Small ubiquitin-like modifier (SUMO) is a member of the superfamily of ubiquitin-like polypeptides that become covalently attached to various intracellular target proteins as a way to alter their function, location, and/or half-life. Here we show that the SUMO conjugation system operates in plants through a characterization of the Arabidopsis SUMO pathway. An eight-gene family encoding the SUMO tag was discovered as were genes encoding the various enzymes required for SUMO processing, ligation, and release. A diverse array of conjugates could be detected, some of which appear to be SUMO isoform-specific. The levels of SUMO1 and -2 conjugates but not SUMO3 conjugates increased substantially following exposure of seedlings to stress conditions, including heat shock, H$_2$O$_2$, ethanol, and the amino acid analog canavanine. The heat-induced accumulation could be detected within 2 min from the start of a temperature upshift, suggesting that SUMO1/2 conjugation is one of the early plant responses to heat stress. Overexpression of SUMO2 enhanced both the steady state levels of SUMO2 conjugates under normal growth conditions and the subsequent heat shock-induced accumulation. This accumulation was dampened in an Arabidopsis line engineered for increased thermostolerance by overexpressing the cytosolic isoform of the HSP70 chaperonin. Taken together, the SUMO conjugation system appears to be a complex and functionally heterogeneous pathway for protein modification in plants with initial data indicating that one important function may be in stress protection and/or repair.

Post-translational modifications of proteins play a critical role in most cellular processes through their unique ability to alter rapidly and reversibly the functions of preexisting proteins, multiprotein complexes, and intracellular structures. Although originally thought to be restricted to small molecules like phosphate and sugars, emerging data now show that several distinct types of polypeptide tags are important modifiers as well (1–4). These polypeptides become covalently attached to various intracellular targets via mechanistically similar ATP-dependent reaction cascades involving activation (E1s)$^1$ and conjugation (E2s) enzymes. Sometimes an additional enzyme (E3s) also participates in target recognition and ligation. Ultimately, a protein conjugate is formed bearing the tag linked via an isopeptide bond between its C-terminal glycine and free lysyl ε-amino groups within the target. Depending on the tag and/or the target protein, the function, location, and/or half-life of the target can be affected. A family of tag-specific proteases also participate in each of the pathways. These proteases help generate the active form of the tag by removing extra residues that cap the C-terminal glycine of the polypeptide and/or are used to disassemble conjugates by cleaving the isopeptide bond between the tag and the target, thus releasing each in an unmodified form.

The most pervasive and best understood tag is the 76-amino acid polypeptide ubiquitin (Ub) (5, 6). Its main function is to become attached post-translationally to proteins destined for degradation. In most cases, a poly-Ub chain is assembled on the target via reiterated rounds of conjugation. These poly-ubiquitin intermediates are then recognized by the 26 S proteasome, a protease complex with broad specificity that degrades the target with the concomitant release of the Ub moieties undigested. In other cases, a single Ub is added. These mono-ubiquitinated intermediates help affect vesicular trafficking and shuttle short lived membrane proteins to the lysosome/vacuole where they are degraded by resident proteases (7).

More recently, a family of polypeptides distantly related to Ub called small ubiquitin-like modifiers (SUMOs; also known as sentrin, Smt3, ULP, and PIC1) has emerged as a second influential modifier (2–4). These —100-amino acid tags are only 8–15% identical to Ub but fold into a similar globular domain engineered for increased thermostolerance by overexpressing the cytosolic isoform of the HSP70 chaperonin. Taken together, the SUMO conjugation system appears to be a complex and functionally heterogeneous pathway for protein modification in plants with initial data indicating that one important function may be in stress protection and/or repair.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank$^TM$/EBI Data Bank with accession number(s) AF510519–AF510626.

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The abbreviations used are: E1, SUMO-activating enzyme; E2, SUMO-conjugating enzyme; E3, SUMO ligase; Ub, ubiquitin; SUMO, small ubiquitin-like modifier; EST, expressed sequence tag; RT, reverse transcribed; HSP, heat shock protein; HSC, heat shock cytosolic protein; HSF, heat shock transcription factor.
Arabidopsis SUMO Conjugation Pathway

**Fig. 1. Description of the SUMO modification pathway.** The pathway begins with processing of the SUMO precursors by ULPs to release the mature forms bearing an exposed C-terminal glycine. The E1 heterodimer SAE1/SAE2 activates the SUMO in an ATP-dependent reaction, resulting in binding of the SUMO moiety through a high energy thioester linkage to a cysteine (C) in the E1. The activated SUMO is then transferred to the active-site cysteine in the E2 SCE1 by transetherification and finally attached to the target protein with the help of E3s like SIZ1. The conjugate is connected by an isopeptide bond between the C-terminal glycine of SUMO and a lysine (K) in the target. ULPs can also release the bound SUMO thus generating both proteins in free unmodified forms.

**EXPERIMENTAL PROCEDURES**

Identification and Analyses of SUMO Pathway Genes in Arabidopsis—Arabidopsis genes encoding SUMOs (SUM1s), SUMO-activating enzyme (E1: SAE1 and -2), SUMO-conjugating enzyme (E2: SCE), SUMO ligase (E3: SIZ1), and SUMO proteases (ULPs) were identified by Blast searches (27) of the A. thaliana DNA database (www.arabidopsis.org). Primary queries were human SUMO-1 (GenBank™ accession number X96585 (28)), SAE1 and SAE2 (GenBank™ accession numbers AF110956 and AF110957 (29)), S. cerevisiae UBC9 (GenBank™ accession number X82538 (11)), SIZ1 (GenBank™ accession number NP_010897 (30)), Smt4 (GenBank™ accession number Q09969 (31)), and ULP1 (GenBank™ accession number Q02724 (32)). Gene structures for SUM1-3, SAE1a-b, SAE2, SCE1a-b, and SIZ1 were determined by comparison of chromosomal DNA sequences with those obtained from full-length cDNAs provided from the expressed sequence tag (EST) database or by reverse-transcribed (RT)-PCR. Sequences not yet described were deposited in the GenBank™ database under accession numbers AF510519 (SUM1), AF510520 (SUM2), AF510521 (SUM3), AF510522 (SUM4), AF510523 (SAE1a), AF510524 (SAE1b), and AF510525 (SAE2). The structures of the remaining loci were predicted from partial cDNAs (when available) and comparisons with Arabidopsis, yeast, and animal relatives.

Sequences were analyzed using the ClustalW algorithm (www2.ebi.ac.uk/clustalw). Multiple sequence alignments and neighbor joining analyses were displayed using SeqVu 1.0 (The Garvan Institute of Medical Research, Sydney, Australia) and the NJplot programs (mgouy@bioserv.univ-lyon1.fr). Multiple protein domains were identified by the Smart prediction program (smart.embl-heidelberg.de), and sequence homology was detected by Block Maker (blocks.f cere.org/blocks/blockname/make_blocks.html).

**Plant Growth Conditions and Stress Treatments**—A. thaliana ecotype Col-0 tissues were obtained from plants grown on soil at 22 °C in continuous light. For the stress treatments, plants were grown at 24 °C in liquid Gamborg's B5 medium (Invitrogen) in continuous light with shaking (100 rpm) after a 4 °C stratification for 2 days. To induce temperature stress, cultures were transferred to shaking water baths preset to the designated temperatures. For chemical treatments, compounds were added directly into the liquid culture medium.

**Immunological Analyses**—Antibodies were raised against full-length recombinant Arabidopsis SUMO1 and -3. The full-length SUM1 and SUM3 cDNAs were generated by PCR using either the cDNA SUM1 or a RT-PCR product from total RNA isolated from A. thaliana Col-0 (SUM3) as the templates. The 5' and 3' primers were designed to introduce NdeI and XhoI sites or NdeI and SmaI sites at the predicted start and stop codons, respectively. The SUM1 cDNA was inserted into NdeI/XhoI-digested pET28b (Novagen, Madison, WI) and mobilized into Escherichia coli strain BL21 (DE3). Following a 3-h induction at 37 °C with 0.5 mM isopropyl-1-thio-galactoside (IPTG), an extract was heated to 90 °C for 30 min and clarified, and the supernatant was subjected to Sephadex G-75 column chromatography (Amersham Biosciences). The SUMO-containing eluate, which represented a nearly homogeneous preparation as determined by SDS-PAGE, was

are synthesized as inactive precursors that require processing by specific protease(s) to expose this glycine. SUMOs then employ an E1 → E2 → E3 conjugation scheme similar to ubiquitination (Fig. 1) but appear to impart unique non-proteolytic functions to the target (2–4). Although most targets are modified by a single SUMO moiety, others can bear chains of SUMOs polymerized by concatenation of the individual SUMOs through a specific lysine in each unit (9).

By analysis of mutation mutants and the identification of specific targets, it has become apparent that SUMOs play an influential role in various cellular processes in yeast and animals by regulating protein-protein interactions and subcellular location or by antagonizing ubiquitination (2–4). For example, both SUMO and all the enzymes in the ligation pathway are essential in yeast (Saccharomyces cerevisiae), and the corresponding temperature-sensitive mutants arrest at the G 2/M transition, implicating a critical role for SUMO in the cell cycle transition, implicating a critical role for SUMO in the cell cycle progression and the heat shock transcription factors (HSF)-1 and -2 (15–17), which SUMO is conjugated, activating the Ran GTPase, a regulator of nuclear protein translocation, or by antagonizing ubiquitination (2–4).

In some cases sumolation localizes the proteins to nuclear port (14). A number of DNA-binding proteins are SUMO targets, including the heat shock transcription factors (HSF)-1 and -2 (15–17), which SUMO is conjugated, activating the Ran GTPase, a regulator of nuclear protein translocation or by antagonizing ubiquitination (2–4).

In this study, we report the identification of many of the core components, including eight genes encoding the full-length SUMO tag and the predicted E1, E2, and E3, and SUMO proteases required for SUMO processing, ligation, and conjugate disassembly. Immunoblot analysis with isoform-specific antibodies against SUMO1/2 and -3 detected different profiles of conjugates in crude Arabidopsis extracts, indicating that the individual SUMOs have distinct targets. The array of SUMO1/2 conjugates are substantially increased in planta by exposing seedlings to heat shock, H 2O 2, ethanol, and canavanine, with the kinetics of accumulation suggesting that sumolation by SUMO1/2 has an early role in the plant stress response.

Inducing xylanase from the fungus Trichoderma viride. This xylanase is a strong elicitor of the rapid defense response in tomato. Orth et al. (26) identified AvrBsT in the plant pathogen Xanthomonas campestris as a potential SUMO protease. AvrBsT enters its hosts via a type III secretion system where it then interferes with the host defense response, possibly by de-sumolating a key defense regulator.

To understand further the roles of sumolation in plants, we have begun to characterize the SUMO pathway in Arabidopsis thaliana (1). Here we report the identification of many of the core components, including eight genes encoding the full-length SUMO tag and the predicted E1, E2, and E3, and SUMO proteases required for SUMO processing, ligation, and conjugate disassembly. Immunoblot analysis with isoform-specific antibodies against SUMO1/2 and -3 detected different profiles of conjugates in crude Arabidopsis extracts, indicating that the individual SUMOs have distinct targets. The array of SUMO1/2 conjugates are substantially increased in planta by exposing seedlings to heat shock, H 2O 2, ethanol, and canavanine, with the kinetics of accumulation suggesting that sumolation by SUMO1/2 has an early role in the plant stress response.
injected directly into rabbits. For SUMO3, the RT-PCR product was cloned into pGEM-<sup>®</sup>-<sup>T</sup> Easy vector (Promega, Madison, WI), released as a NdeI 18681 fragment, and then introduced into the pTBY2 vector linearized with SmaI and NdeI for expression in E. coli as an intein/chitin-binding protein fusion (IMPACT I, New England Biolabs). Following a 3-h induction at 37 °C, the BL21(DE3) cells were lysed, and total soluble protein was applied to a chitin column according to manufacturer’s recommendations (New England Biolabs). The bound protein was treated with 30 mM dithioerythritol for 12 h at 4 °C. The intein cleavage product was eluted, heated for 30 min at 90 °C, and separated from precipitated proteins by centrifugation. The supernatant was applied to a Sephadex G-75 column; the SUMO3-containing fractions were used directly as the antigen.

Anti-SUMO1 antibodies were affinity-purified from serum by adsorption to SUMO1 protein coupled to Affi-Gel 10 beads (Bio-Rad). Anti-SUMO3 antibodies were purified by protein A chromatography (Sigma). The anti-Ub antibody, anti-PBAI antiseraum, and anti-HPPI01 antiseraum were described previously (33, 34).

Immunoblot analyses were performed as described (33), using proteins subjected to SDS-PAGE and transferred to nitrocellulose membranes (Hybond-C Extra, Amersham Biosciences). The membranes were blocked using 10% milk powder and probed with antibodies diluted in phosphate-buffered saline containing 1% milk powder. Detection employed either alkaline phosphatase- or peroxidase-labeled goat anti-rabbit immunoglobulins (Kirkegaard & Perry Laboratories, Gaithersburg, MD) in conjunction with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate or Super Signal chemiluminescence (Pierce) and X-Omat autoradiographic film (Eastman Kodak), respectively.

Immunolocalization was performed as described using thin sections of developing Arabidopsis embryos (35). Sections were probed with preimmune antiseraum or anti-SUMO1 antibodies followed by fluorescein isothiocyanate-labeled goat antibodies against rabbit IgGs (Jackson ImmunoResearch Laboratories, West Grove, PA) and visualized by fluorescence confocal microscopy. DNA was detected by staining the sections with the TO-PRO 3 (Molecular Probes, Eugene, OR).

In Vitro Conjugation Reactions—Purified recombinant SUMO1 was 32P-labeled using protein kinase A (Sigma) as described (36) and separated from unincorporated 32P by filtration on a NAP10 column (Amersham Biosciences). Plant extracts were prepared by homogenizing pulverized frozen tissue in 50 mM Tris-HCl (pH 8.0), 1 mM Na<sub>4</sub>EDTA, and 0.1% Triton X-100. 20 μg of each extract was digested with 50 μl of 500 U of plant SUMO protease (Sigma) in the presence of ATP and ATP-regenerating system (37). After 1-h incubation at 25 °C, the reactions were assayed by SDS-PAGE and autoradiography.

Identification of SUMO Genes—The full-length cDNA sequences were amplified from cDNA libraries (Fig. 2A) and sequenced directly from the inserts of the phagemid DNA inserts. Our initial search identified three SUMO genes (Fig. 2A) and one EST encoding a SUMO-like protein (Fig. 2B). Of these, two were confirmed as genuine SUMO genes by sequence analysis and expression in yeast, thereby confirming the Arabidopsis SUMO family.

| Protein activity | Gene | Chromosome | AGI no. | Expression ESTs<br> |
|------------------|------|------------|----------|----------------------|
| SUMO             | AtSUM1 | 4 | At5g268840 | Yes (12) |
| SUMO             | AtSUM2 | 5 | At5g55160 | Yes (9) |
| SUMO             | AtSUM3 | 5 | At5g55170 | Yes (0) |
| SUMO             | AtSUM4 | 5 | At5g48170 | No |
| SUMO             | AtSUM5 | 2 | At2g32760 | Yes (3) |
| SUMO             | AtSUM6 | 5 | At5g18700 | No |
| SUMO             | AtSUM7 | 4 | NA | No |
| SUMO             | AtSUM8 | 4 | NA | No |
| SUMO             | AtSUM9 | 4 | NA | No |
| E1               | AsSAE1a | 4 | At4g24940 | Yes (6) |
| E1               | AsSAE1b | 5 | At5g50580 | Yes (13) |
| E1               | AsSAE2 | 2 | At2g21470 | Yes (4) |
| E2               | AsSCE1a | 3 | At3g57870 | Yes (9) |
| E2               | AsSCE1b | 5 | At5g02240 | No |
| E3               | AsSIZI | 5 | At5g60420 | Yes (9) |
| SUMO protease    | AtULP1a | 3 | At3g06910 | Yes (1) |
| SUMO protease    | AtULP1b | 4 | At4g00690 | No |
| SUMO protease    | AtULP1c | 1 | At1g10570 | Yes (1) |
| SUMO protease    | AtULP1d | 1 | At1g60220 | Yes (4) |
| SUMO protease    | AtULP2a | 2 | At2g07400 | NT |
| SUMO protease    | AtULP2b | 2 | At2g24300 | NT |
| SUMO protease    | AtULP2c | 4 | At4g08430 | NT |
| SUMO protease    | AtULP2d | 4 | At4g01430 | NT |
| SUMO protease    | AtULP2e | 4 | At4g19310 | NT |
| SUMO protease    | AtULP2f | 5 | At5g13290 | NT |
| SUMO protease    | AtULP2g | 2 | At5g05560 | NT |
| SUMO protease    | AtULP2h | 2 | At2g16180 | NT |

a AGI, Arabidopsis Genome Initiative; NA, not yet annotated.

b Expression detected by the presence of ESTs (number shown in parentheses) in the Arabidopsis database (www.arabidopsis.org) and/or by RT-PCR of Arabidopsis total RNA.

c Annotated as part of this open reading frame.

TABLE I Components of the SUMO pathway in Arabidopsis

yeast and animal genes encoding various SUMO pathway components as queries to search for orthologs in the protein and DNA databases. Our initial search identified nine Arabidopsis SUM genes that encode all or part of the canonical SUMO sequence (Table I). The similarity of their intron/exon structures suggested that the Arabidopsis SUM family evolved recently from a common progenitor. With the exception of SUM5 and SUM9, each contains two introns interrupting the coding region at the same location (Fig. 2A). For SUM5, the first intron is absent. For SUM9, the first exon is absent; the remaining coding region contains a number of in-frame stop codons, suggesting that this locus is a pseudogene. Interestingly, six of the nine SUMO genes are arranged in tandem pairs in the genome with apparently intervening genes (Fig. 2A).

Amino acid sequence comparisons showed that the Arabidopsis SUMOs are 32–86% similar to each other but only 17–25% similar to Arabidopsis Ub and RUB1. When compared with yeast Smt3 and human SUMO-1 (9, 12), the Arabidopsis SUMO family is 31–54% similar. Phylogenetic analysis clustered the eight full-length Arabidopsis SUMO proteins into five subfamilies: SUMO1/2, SUMO3, SUMO5, SUMO4/6, and SUMO7/8 (Fig. 2B). Given that SUM4/6 and SUM7/8 genes are arranged in tandem, it is likely these pairs arose from regional duplications of the respective chromosomes. Clustal analysis failed to group any of the Arabidopsis SUMO proteins with animal and yeast versions (Fig. 2B). For example, SUMO1 is equally related to human SUMO-1, -2, and -3 (42, 42, and

Results

A Family of Genes Encode Arabidopsis SUMO—Previous studies by us and others (1, 25) indicated that plants express SUMOs and likely much of the associated conjugation/deconjugation pathway. For a more comprehensive analysis, we used

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<sup>a</sup> D.-Y. Sung and C. L. Guy, submitted for publication.
Acids are shown in reverse type involved in SUMO attachment. Other identical amino acids are boxed.

SUMO denotes the DNA sequence separating two tandemly arranged homolog sequences. The amino acid sequence similarity and sequence divergence. As with yeast and animal SUMOs, we observed that presumably represent SUMO1/2 conjugates. A similar diverse array of conjugates was also detected in other plant species, including corn, oats, pea, tomato, tobacco, and alfalfa (data not shown). Consistent with studies using yeast, animal, and tobacco cells (2, 20, 21, 25), we detected by confocal microscopic immunolocalization SUMO1/2 in the cytoplasmic and nuclear compartments of Arabidopsis cells. As shown in Fig. 4 for heart stage embryos, both punctate staining coincident with nuclear compartments was evident that could not be seen with preimmune antiserum (data not shown). Following centrifugal separations of crude extracts, conjugates could be detected by the anti-SUMO1 antibodies in the soluble and particulate fractions, indicating that both soluble and membrane-associated proteins are sumolated by SUMO1/2.

The most divergent region among SUMOs encompasses the N-terminal 14–30 amino acids just proximal to the Ub fold.

Structural analysis of rat SUMO-1 suggests that this hydrophilic region assumes a flexible solvent-exposed domain that extends from the body of the protein (8). Although its function is unknown, one possibility is that this extension imparts unique properties/functions to the individual members of the SUMO family. Consistent with this notion, we identified the consensus SUMO attachment site (VKXE) (2) within the N-terminal extensions of Arabidopsis SUMO4 and -6, in each case represented by the same VKME sequence (Fig. 2C). Its presence suggests that both SUMO4 and -6 can form poly-SUMO chains, using the lysine as the connection site (9).

We detected transcripts for four of the eight intact Arabidopsis SUM loci. SUM1, SUM2, and SUM5 cDNAs are in the Arabidopsis EST data bases (www.Arabidopsis.org/Blast), and an mRNA for SUM3 was identified by RT-PCR of total RNA from young seedlings. RNA gel blot analyses of SUM1 and SUM2 showed that both genes are expressed in light- and dark-grown seedlings and in all organs tested, indicating the corresponding proteins are present in most Arabidopsis tissues/cells (data not shown). Both the analyses of ESTs and the signal strength on RNA blots suggested that mRNA levels for SUM1 is higher than SUM2 (Table I and data not shown). We could not detect mRNAs for SUM4 and SUM6–8 either by RT-PCR or RNA gel blot analysis of total RNA isolated from 6-day-old seedlings, suggesting that they are either not expressed, expressed at levels below detection, or that their expression is confined to specific cell types or developmental stages not examined here.

The SUMO Conjugation Pathway Is Present in Arabidopsis—To confirm that the Arabidopsis SUMOs become attached to other proteins, we used immunoblot analysis with anti-SUMO1 and -3 antibodies to detect the free and conjugated forms in crude extracts. The antibodies were generated against bacterially expressed antigens containing all residues but those following the C-terminal glycine (Fig. 2C). The anti-SUMO3 antibodies showed a slight cross-reaction with SUMO1, its closest relative by phylogenic analysis (Fig. 3A). In contrast, the anti-SUMO1 antibodies appeared to be specific to the SUMO1/2 group; it easily detected SUMO1 and its closest relative SUMO2 (83% identity) expressed in planta but failed to detect SUMO3 (42% identity) (Fig. 3A and below). Although not tested, we consider it unlikely that the anti-SUMO1 and -3 antibodies recognize SUMO4–8 given their even greater phylogenetic distance from these two antigens.

When both SUMO antibodies were used to probe crude Arabidopsis extracts, an array of immunoreactive proteins was evident (Fig. 3A). The anti-SUMO1 antibody recognized an abundant species of 14 kDa that co-migrated with recombinant SUMO1; this species likely represents free SUMO1 and -2. In addition, a heterogeneous smear of high apparent molecular mass proteins, with many larger than 80 kDa, was also detected that presumably represent SUMO1/2 conjugates. A similar diverse array of conjugates was also detected in other plant species, including corn, oats, pea, tomato, tobacco, and alfalfa (data not shown). Consistent with studies using yeast, animal, and tomato cells (2, 20, 21, 25), we detected by confocal microscopic immunolocalization SUMO1/2 in the cytoplasmic and nuclear compartments of Arabidopsis cells. As shown in Fig. 4 for heart stage embryos, both punctate staining coincident with DNA staining within the nucleus and more diffuse staining of the cytoplasm were evident that could not be seen with preimmune antiserum (data not shown). Following centrifugal separations of crude extracts, conjugates could be detected by the anti-SUMO1 antibodies in the soluble and particulate fractions, indicating that both soluble and membrane-associated proteins are sumolated by SUMO1/2.
and either stained for protein (left panel) from young recombinant SUMO1, SUMO3 (rS1/rS3), and a crude protein extract Arabidopsis activity in plants. 

SUMO1, a heterogeneous profile of SUMO1 conjugates was synthesized in both extracts by an ATP-dependent reaction.

To test if we could recapitulate this sumolation in vitro, $^{32}$P-labeled SUMO1 was added to crude plant extracts under conditions that promote SUMO conjugation (+ATP). In addition to Arabidopsis, wheat germ was tested based on previous studies (38) demonstrating that it represents a rich source of SUMO activation is a heterodimer, structurally and functionally related to the single E1 (or UBA1) polypeptide required for Ub activation (Fig. 5A). The SUMO Activating Enzyme (SAE)-1 subunit corresponds to the N-terminal half of UBA1 and likewise contains a Thiamine Biosynthesis Protein F (ThiF) family domain present in members of the ThiF/Meob/ HesA family (Pfam accession number PF00899). The SAE2 subunit corresponds to the C-terminal part of UBA1. It contains a second ThiF domain with a GSGGATA extension (see Fig. 2).

Identification of Enzymes Required for SUMO Processing, Conjugation, and Disassembly—Like ubiquitination, sumolation requires a number of accessory proteins as follows: (i) for processing the SUMO precursors, (ii) for the ATP-dependent multistep enzymatic cascade to attach SUMOs to various targets, and (iii) for ultimately removing the bound SUMOs (see Fig. 1). By using yeast and animal genes as queries, we identified by Blast one or more Arabidopsis loci predicted to encode each of these required catalytic steps (Fig. 5).

In animals and yeasts, the E1 responsible for ATP-dependent SUMO activation is a heterodimer, structurally and functionally related to the single E1 (or UBA1) polypeptide required for Ub activation (Fig. 5A). The SUMO Activating Enzyme (SAE)-1 subunit corresponds to the N-terminal half of UBA1 and likewise contains a Thiamine Biosynthesis Protein F (ThiF) family domain present in members of the ThiF/Meob/HesA family (Pfam accession number PF00899). The SAE2 subunit corresponds to the C-terminal part of UBA1. It contains a second ThiF domain with a GSGGATA motif that likely participates in ATP binding. This domain is followed by a positionally conserved cysteine in the consensus KXXP/V/GCTXXXXX motif (29) that activates SUMOs by forming the ATP-dependent thiol-ester linkage with the tag. In addition, SAE2 has a UBA-C-terminal (UBACT) domain (Pfam accession number PF02134). This domain of unknown function is also present in Ub-activating enzymes and is located downstream of the active-site cysteine. Our search identified two Arabidopsis genes encoding SAE1 proteins of 81% amino acid sequence identity to each other and 28 and 32% identity to their Drosophila and humans orthologs, respectively (Fig. 5A).

The presence of ESTs and RNA gel blot analyses indicate that both SAE1a and SAE1b are expressed (Table I and data not shown). Only one gene was detected that encodes the second subunit, SAE2 (Fig. 5A). It is 28–33% identical to its animal orthologs and likewise contains the active-site cysteine, the UBA-CT domain, and a C-terminal motif (amino acids 646–650) proposed to function as a nuclear localization signal. SAE2 is expressed, but both the scarcity of ESTs and weak signals by RNA gel blot analysis suggest that its expression is low as compared with SAE1a and -b (Table I and data not shown).

Following activation, SUMOs are transferred to the SUMO-conjugating enzyme (E2), identified as UBC9 in yeast (10, 11). We identified two Arabidopsis loci encoding related proteins designated here as $\text{SUMO Conjugating Enzyme (SCE)-1a and}$
SUMO proteases specifically remove amino acids distal to the C-terminal glycine important for conjugation and thus are essential for processing SUMO precursors to generate the active polypeptides and for releasing bound SUMOs to regenerate targets and SUMOs in unmodified forms (2, 4) (Fig. 1). Two types of SUMO proteases have been described thus far in animals related to yeast Ub-Like Protease-1 (ULP1) and ULP2/Smt4 (31, 32). Both types contain a conserved 200-amino acid ULP1-Catalytic (ULP1-C) domain that surrounds a catalytic triad of histidine, aspartate, and cysteine residues (31, 32). Searches of the Arabidopsis database identified 12 genes (designated here as ULP1a–d and ULP2a–h) that encode the consensus UBP1-C domain with the His/Asp/Cys catalytic triad (Table I and Fig. 5, A and B). Phylogenic analysis of the ULP1-C domain clustered the 12 into three subfamilies with two subfamilies more related to yeast ULP1 and the third more related to yeast ULP2/Smt4 (Fig. 5B). Outside of the ULP1-C domain, the Arabidopsis ULPs bear little sequence similarity, suggesting that they attack different targets. In addition, we detected a number of ULP-like proteins with the ULP1-C signature but missing one or more of the essential amino acids that form the catalytic triad (data not shown). Whether these are functional ULPs is not yet known.

Stress-induced Accumulation of SUMO Conjugates—Given the observations that sumolation is activated by stress and that some SUMO targets are part of the stress response in animals (2, 20–23), we tested if SUMOs play a comparable role in plants. Here, we subjected young Arabidopsis seedlings grown in liquid culture to various stress conditions, and we measured the levels of free and conjugated SUMO1/2 and SUMO3 by immunoblot analysis of crude seedling extracts. Stress conditions included exposure to heat (28–47 °C) and cold shock...
SUMO1 (to SDS-PAGE and immunoblot analysis with antibodies against temperature upshift (Seedlings were collected at various time intervals after the start of the temperature shift from 24 to 37 °C, a dramatic rise in SUMO1/2 conjugates was evident as compared with PBA1, a 26 S proteasome subunit used to verify equal protein loads (33) (Figs. 6 and 7 and data not shown).

Although many stress conditions had little or no effect on the pools of free and conjugated SUMO1/2 and -3, exposure to heat, H2O2, ethanol, and canavanine induced a dramatic increase in SUMO conjugates that was specific for the SUMO1/2 isoforms (Figs. 6 and 7). For example within 30 min from the beginning of a temperature shift from 24 to 37 °C, a dramatic rise in SUMO1/2 conjugates was evident as compared with PBA1, a 26 S proteasome subunit used to verify equal protein loads (33) (Fig. 6). This increase preceded the accumulation of HSP101, a chaperone whose synthesis is rapidly up-regulated following heat shock (48), suggesting that the response is fast. From quantitations of the blots, we estimated that the levels of SUMO1/2 conjugates increased ~6-fold during the heat stress. In contrast, the levels of SUMO3 conjugates remained constant during the heat stress, whereas the levels of Ub conjugates, previously shown to accumulate during heat stress (49, 50), displayed a slower and more modest increase (Fig. 6).

Concomitant with the rise in SUMO1/2 conjugates was a significant drop in the levels of the free form raising the possibility that free SUMO1/2 became limiting during the heat stress. To examine this possibility and prove that SUMO2 is also involved in the response, we tested transgenic Arabidopsis lines overexpressing SUMO2 under the control of the 35S promoter. As can be seen in Fig. 6, these lines had increased levels of free and conjugated SUMO1/2 under non-stressed conditions. When subjected to heat shock, the levels of SUMO1/2 conjugates rose even further to levels beyond that seen for wild-type plants. Only a modest drop in free SUMO1/2 was observed, suggesting that these transgenic plants were now saturated for the free forms. Thus under acute heat stress, free SUMO1/2 and not their targets, appears to be limiting in wild-type plants. Despite such changes, the phenotype of the 35S-SUMO2 plants were indistinguishable from wild type when grown under a variety of conditions including elevated temperatures (data not shown). They also showed no change in the induction of HSP101 accumulation during heat stress, suggesting that the heat shock response was not altered as well (Fig. 6).

In a similar fashion to heat shock, exposure to 7% ethanol or increasing concentrations of H2O2 and canavanine dramatically increased the levels of SUMO1/2 conjugates (Fig. 7). The effects of ethanol and H2O2 were rapid, with SUMO1/2 conjugates rising within 10 min after their addition to the culture medium (data not shown). In contrast, the effect of canavanine was much slower, requiring hours to elicit a response. This slower response is consistent with the fact that this amino acid analog must be taken up by the plant and translationally incorporated into protein before inflicting cellular damage. Like heat shock, these stresses substantially changed the amount but not the profile of SUMO1/2 conjugates, suggesting that the same array of targets was saturated with the stress simply increasing the percentage in the modified form. The only major difference among the four stress treatments was an abundant 31-kDa conjugate that specifically appeared during a 24-h exposure to canavanine (Fig. 7). In contrast to heat shock, we did not observe a drop in free SUMO1/2 suggesting that the other stresses consumed less of the free pool when forming conjugates. Whereas the ethanol and H2O2 treatments failed to induce the expression of HSP101 within the 60 min that we observed an increase in SUMO1/2 conjugates, an increase in HSP101 levels was evident following a 24-h exposure to canavanine.

To examine the heat-stress response in more detail, we varied the duration and magnitude of the treatment and examined their effects on the accumulation of SUMO1/2 conjugates. As shown in Fig. 8 using increasing lengths of the heat shock, the response is transient with the duration dependent on pulse length. A 2-min incubation of the cultures at 37 °C induced only a mild accumulation of SUMO1/2 conjugates that subsequently returned to normal levels ~30 min later. Progressively longer incubations increased not only the amount of conjugates but also their duration such that a 120-min exposure to 37 °C generated an increased pool of SUMO1/2 conjugates that remained in the seedlings for more than 2 h after their return to 22 °C. The rise and fall of SUMO1/2 conjugates were paralleled...
A decline and reappearance of free SUMO1/2 (Fig. 8). This inverse relationship suggested that the SUMO1/2 modifier was not consumed following conjugation but was instead released from sumolated proteins after the heat shock was over. Like other heat shock responses (48, 51), the Arabidopsis seedlings required temperatures \( > 34 \) °C to induce a significant increase in SUMO1/2 conjugates with the maximal response at 40 °C (Fig. 9). The response was also remarkably rapid. We detected a rise in SUMO1/2 conjugates within 2 min after transferring the cultures to the 40 °C water bath (Fig. 9), which was even more impressive when considering the fact that the medium needed almost 8 min to reach this heat shock temperature under our experimental conditions.

Surprisingly, heat-induced sumolation appeared to adapt to the stress. Plants subjected to a first heat treatment at 37 °C showed a substantial but transient accumulation of SUMO1/2 conjugates (Fig. 10). However, only a slight increase in conjugates was evident for a second heat shock if given within 5 h of the first. This dampened response required almost 20 h to fully dissipate. A comparable effect for the HSP101 protein was not observed. Instead HSP101 accumulated following the first heat shock and remained at high levels for over 24 h with or without a second heat treatment (Fig. 10).

The rapid and temperature-dependent nature of the response along with its transient effect and adaptability implied that stress-induced sumolation by SUMO1/2 has an important regulatory role in the plant stress response. To help confirm this role, we examined the SUMO1/2 conjugate patterns in Arabidopsis lines constitutively expressing high levels of a cytosolic isoform of HSP70, designated HSC70-1. The HSC70-1 gene is expressed in most Arabidopsis tissues and is mildly up-regulated by heat shock (52). Similar to the behavior
of comparable Drosophila and mammalian mutants (53, 54), these HSC70-1-overexpressing lines are more thermotolerant, exhibiting an increased survival to an acute 44 °C heat shock.2 Young homozygous HSC70-OX seedlings contained 3–4 times more HSC70 protein as compared with wild type or a weak expresser but normal levels of total protein and a variety of control proteins such as a PBA1 (Fig. 11 and data not shown). When subjected to a 37 °C heat stress for 30 min, these overexpressing seedlings accumulated substantially less HSP101 protein, presumably caused by a lower demand for other types of chaperones. In addition, these HSP70-OX plants accumulated fewer SUMO1/2 conjugates during the heat shock and concomitantly retained more of the free forms (Fig. 11). Both the peak amount of SUMO1/2 conjugates and their duration within the plants were dampened (e.g. compare levels at the 120-min time point), suggesting that the accumulation of SUMO1/2 conjugates is physiologically relevant to thermotolerance.

**DISCUSSION**

In addition to Ub, it is now becoming clear that plants use a variety of other polypeptide tags to post-translationally modify and thus regulate numerous intracellular proteins (1, 55). Here we show that the family of SUMO proteins recently discovered in yeast and animals represents another class of influential modifiers. Through searches of the Arabidopsis genome databases, we discovered loci predicted to encode all components of the SUMO modification system, including the SUMO tag and release, are the largest collection of isozymes in Arabidopsis. The size of this group argues that de-sumolation must be an important step in SUMO function and regulation in plants, possibly by creating a reversible modification cycle akin to phosphorylation/dephosphorylation (4, 58).

**Arabidopsis SUMO Conjugation Pathway**

Of the nine Arabidopsis SUM loci, eight (SUM1–8) are predicted to encode the expected full-length proteins of 94–103 amino acids, making this family the largest known so far in a eukaryote. By comparison, yeast encodes a single SUMO (Smt3), whereas humans encode only three, SUMO1, -2, and -3 (23). Like their yeast and animal counterparts, the Arabidopsis proteins bear a long N-terminal region extending beyond the Ub fold and terminate in a 4–14 amino acid sequence that caps the C-terminal glycine essential for attachment. Two (SUMO4 and -6) have the consensus sumolation site (ProXKXE) near their N termini, suggesting they may be involved in forming polymeric SUMO chains (9). Expression studies indicate that at least four of the Arabidopsis SUM genes are transcribed, implying that a complex assortment of SUMO isoforms may exist in each cell type. In contrast, SUM9 appears to be a pseudogene. Such pseudogenes have been described in other organisms; for example, there are three predicted SUMO-1 pseudogenes in humans and two in mice (56).

The SUMO pathway biochemically resembles the more thoroughly studied Ub pathway (5, 6). Structurally, the most prominent difference between these two pathways is the heterodimeric nature of the SUMO E1 (2, 57). Like its yeast and mammalian orthologs, the Arabidopsis SUMO E1 subunits SAE1 and SAE2 show similarity to N- and C-terminal parts of the monomeric E1 in the Ub pathway (Fig. 5A). In contrast to all other organisms studied to date where both SAE subunits are encoded by a single gene, the Arabidopsis SAE1 subunit is encoded by two highly similar genes. Given that SAE1 could have a regulatory role by influencing the active site in SAE2, the use of two distinct isoforms could provide a way to modulate sumolation or selectively activate specific SUMO isoforms. Clearly, analyses of tissue-, cell cycle-, or stress-related expression patterns of the SUM and SAE1 genes and enzymatic characterizations of the resulting proteins are needed. The ULPs, presumably involved in SUMO processing and/or release, are the largest collection of isozymes in Arabidopsis. The size of this group argues that de-sumolation must be an important step in SUMO function and regulation in plants, possibly by creating a reversible modification cycle akin to phosphorylation/dephosphorylation (4, 58).

Previous studies with human SUMOs (9, 23) and our phylogenetic analysis of the Arabidopsis SUMOs suggested that several functionally distinct isoforms exist. Our immunological studies of Arabidopsis SUMO1/2 and -3 support this notion by showing that each is conjugated to a distinct set of targets. SUMO3 becomes attached to only a few proteins with a range of molecular masses with little of the free form evident. However, a substantial percentage of SUMO1/2 exists in the free form with the remaining present in a heterogeneous collection of conjugates with masses >80 kDa. Whereas the levels and distribution of SUMO3 conjugates appear unaffected by stress, the levels of SUMO1/2 conjugates are dramatically but transiently increased by various types of cellular stress, including exposure of the plants to heat shock, H2O2, ethanol, and canavanine. The increase and decrease in conjugates following heat shock are paralleled by a substantial decrease and increase in the free SUMO1/2 pool, suggesting that the heat-induced conjugation is reversible. The remarkable feature of this response is its speed, occurring within minutes of heat shock, thus implicating a conjugation cascade that can rapidly respond to stress signals.

Because several types of stress elicit the same accumulation of SUMO1/2 conjugates, it is likely that a more general outcome, such as the accumulation of denatured/damaged proteins, rather than the stimulus is the signal. A role for damaged proteins is consistent with our studies with Arabidopsis plants...
engineered to constitutively express HSC70-1, a cytosolic version of HSP70 that helps refold damaged proteins (52, 59). Similar to mammalian cells and Drosophila overexpressing Hsp70 (53, 54), these plants have increased thermotolerance presumably due to their increased capacity to repair damaged proteins that accumulate during heat stress. Here, we found that such HSC70-0X lines also have a dampened accumulation of SUMO1/2 conjugates, implying that this accumulation is triggered either directly or indirectly by damaged proteins. It is tempting to speculate that signaling pathway(s) initiated by proteins that accumulate during heat stress. Here, we found presumably due to their increased capacity to repair damaged Hsp70.

Similar to mammalian cells and Drosophila, Arabidopsis overexpression of HSP70 that helps refold damaged proteins (52, 59). The differential actions of SUMO1/2 and SUMO3 are functionally analogous to human SUMO-2/3 and SUMO-1, respectively (9, 23). Like that for Arabidopsis SUMO3, the SDS-PAGE profile of human SUMO-1 conjugates showed that little is in the free form with most conjugated to a limited set of targets whose abundance is unaffected by stress (23). One of the main targets of animal SUMO-1 is RanGAP (14). Consistent with this potential similarity, we detected an Arabidopsis SUMO3 conjugate of near 80 kDa that matches the size of Arabidopsis RanGAP plus one SUMO3. However, it should be noted that the recent cloning of plant RanGAP genes revealed that the encoded proteins are missing the consensus sumolation domain found in their animal counterparts (61). In contrast, the SDS-PAGE profile of human SUMO-2/3 conjugates, like that for Arabidopsis SUMO1/2, showed that a substantial percentage is in the free form with the rest present in a heterogeneous array of conjugates. The levels of these conjugates are dramatically increased by various stresses, including exposure of the cell cultures to heat shock, ethanol, H2O2, N-ethylmaleimide, osmotic stress, and DNA-damaging agents such as UV light and cisplatin (22, 23). The only notable difference between Arabidopsis SUMO1/2 and human SUMO-2/3 is the absence of a consensus sumolation site in the Arabidopsis forms.

The differential actions of SUMO1/2 and -3 raise the possibility that the other Arabidopsis SUMOs also have unique functions. Based on phylogenetic clustering predictions alone, this plant has five distinct SUMO types (SUMO1/2, -3, -4/6, -5, and -7/8 (Fig. 2B)) that each could become attached to a different set of targets. For the clade comprising SUMO4 and -6, one obvious possibility based on the presence of the consensus sumolation site (ΨKXE) is that this type participates in forming poly-SUMO chains. As a result sumolation could have a number of diverse roles in plant cell regulation beyond that seen here for SUMO1/2 and -3. How would each of these SUMO isoforms become preferentially attached to their respective targets? To date, we identified two types of E1s (based on use of the SAE1b and -b isoforms), only one functional E2 (SCE1), and only one E3 (SIZ1). Given that this small number seems insufficient to attach selectively the various SUMO isoforms, it is likely that additional activities are necessary, especially with respect to the E3 activities required for ligation. One additional candidate for an E3 could be an Arabidopsis ortholog of RanBP2 which appears to be a SUMO-1 ligase in mammalian cells (57).

Why are Arabidopsis SUMO1/2 conjugates and their potential counterparts in animals increased upon stress? The transient nature of the increase in Arabidopsis and the fact that the free SUMO1/2 pool recovers after the stress imply that SUMO1/2 is in a dynamic equilibrium between the free and conjugated forms and that the SUMO moiety is not consumed during the cycle. That the levels of conjugates under both non-stressed and stressed conditions can be elevated by over-expressing SUMO2 indicates that the pool size of free SUMO1/2 is a major limitation in forming these conjugates. An intriguing aspect of this heat-induced sumolation is our observation that the plants adapt transiently to the stress. Whereas the response is robust initially, subsequent heat shocks are less effective if given shortly after the first, despite an apparently adequate supply of free SUMO1/2. This damping implies that either the SUMO conjugation system is attenuated and/or that the availability/abundance of targets is substantially reduced following the first heat stress. Under our experimental conditions, almost 20 h were needed to fully reactivate the response and/or replenish the substrates.

One possibility is that SUMO1/2 conjugation plays a regulatory role in the stress response. Here attachment of SUMO1/2 could alter the activity or location of critical effector(s) of the stress response. Consistent with this possibility are the facts that a majority of Arabidopsis SUMO1/2 (Fig. 4) and the heat up-regulated SUMO conjugates in mammalian cells (22) are nuclear localized and that a number of nuclear DNA-binding proteins in mammalian cells such as p53, c-Jun, c-Myb, AP-2, and the androgen receptor are SUMO targets (15–19). In several cases this sumolation appears to alter the association of these factors with cognate DNA-binding sites, either positively or negatively depending on the target. Two notable SUMO targets are HSF1 and -2, which are responsible for up-regulating many HSP genes during heat shock and other stresses (20, 21).

Importantly, sumolation of these proteins increases their affinity for heat shock DNA elements. Whereas HSF2 is constitutively sumolated, the level of HSF1-SUMO conjugates is dramatically up-regulated by heat shock supporting this modification as an activator of HSF1 function (20, 21).

In a similar fashion, it is possible that the SUMO1/2 conjugation in Arabidopsis helps activate (deactivate) a battery of nuclear regulatory proteins whose activities are needed at low (high) levels in non-stress plants and at high (low) levels when the plants are exposed to various stress signals. Potential targets under negative regulation could include a battery of factors that promote cell division and other general physiological processes that should be repressed as plants cope with adverse environments. Potential targets under positive regulation could include the 21 HSF-like proteins present in the Arabidopsis genome (www.uni-frankfurt.de/fb15/botanik/AFGGnvr.html) whose activation promotes the stress response (46). Consistent with this notion, we showed here that overexpression of HSC70, which should lessen demand for the targets of HSFs, reduces the heat-induced accumulation of SUMO1/2 conjugates. Such a regulatory role is also supported by the observations that plant defense responses to pathogens may involve SUMO conjugation (25, 26). In particular, one can imagine that AvrBsT from the plant pathogen X. campestris, which may act as a SUMO protease (26), interferes with the stress response that attenuates pathogen invasion by de-sumolating and thus deactivating an important stress regulatory factor. For other targets in animals, sumolation appears to control cytoplasmic/nuclear partitioning and location within the nucleus (2–4). For example, sumolation of RanGAP helps control cytoplasmic/nuclear partitioning and location within the nucleus (2–4). For example, sumolation of RanGAP helps control cytoplasmic/nuclear partitioning and location within the nucleus (2–4).
to the antagonistic role of sumolation in Ub-mediated IκBα degradation (24), heat-induced sumolation could block the turnover of proteins by masking their Ub attachment sites. These targets could be regulatory proteins whose stabilization is needed to elicit a robust stress response or could be unfolded proteins destined for repair after relief from the stress condition. Alternatively, SUMO once attached could by itself protect native proteins from unfolding or stabilize unfolded proteins from aggregation or further denaturation and thus work cooperatively with proteins like HSC70. Such a mass action is supported by our observations that the level of SUMO1/2 and not the targets appear to be limiting during heat shock. Given the dynamic nature of the SUMO tag, it is also possible that SUMO1/2 actually promotes degradation. Here, SUMO1/2 conjugation could increase the solubility of denatured proteins formed during stress and thus enhance their removal by proteolytic pathways such as the Ub26-S proteasome system. Whatever their role, an increase in SUMO1/2 conjugates is by itself insufficient to enhance stress tolerance in Arabidopsis.

Despite the fact that transgenic plants overexpressing SUMO2 had increased levels of free SUMO2 and SUMO1/2 conjugates, they exhibited the same sensitivity to heat shock as wild-type plants by several diagnostic parameters (51), including reduced hypocotyl growth and cotyledon expansion and increased chlorosis (data not shown).

Collectively our data point to sumolation as an important post-translational modification in plants that may have a number of roles created by the use of distinct SUMO isoforms. In Arabidopsis, it is clear that an array of proteins become modified by this conjugation system. The rapid and reversible formation of SUMO1/2 conjugations following exposure of seedlings to stress points to sumolation as an important regulator of the stress response. Determining how these stresses activate the conjugation cascade and identifying the various targets will be paramount to defining how sumolation ultimately participates in stress protection and/or recovery.

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REFERENCES
1. Vierstra, R. D., and Callis, J. (1999) Plant Mol. Biol. 41, 435–442
2. Melchior, F. (2000) Annu. Rev. Cell Dev. Biol. 16, 591–626
3. Hay, R. T. (2001) J. Cell. Physiol. 190, 151–178
4. Pickart, C. M. (2001) Mol. Cell 7, 1257–1266
5. Becher, M., and Becker, J. (1998) J. Mol. Biol. 280, 275–286
6. Noller, H. F., Scharf, K. D., Galiardi, D., Vergne, P., Czarnecka-Verner, E., and Garbaczy, O.-K. (2001) J. Biol. Chem. 276, 1857–1844
7. Schaller, M. K. (1999) Cell Stress Chaperones 4, 1–12
8. Arndt, T., Baier, S., and Mayor, P. (2000) J. Biol. Chem. 275, 1899–1909