Membrane	Phospholipid-binding protein	8,12
BIR1	*bir1-1* *bir1-2*	++++	Partially	Plasma membrane	Receptor like kinase	13,17
SRFR1	*srfr1-3* *srfr1-4* *srfr1-5*	++++	Partially	Cytoplasm and nucleus	TPR1 domain protein	24,26
CPR1	*cpr-1* *cpr1-2 (cpr30-1)* *cpr1-3 (cpr30-2)*	++	Fully	Cytoplasm and nucleus	F-box Protein	20–23
MKP1	*mkp1*	+	Partially	Cytoplasm	MAPK phosphatase	27

Note: more "+" means smaller size.

of BRI1 (BRASSINOSTEROID INSENSITIVE 1) and FLS2 (FLAGELLIN SENSITIVE 2).[14-16] BAK1 was also found to interact with BON1.[17] The *bir1* mutant shows a more severe growth defect than *bon1-1* and can be partially rescued by *BON1* overexpression. In addition, the *bir1* mutant phenotype can be suppressed by a *SNC1* loss-of-function mutant *snc1-11*, indicating that BIR1 and BON1 can function together to regulate SNC1.[17] BAK1 is involved in PTI as well as in brassinosteroid signaling in development. The *BAK1* loss-of-function mutant does not exhibit a severe growth defect but the double mutant of *BAK1* and its homolog *BKK1* has a very severe dwarf phenotype and cell death.[18] It will be interesting to test if *SNC1* contributes to this phenotype.

Although BIR1, BAK1 and BON1 could interact with each other in vitro and are all plasma membrane associated, the in vivo interaction is yet to be verified and the biological relevance is yet to be revealed. BIR1 has extracellular leucine-rich-repeats, and is presumably a receptor for an unknown ligand. As overexpression of BON1 and BIR1 can mutually partially suppress the loss-of-function mutant phenotypes of each other,[17] they two probably do not work in a linear signaling pathway but rather in a protein complex or in parallel. BAK1 can interact with multiple RLKs including BRI1 and FLS2,[14-16,19] and therefore could transduce multiple signals such as the pathogen signature flagellin and the plant hormone brassinolides. As BAK1 is shown to phosphorylate BON1 in vitro, BON1 could potentially be modified by multiple signals through BAK1 if indeed this phosphorylation has biological relevance. BON1 does not appear to have a kinase activity[11] and therefore unlikely will serve as a phosphorylation transducer. Two other activities are shown to be critical for BON1 activity: its calcium binding and its interaction with BAP1.[11] Therefore its modification (such as phosphorylation) by its interacting proteins could potentially regulate its calcium binding activities and/or BAP1 interacting activities. The output of BON1 activity could be a calcium signal and/or the BAP1 activity.

It is not entirely clear how the plasma-membrane localized proteins BON1 and BIR1 and the membrane associated BAP1 regulate *SNC1* activity. In the *bon1-1* and *bap1-1* mutants, the transcript of *SNC1* is upregulated, which is sufficient to cause an autoimmune phenotype. The upregulation is largely due to a feed-forward regulation by salicylic acid (SA) and PAD4/EDS1 as the *SNC1* transcript level is greatly reduced in the *NahG*, *pad4*, or *eds1* background.[10,12] It therefore remains unclear if an initial small change of transcript level or protein activity leads to the final increase of *SNC1* transcript. In either case, the regulation of these proteins on *SNC1* is likely to be indirect as no physical interaction between BON1 and SNC1 has been detected (unpublished results).

CPR1/CPR30 and Possibly SRFR1 Regulate the SNC1 Protein Stability

cpr1 is one of the first auto-immune Arabidopsis mutants isolated in a screen of *constitutive expressor of PR genes (cpr)* mutants.[20] *cpr30* was more recently identified as a *cpr* like mutant from a background mutation in a T-DNA line, and recently *CPR1* and *CPR30* were found to be the same gene.[21] *CPR1/CPR30* encodes a functional F-box protein that interacts with multiple SKP1 homologs,[22] and the *cpr1* phenotype is *SNC1*-dependent. The SNC1 protein is upregulated in both mutants and down-regulated in *CPR1* overexpression lines. CPR1 can physically interact with SNC1 and affect SNC1 protein accumulation through the 26S proteasome mediated protein degradation.[21,23] Therefore, CPR1 regulates the protein stability of SNC1. The *SUPPRESSOR OF rps4-RLD* (*SRFR1*) likely regulates SNC1 at the protein level as well. The *srfr1* mutants in Col-0 background show a *SNC1*-dependent auto-immune phenotype similar to that of *bon1-1*.[24,25] *SRFR1* encodes a conserved TPR domain-containing protein, and it can physically interacts with SGT1 (SUPPRESSOR OF THE G2 ALLELE OF SKP1) proteins that are involved in the control of stability of multiple R proteins.[25] The SNC1 protein level is elevated in both *srfr1* and *sgt1b* mutants, suggesting a regulation of SNC1 protein stability by SRFR1 and SGT1 proteins.[26]

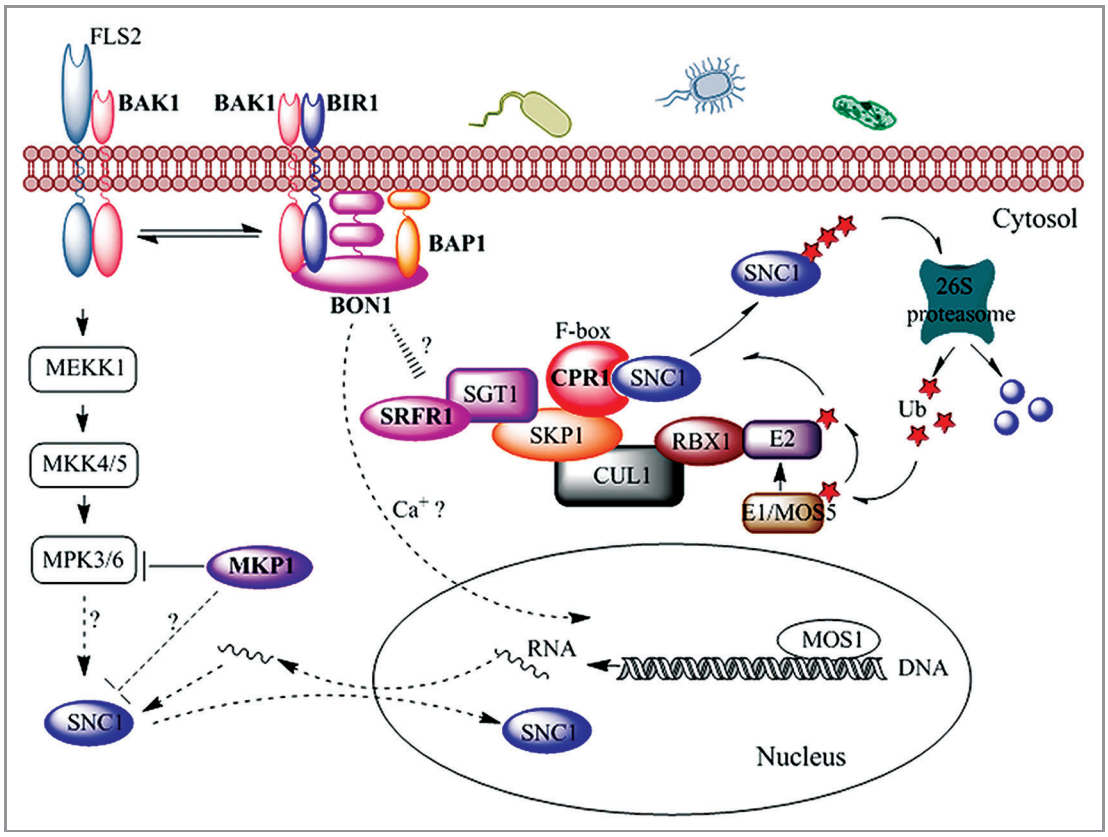

Figure 1. Working model for the regulation of SNC1.

Negative Regulation of SNC1 by MKP1

MKP1 (MAP KINASE PHOSPHATASE 1) and PTP1 (PROTEIN TYROSINE PHOSPHATASE 1) both dephosphorylate MPK6 (MAP KINASE6). The *mkp1* null mutant in Col-0 but not in Ws exhibits auto-immune mutant phenotype that is enhanced by *ptp1* and dependent on *MPK3* and *MPK6*, key signaling components in PTI.[27,28] The *mkp1* and the *mkp1ptp1* phenotypes could be partially suppressed by *snc1-11*, suggesting that *MKP1* is another negative regulator of *SNC1*. Because the *SNC1* transcription level is not significantly changed in the *mkp1* mutant,[27] *MKP1* might regulate *SNC1* at post-transcriptional level. It will be of interest to determine the relevance between MPK3 and MPK6 dephosphorylation and *SNC1* negative regulation, which might shed light on the link between PTI and ETI.

Model for the Complex Regulation of SNC1 by Multiple Proteins

The identification of multiple regulators of *SNC1* indicates a complex regulation of *SNC1* gene function at different levels as summarized in the working model (**Fig. 1**). *SNC1* is repressed in the wild type in the absence of pathogen invasion. Perturbation in various host genes induces activation of *SNC1* and this could be at both the transcriptional and posttranscriptional levels. Some of them directly regulate the stability of SNC1 protein. SNC1 is targeted by the F-box protein CPR1, ubiquitinated, and degraded by the 26S proteasome. SGT1 proteins, which interact directly with the SKP1 proteins, might affect SNC1 activity through its regulation of the SCF complex stability. SRFR1, which interacts directly with SGT1, might also regulate SNC1 by affecting the stability of the SCF complex. Other host genes might modulate SNC1 expression or activity indirectly. Signals generated from the loss of BON1 and BIR1 at the plasma membrane could be transduced through BAP1 or a secondary messenger like Ca^{2+} to regulate the *SNC1* transcription in the nucleus. Indeed, *MOS1*, identified as a suppressor of *snc1*, regulates the transcript level of SNC1 possibly through chromatin remodeling and DNA methylation.[29] However, it cannot be excluded at this point that BON1 and its interacting proteins could affect the stability of SCF complex that regulates SNC1 in a yet unkown mechanism. MKP1 is a negative regulator of MPK3 and MPK6 as well as SNC1, suggesting a cross regulation of both PTI and ETI. It will be interesting to reveal whether the regulation of SNC1 by MKP1 is dependent on its regulation on MPK3 and MPK6.

The pressing question is to further understand the roles, if any, of these regulators of *SNC1* in plant immunity. Presumably, they are involved in the perception and signaling of pathogen invasions if they do not function only in general production, processing and degradation of transcripts and proteins. One interesting observation is that some of the negative regulators of

SNC1 including *BON1*, *CPR1*, and *BAP1* are induced upon pathogen invasion.[12,23,30] Presumably, SNC1 activity will be consequently reduced during pathogen invasion. It will be interesting to determine if SNC1 is initially activated and then repressed during pathogen invasion, which would indicate a fine tuning of defense responses by modulating R activities. In sum, SNC1 activities are under tight controls by different signaling components. Further study of these components and their pathways will enhance our understanding of complex regulation of plant immunity.

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