# Sumoylation, a post-translational regulatory process in plants

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The reversible conjugation of the small ubiquitin-related modifier (SUMO) peptide to protein substrates (sumoylation) is emerging as a major post-translational regulatory process in animals and other eukaryotes, including plants. Database annotation, and genetic and biochemical analyses indicate that components of the SUMO conjugation and deconjugation systems are conserved in plants such as *Arabidopsis*, rice, tomato, and *Medicago*. Specifically, *Arabidopsis* AtSUMO1/2 and SUMO E2 conjugation enzyme AtSCE1a are implicated in abscisic acid (ABA) responses and the ubiquitin-like SUMO protease 1 (ULP1) AtESD4 in flowering time regulation. The AtSIZ1 SUMO E3 ligase functions in phosphate starvation responses, cold tolerance, basal thermotolerance, salicylic acid (SA)-dependent pathogen defense, and flowering time regulation. Following is a brief overview of the current understanding of SUMO conjugation and deconjugation determinants, and biological processes that are regulated in plants.

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## Introduction

Sumoylation is categorized as an ubiquitin-like protein (UBL) conjugation process because of similarities between ubiquitin and SUMO, and the biochemical steps that catalyze SUMO conjugation and deconjugation of protein substrates [1]. SUMO is similar to ubiquitin in 3D structure but differs in primary sequence (about 20% similarities) and contains ~15 additional N-terminal amino acid (aa) residues; SUMO and ubiquitin are ~92–103 and 76 aa, respectively [2]. SUMO is reversibly conjugated to target proteins like ubiquitin but the modification does not appear to facilitate, but may attenuate, protein degradation [1,3•,4]. SUMO was discovered in 1996 as a peptide that is conjugated to RanGAP1, a small GTPase, which is localized to the nuclear pore complex and promotes nucleocytoplasmic trafficking [5]. Sumoylation is associated with cell cycle activity, DNA repair, subnuclear localization, enzymatic activity and stability, and innate immunity [3•,6]. Sumoylation has recently been linked causally to major human maladies such as Alzheimer's and Huntington's diseases [7], viral infection [8], and cancer [9,10], and is required for viability of eukaryotes including yeast, nematodes, vertebrates, and plants [11–13,14••].

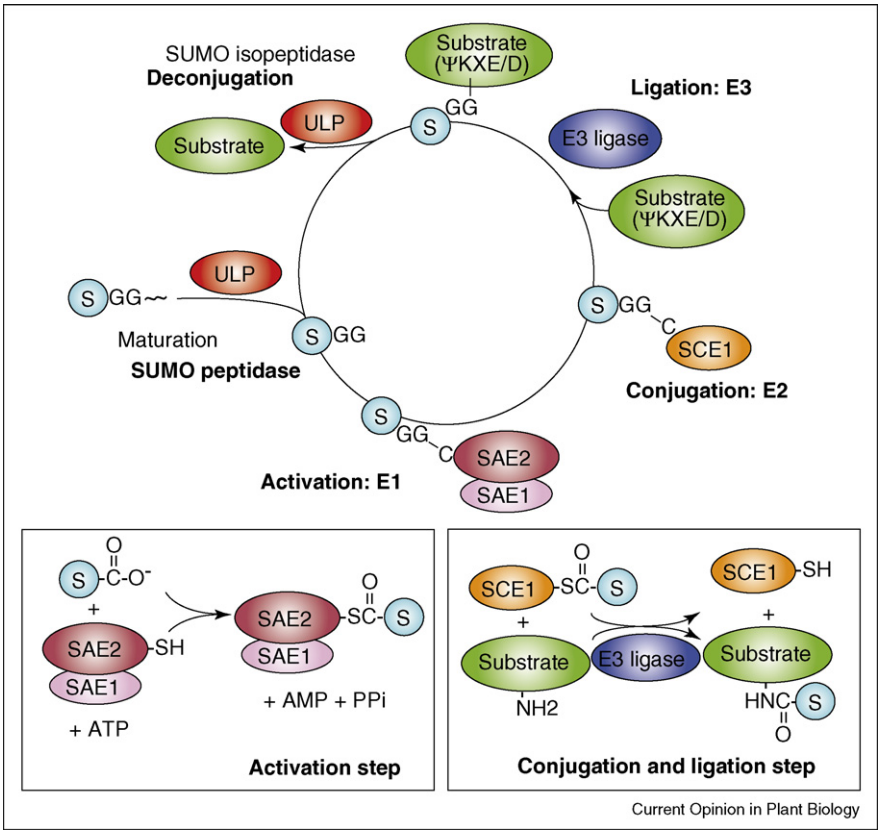
Most notably, SUMO modification modulates transcription factor activity to coordinate gene expression that is necessary for development and hormonal and environmental responses of animals and plants [3•,6,15,16,17••–19••]. Sumoylation results in repression or activation of transcription factor activity, and desumoylation mediates the opposite response [3•,20]. That is, SUMO conjugation and deconjugation of transcription factors or components of coregulatory complexes function in concert to facilitate precise regulation of gene regulons [3•,6,21]. Current models hypothesize that sumoylation regulates gene expression through chromatin remodeling directly and/or through subnuclear compartmentalization of transcriptional coregulators [22].

The biochemical components of the sumoylation and desumoylation systems are conserved in *Arabidopsis* and other plants based on genome sequence information [16,23]. This infers that post-translation modification by sumoylation is a major regulatory process in plants, as it is in other eukaryotes [3•,20], and evidence is emerging to substantiate this likelihood [23]. Current research in plants is identifying the biological processes that are regulated by sumoylation, and dissecting the sumoylation and desumoylation processes and the gene expression mechanisms by which the post-translational process regulates gene expression.

## The SUMO conjugation and deconjugation pathway

Sumoylation occurs in a series of biochemical steps referred to as SUMO E1 activation, E2 conjugation, and E3 ligation (Figure 1). Deconjugation of SUMO substrates is catalyzed by ubiquitin-like SUMO-specific proteases (ULP) (Figure 1). The sumoylation and desumoylation effector proteins are conserved in plants, though there is variation in gene complexity for each in different species based on genome sequence information (Table 1). Recently, *Arabidopsis* sumoylation and desumoylation components have been established by direct biochemical evidence [15,16,17••,24,25••,26••].

**Figure 1**




The sumoylation and desumoylation cycle of plants and other organisms. *Maturation* — SUMO isoforms are encoded as precursor proteins. SUMO precursors are processed by SUMO-specific cysteine proteases (ULP, ubiquitin-like protein protease) with SUMO peptidase activity that carboxyl-terminally truncates the preprotein to the diglycine (GG) motif. *Activation* (*E1*) — the SUMO carboxyl-terminal G is linked to AMP (SUMO-AMP) catalyzed by the heterodimeric E1 SUMO activating enzymes 1 and 2 (SAE1 and SAE2) in an ATP-dependent reaction. Subsequently, the G of SUMO is coupled to a cysteine (C) residue in SAE2 via a high-energy thioester bond. *Conjugation* (*E2*) — SUMO is transferred to a C residue of the E2 SUMO-conjugating enzyme (SCE1) by transesterification that is catalyzed by SCE1. *Ligation* (*E3*) — SUMO is transferred to the ε-amino group of a lysine (K) side chain in the sumoylation consensus motif (ΨKXE/D; Ψ, a large hydrophobic residue; X, any amino acid; E/D, glutamate or aspartate) of the substrate protein, forming an isopeptide bound between the carboxyl of G in SUMO and ε-amino of K in the substrate, a process that requires the E3 SUMO ligase *in vivo*. *Deconjugation* — a SUMO-specific proteases with isopeptidase activity cleaves the isopeptide bond and SUMO is recycled through the conjugation system (adapted with permission [6]. Copyright 2003, Macmillan Publishers Ltd.).

Mature SUMOs are generated from precursor proteins of 95–111 aa by ULPs with SUMO peptidase activity, which recognize a carboxyl-terminal diglycine (GG) motif and delete about 10 aa directly after the GG motif (Figure 1) [21]. The *Arabidopsis* and the rice genomes are predicted to encode nine and three SUMOs, respectively, based on sequence similarity with proteins in animals and fungi, ~40% [16,23] (Table 1). AtSUMO1/2 and OsSUMO1/2 seem to be orthologs of human SUMO2/3 based on sequence similarity. In addition, conjugation of AtSUMO1/2 and human SUMO2/3 are induced by heat, ethanol, and oxidative stresses [16,27].

SUMO is activated by an E1 heterodimer containing SAE1 and 2 (SUMO E1 activating enzyme) that catalyzes ATP hydrolysis to form SUMO-AMP [21]. SAE1 and SAE2 both contain the ThiF domain that participates in ATP binding [28]. Subsequently, AMP is released from SUMO-AMP with the formation of a high-energy thioester bond between the sulfhydryl group of the catalytic cysteine (C) residue in SAE2 and the carboxyl group of G in SUMO (Figure 1).

Activated SUMO is transferred from SAE2 to a C residue in SCE1 (SUMO E2 conjugating enzyme) in a transesterification reaction to form a SUMO–SCE1 thioester complex (Figure 1) [21]. C94 is the conjugated residue in AtSCE1 [15]. SUMO is transferred from SUMO–SCE1 and is linked to a substrate protein via an isopeptide bond between the SUMO carboxyl-terminal G and the ε-amino group of lysine (K) in the SUMO consensus motif, ΨKXE/D (Ψ, a large hydrophobic residue; K, the acceptor lysine; X, any amino acid; E/D, glutamate or aspartate) in the substrate (Figure 1).

**Table 1**

**Sumoylation and desumoylation determinants in *Arabidopsis* and rice**

| Protein activity | Gene | Chr. locus *Arabidopsis* | Chr. locus rice |
|---|---|---|---|
| SUMO | SUM1 | At4g26840 | Os01g0918300 |
| | SUM2 | At5g55160 | Os01g0918200 |
| | SUM3 | At5g55170 | Os07g0574500 |
| | SUM4 | At5g48710 | |
| | SUM5 | At2g32765 | |
| | SUM6 | At5g48700 | |
| | SSUM7 | At5g55855 | |
| | SUM8 | NA (Chr. 5) | |
| | SUM9 | NA (Chr. 4) | |
| SUMO E1 | SAE1a | At4g24940 | Os11g0497000 |
| | SAE1b | At5g50580/ At5g50680 | |
| | SAE2 | At2g21470 | Os07g0586500 |
| SUMO E2 | SCE1a | At3g57870 | Os03g0123100 |
| | SCE1b | NA (Chr. 5) | Os10g0536000 |
| | SCE1c | | Os04g0580400 |
| SUMO E3 | SIZ1 | At5g60410 | Os05g0125000 |
| | SIZ2 | | Os03g0719100 |
| | NSE2/ MMS21 | At3g15150 | Os05g0563500 |
| SUMO protease | ULP1a | At3g06910 | Os01g0355900 |
| | ULP1b | At4g00690 | Os03g0410100 |
| | ULP1c | At1g10570 | Os03g0344300 |
| | ULP1d | At1g60220 | |
| | ESD4 | At4g15880 | |
| | ULP2a | At4g33620 | Os05g0207900 |
| | ULP2b | At1g09730 | Os01g0738100 |

SUMO: small ubiquitin-related modifier; E1: two subunits of SUMO activation enzyme SAE1 and 2; E2: single subunit of SUMO conjugating enzyme SCE1; E3: two types of SUMO ligase, SIZ and NSE2/MMS21; protease: two types of ubiquitin-like SUMO protease ULP1 and 2; NA: not annotated.

SUMO E3 ligases are SCE1-interacting proteins that are necessary for the transfer of SUMO from SCE1 to the substrate proteins *in vivo* [21]. SIZ/PIAS (SAP and MIZ/protein inhibitor of activated STAT) transcriptional coregulators were the first identified and are the most characterized SUMO E3 ligases [3•,21,29,30,31•]. The SAP, PINIT, SP-RING, SUMO binding, and NLS domains (see Figure 2 legend for acronym definition) are conserved in all SIZ/PIAS proteins including those in plants [31•]. SP-RING is a Zn-finger domain containing $C_2HC_3$ in SIZ/PIAS or $C_2HC_2$ in NSE2/MMS21 (see below) (Figure 2) [31•]. The PINIT and SP-RING domains are necessary for SUMO E3 ligase activity of SIZ/PIAS proteins, and the SP-RING facilitates binding of SIZ/PIAS to SCE1 [32]. The SAP domain forms a helix-extended loop-helix structure that binds to DNA [33]. Specifically, the SAP domain is required for transrepression activity of PIASy, which attenuates transcription factor activity of STAT1 and the androgen receptor [34,35]. Only plant SIZ/PIAS homologs contain a plant homeodomain (PHD), that is, a $C_4HC_3$ Zn-finger [36]. PHD domains are present in nuclear proteins and are associated with phosphoinositide recognition, chromatin remodeling, and ubiquitin E3 ligase activity [36–38]. However, the specific function of the PHD domain in plant SIZ proteins is unknown. SIZ/PIAS proteins are also implicated as transcriptional coregulators, independent of E3 ligase activity, through processes including subnuclear relocalization of substrates and interaction with transcriptional coregulatory complexes [31,35].

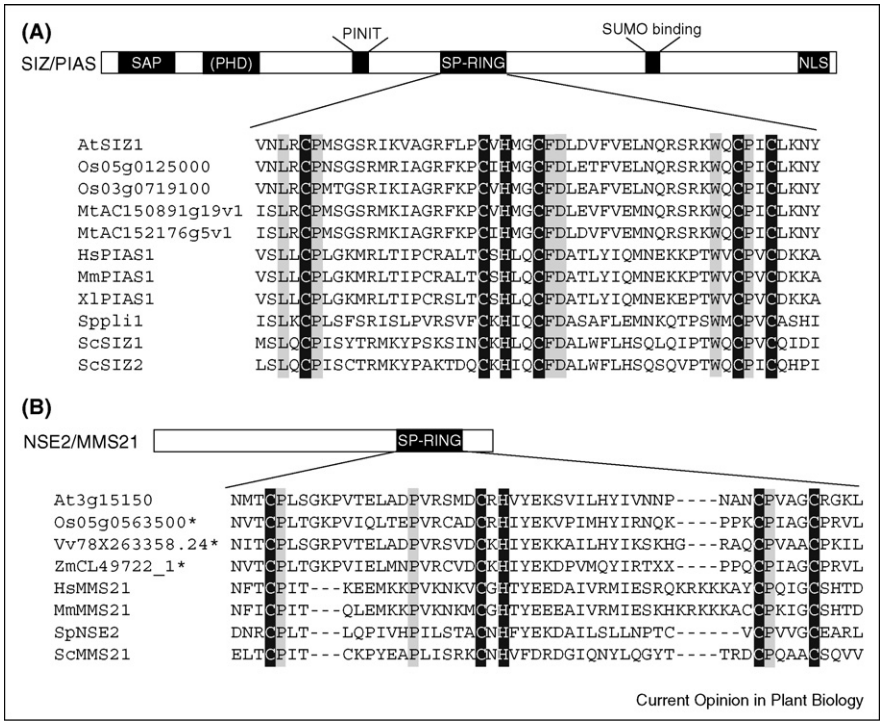
Three additional types of SUMO E3 ligeses have been identified in other organisms and are named for the prototypical proteins: RanBP2 (Ran binding protein), Pc2 (polycomb group), and NSE2/MMS21 (non-SMC element/methyl methanesulfonate sensitive) [3•,20]. None of these E3 ligases have been functionally identified in plants. However, putative orthologs of NSE2/MMS21, but not RanBP2 or Pc2, can be inferred in *Arabidopsis* (At3g15150) and rice (Os05g0563500) based on sequence annotation (Table 1). NSE2/MMS21 orthologs are characterized by SP-RING domains that are necessary for SUMO E3 ligase activity (Figure 2B) [39].

SUMO conjugates are recycled from substrates by ULPs with SUMO isopeptidase activity (Figure 1) [40]. *Arabidopsis* AtULP1a, AtULP1c, AtULP1d, and AtESD4 have been functionally characterized as SUMO proteases (Table 1), which have both SUMO peptidase and isopeptidase activities that are required for SUMO precursor protein processing and substrate deconjugation, respectively [24,25••,26••]. The catalytic cores of the *Arabidopsis* AtULP1 proteases have sequence similarity to each other and to yeast Ulp1 but the amino-terminal regions have limited similarity [26••]. The amino-terminal region is presumed to be responsible for the specificity of SUMO-protein conjugate recognition and for modulation of enzymatic activity. AtULP1a, but not AtULP1c or AtESD4, cleaved AtSUMO3 conjugates *in vitro* [26••], and AtULP1a showed greater SUMO peptidase than isopeptidase activity, whereas AtESD4 had greater deconjugation activity [26••]. Interaction between NUA (nuclear pore anchor) and AtESD4 at the nuclear envelope is required for deconjugation of SUMO substrates *in planta* [41•].

## Sumoylation and biological processes in plants

Loss-of-function and gain-of-function analyses have resulted in an emerging paradigm that sumoylation (and desumoylation) is a post-translational process that regulates plant signaling, development, and responses to hormonal and environmental cues through transcriptional regulation of determinant gene expression [14••,15,16,17••–19••,24]. An insertional mutation in *AtSAE2* or *AtSCE1* or the double mutations in *AtSUMO1* and *AtSUMO2* cause embryo lethality [14••]. However,

**Figure 2**




Sequence structures and alignment of SP-RING domains in SIZ/PIAS proteins **(A)** and in MMS21/NSE2 proteins **(B)**. The domains are: SAP (scaffold attachment factors SAF-A/B, Acinus, PIAS, a helix-extended loop-helix [33]), PINIT (Pro-Ile-Asn-Ile-Thr), SP-RING (SIZ/PIAS-RING, $C_2HC_3$, or $C_2HC_2$-type Zn-finger [32,39]), SUMO binding domain (Ser-X-Ser), and NLS (nuclear localization sequence). Note that only plant SIZ proteins contain the PHD (plant homeodomain, $C_4HC_3$-type Zn-finger). The putative $Zn^{2+}$-coordinating cysteine and histidine residues are highlighted, and conserved residues are shaded. Alignment was performed using the ClustalW program (http://clustalw.ddbj.nig.ac.jp/top-e.html). The species identifying code of each protein is as follows: *Arabidopsis thaliana* (At), *Oryza sativa* (Os), *Medicago truncatula* (Mt), *Vitis vinifera* (Vv), *Zea mays* (Zm), *Homo sapiens* (Hs), *Mus musculus* (Mm), *Xenopus laevis* (Xl), *Schizosaccharomyces pombe* (Sp), and *Saccharomyces cerevisiae* (Sc). *, these genes are predicted with GeneMark program (http://exon.gatech.edu/GeneMark/).

the biochemical and molecular genetic mechanisms by which SUMO conjugation modulates transcription factor activity in plants are yet resolved.

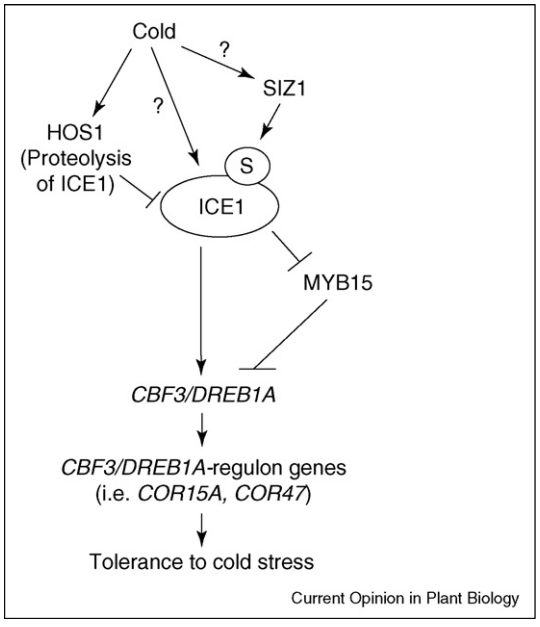
## Responses to the environment

AtSUMO1/2 conjugation to substrate proteins was induced by cellular stresses such as high temperature and $H_2O_2$, inferring that sumoylation is involved in stress responses, perhaps adaptation [14••,16,17••,18••]. Stress-responsive SUMO conjugation is mediated mainly by the AtSIZ1 SUMO E3 ligase [14••,17••]. AtSIZ1 facilitates basal thermotolerance independent of SA but does not appear to affect heat shock factor (HSF) regulon expression, including heat shock proteins (HSPs) despite the fact that sumoylation of HSFs regulates expression of *HSPs* and acquired thermotolerance in other eukaryotes, including mammals [42,43]. Animal HSFs contain a phosphorylation-dependent sumoylation motif (PDSM, ΨKXEXXSP; S, serine; P, proline), which is composed of a SUMO consensus site and a proline-directed phosphorylation site, which biochemically links the two post-translational modification processes [43].

AtSIZ1-mediated sumoylation of the MYC-like transcription factor ICE1 (for inducer of *CBF/DREB1* expression 1 [44]), at K393, is required to activate *CBF3/DREB1A*-dependent cold signaling and freezing tolerance in *Arabidopsis* (Figure 3) [19••]. ICE1 K393R variant protein repressed the expression of *CBF3/DREB1A* and its regulon genes, including the cold-responsive genes *COR15A* and *COR47*, and reduced freezing tolerance. Sumoylation of ICE1 blocked poly-ubiquitination of the transcription factor, presumably by HOS1 RING-type ubiquitin E3 ligase (high expression of osmotically responsive genes1) that leads to proteasome degradation [19••,45], suggesting that sumoylation also regulates freezing response by stabilizing ICE1 activity (Figure 3) [4,19••]. SUMO conjugation to ICE1 resulted in down-regulated expression of *MYB15*, which is a negative regulator of *CBF3/DREB1A* [46]. Together, sumoylation of ICE1 facilitates *DBF3/DREB1A* expression directly by enhancing the activity of the transcription factor, or indirectly by repressing *MYB15* expression [19••].

AtSIZ1 SUMO E3 ligase is a negative regulator of Pi starvation signaling and links two prototypical responses,

**Figure 3**




A model of ICE1-dependent cold signaling regulated by SIZ1-mediated sumoylation. SIZ1 SUMO E3 ligase facilitates SUMO conjugation to ICE1, a MYC-like transcription factor [44], at K393 [19••]. Sumoylated ICE1 induces *CBF3/DREB1A*, represses expression of *MYB15*, which is a negative regulator of *CBF3/DREB1A* [46], and blocks HOS1 ubiquitin E3 ligase-mediated poly-ubiquitination of ICE1 [19••]. Localization of HOS1 to nucleus and proteolysis of ICE1 are enhanced by cold [45]. In total, sumoylation of ICE1 enhances expression of cold-responsive genes, such as *COR15A* and *COR47*, and cold tolerance. Thus, sumoylation of ICE1 plays a key role in cold signaling and tolerance (adapted with permission [19••]. Copyright 2007, American Society of Plant Biology).

root architecture remodeling and determinant gene expression, which are necessary for acquisition of the nutrient [17••]. SIZ1 negatively regulates phosphate uptake, and transporter (*PHT1;4*) and phosphatase (*AtPS2*) gene expression, probably through sumoylation of the MYB transcription factor PHR1 [17••]. PHR1 positively controls expression of Pi transporter and recycling determinant genes [47].

### Hormonal responses

Sumoylation is implicated in ABA responses through the determinants AtSUMO1/2, AtSCE1, and AtSIZ1 [15] (K Miura *et al.*, unpublished). Overexpression of AtSUMO1 or AtSUMO2 reduced, and cosuppression of AtSCE1 or the *siz1* mutation enhanced sensitivities to the ABA-mediated inhibition of root growth [15] (K Miura *et al.*, unpublished). AtSUMO1/2-overexpression induced expression of the ABA-responsive genes *RD29A* and *AtPLC1* [15].

AtSIZ1 negatively regulates primary root growth cessation and lateral root development that occur as responses to Pi starvation and are attributed to the redistribution of auxin in roots [17••,48–50]. *siz1* mutations caused Pi starvation hypersensitivity for root architecture remodeling [17••]. *siz1* promoted auxin remobilization from primary root tips into lateral roots and primordia in response to Pi starvation based on monitoring of the auxin responsive reporter *DR5::GUS* (K Miura *et al.*, unpublished). Furthermore, application of brefeldin A and naphthylphthalamic acid, inhibitors of vesicle trafficking and auxin efflux, respectively, inhibited Pi starvation induced hyper-responsive root architecture responses caused by the *siz1* mutations (K Miura *et al.*, unpublished). These results suggest that AtSIZ1-mediated sumoylation may regulate auxin signaling and/or auxin transport in response to Pi starvation.

AtSIZ1 functions in salicylic acid (SA) signaling in both plant innate immunity and development [51••,52]. *siz1* mutations caused hyperaccumulation of SA that is associated with dwarfism and *NahG* expression in *siz1* plants suppressed dwarfism and SA accumulation. It is not clear if sumoylation regulates SA synthesis and/or catabolism.

### Phytopathogen infection and pathogen defense

Sumoylation was first implicated in phytopathogen infection based on observations that the bacterial pathogen *Xanthomonas campestris* introduces virulence factors, AvrXv4, AvrBsT, and XopD, into plant cells that are SUMO proteases [53–55]. Presumably, these proteases inhibit SUMO-substrate conjugation or enhance deconjugation that compromises the host defense system [56]. *Xanthomonas* XopD can specifically hydrolyze tomato and *Arabidopsis* precursor SUMOs and SUMO substrates but not mammalian or yeast SUMO, indicating that the pathogen proteases has a high degree of specificity for plant proteins [54,57•]. *Ralstonia solanacearum* effector protein PopP2, which shows similarity with AvrXv4 and AvrBsT, physically interacts with the *Arabidopsis* TIR-NBS-LRR type R protein RRS1 in a yeast two-hybrid assay [58], inferring that plant R proteins may be targets for desumoylation.

Current evidence provides foundation that SUMO conjugation directly regulates innate defensive responses of plants [51••,52]. Plants harboring *siz1* mutations constitutively accumulated SA but not JA-induced defensive proteins and exhibited tolerance to biotrophic but not necrotrophic fungal pathogens [51••]. AtSIZ1 negatively regulates SA-mediated defense signaling through PAD4 that facilitates PR protein expression and basal immunity [51••].

### Control of flowering

Mutations to *AtESD4* or *AtSIZ1* caused early short-day flowering that is associated with down-regulation of the floral suppressor *FLC* [24,59] (JB Jin *et al.*, unpublished). That is, AtSIZ1 and AtESD4 both are negative regulators of flowering even though these proteins facilitate sumoy-

lation and desumoylation, respectively [24] (JB Jin *et al.*, unpublished). Genetic analyses revealed that *AtESD4* functions either downstream or in parallel with the autonomous flowering pathway [59], whereas *AtSIZ1* functions up-stream of an autonomous pathway gene *FLD* (JB Jin *et al.*, unpublished). SIZ1-mediated sumoylation of FLD represses its activity to deacetylate histones in the *FLC* chromatin and repress expression (JB Jin *et al.*, unpublished). These data indicate that sumoylation/desumoylation determinants are involved in the control of flowering. Moreover, *NUA*, a regulator of ULP1, also negatively regulates flowering [41].

## Conclusions

While still in an early stage of discovery, research is revealing that sumoylation/desumoylation functions to regulate several important biological processes in plants as it does in other organisms. Several signaling pathways are regulated but questions remain to be answered about how sumoylation is regulated, and how the post-translational process functions to integrate signal pathway networks. Another focal question is the extent to which SUMO modification, and the biochemical determinants that mediate the process, are involved in epigenetic control of gene expression, and what are the mechanisms that are involved. In the near term, research will likely identify the regulators of sumoylation/desumoylation and the regulons that are controlled.

## Acknowledgements

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