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REVIEW

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SUMO conjugation in plants

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Abstract Covalent attachment of small proteins to substrates can regulate protein activity in eukaryotes. SUMO, the *s*mall *u*biquitin-related *mo*difier, can be covalently linked to a broad spectrum of substrates. An understanding of SUMO's role in plant biology is still in its infancy. In this review, we briefly summarize the enzymology of SUMO conjugation (sumoylation), and the current knowledge of SUMO modification in *Arabidopsis thaliana* (L.) Heynh. and other plants, in comparison to animals and fungi. Furthermore, we assemble a list of potential pathway components in the genome of *A. thaliana* that have either been functionally defined, or are suggested by similarity to pathway components from other organisms.

Keywords *Arabidopsis* · Protein modification · Stress response · SUMO · Transcriptional regulation · Ubiquitin

Abbreviations SAE: SUMO-activating enzyme · SCE: SUMO-conjugating enzyme · SUMO: Small ubiquitin-related modifier

Introduction

SUMO proteins are "*s*mall *u*biquitin-related *mo*difiers" and are approximately 100 amino acids in length. Alternative names for SUMO are Sentrin and Smt3, and, in earlier publications, UBL1, PIC1, GMP1 or SMT3C (for reviews on SUMO in animals and fungi, see Müller et al. 2001, 2004; Gill 2003; Melchior et al. 2003; Seeler and Dejean 2003; Verger et al. 2003; Johnson 2004). SUMOs have limited sequence similarity to ubiquitin and adopt a ubiquitin-like fold. The characteristic amino-terminal extension is variable in sequence and conformationally flexible and, like the somewhat shorter carboxyl terminus, extends from the compact core (Bayer et al. 1998). Similar to ubiquitin, SUMO is a protein modifier that can be covalently linked to other proteins by a set of enzymes specifically devoted to this task (Fig. 1). Sumoylation occurs in the nucleus and in the cytoplasm. The reaction has been associated, among others, with stress response, transcriptional regulation, and genome maintenance functions. In the following paragraphs, we try to relate insights obtained in fungi or animals to the current state of knowledge in plants.

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SUMO

Arabidopsis thaliana has nine genes with significant similarity to animal and fungal SUMO proteins (Table 1; Kurepa et al. 2003). One of them, SUM9, is a pseudogene and does not encode a complete protein. The SUMO gene family is potentially derived from genome rearrangements. For instance, SUM2 and SUM3, as well as SUM4 and SUM6, are closely linked and are listed as examples of tandem duplication (http://www.tigr.org/tdb/e2k1/ath1/TandemDups/duplication_listing.html). The same probably holds true for SUM7 and SUM8. SUM1, on the other hand, is part of a segmental genome duplication between chromosomes 4 and 5, with SUM2/SUM3 present at the equivalent position of chromosome 5 (http://www.tigr.org/tdb/e2k1/ath1/duplication_listing.html). Sequence comparison shows that SUM1/SUM2, SUM4/SUM6, and SUM7/SUM8, respectively, are very similar to each other (Fig. 2). SUM5 is sequentially most distinct from all other *Arabidopsis* SUMO proteins. ESTs (expression sequence tags) exist for SUM1, SUM2, SUM3 and SUM5, providing evidence for expression in vivo. The

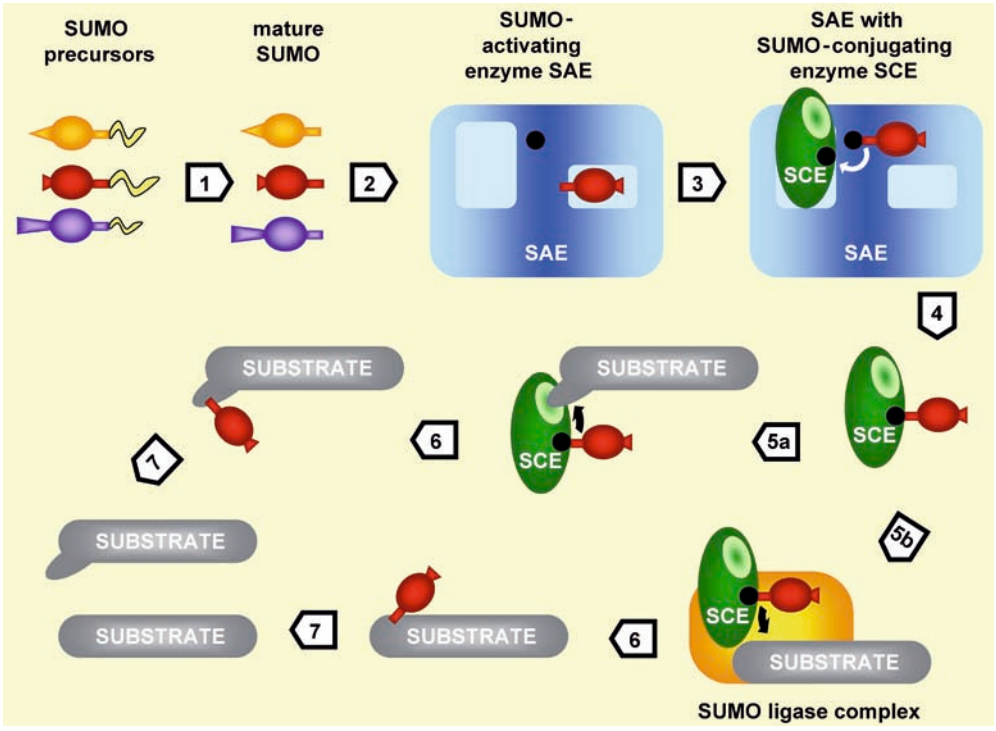

Fig. 1 The sumoylation cycle. Multiple isoforms of SUMO exist in plants. All are encoded as precursors that need to be cleaved close to their carboxyl terminus by SUMO-specific proteases (*step 1*). Mature SUMO is activated by SUMO-activating enzyme (*SAE*), a heterodimer that has two large cavities (*light blue boxes*). One of the cavities can bind SUMO for activation (*step 2*). The carboxyl-terminal Gly of mature SUMO is activated by linkage to ATP, forming an AMP–SUMO intermediate. The SUMO carboxyl terminus is subsequently coupled to a Cys residue of SAE (symbolized by a *black dot*) in a thioester linkage (*step 3*). The second cavity of SAE can hold SUMO-conjugating enzyme (*SCE*). SUMO is transferred to the active-site Cys residue of SCE, which dissociates from the complex (*step 4*). SCE can directly bind to substrates that contain a sumoylation consensus sequence (ΨKXE/D) in an accessible position (*step 5a*; so far, this sequence of events is mainly supported by in vitro data). Alternatively, SUMO protein ligases form a ternary complex with SCE and substrate, to catalyze sumoylation of substrate proteins at ϵ-amino groups of internal Lys residues (*step 5b*). The sumoylated substrates are released (*step 6*). SUMO-specific proteases cleave off SUMO for re-use and restore the default state of the substrate (*step 7*)

expression levels of SUM4, SUM6, SUM7, and SUM8, if they do not represent pseudogenes, are presumably much lower. Forced expression of an intron-containing SUM7 construct allowed detection of mRNA (R.B. and A.B., unpublished). cDNA isolation indicated the formation of two splice variants, SUM7 and SUM7v. The latter has a three-amino-acid insertion (Glu-Leu-Gln) at the position of the second intron (see Fig. 2). Forced expression of SUM6 confirmed the intron–exon structure predicted by computer algorithms.

Antibodies directed against SUM1/SUM2 (Kurepa et al. 2003; Lois et al. 2003; Murtas et al. 2003), and those directed against SUM3 (Kurepa et al. 2003; Yong-Fu Fu and G.C., unpublished), indicate that these proteins form conjugates in vivo. Similarly, expression of epitope-tagged SUM5 allows detection of conjugates with this protein (R.B. and A.B., unpublished). Thus, all highly expressed SUMO forms in *Arabidopsis* are engaged in conjugation reactions. At this point, it is an open question whether or not the various isoforms have a different spectrum of substrates.

Like ubiquitin, SUMOs are encoded as precursor proteins. A short peptide extension is proteolytically removed to generate the mature forms (Fig. 1; Johnson et al. 1997). Cleavage occurs after a conserved Gly residue (position 108 in Fig. 2). Whereas most plant SUMO proteins have the same Gly-Gly motif at the cleavage site as present in animal and fungal SUMOs, the carboxyl termini of SUM4, SUM6 and SUM7 deviate at the penultimate position. SUM7 has Ala-Gly, while SUM4 and SUM6 have Ser-Gly instead. Interestingly, SUM1 fusion proteins with Ala-Ala instead of Gly-Gly at the corresponding position cannot be processed by the SUMO-specific protease ESD4 (Murtas et al. 2003). However, when expressed in *Arabidopsis*, mature SUM1 carrying an Ala-Gly at this position is still conjugated to substrates (R.B. and A.B., unpublished), indicating that the changes present in SUM4, SUM6 and SUM7 do not necessarily compromise functionality, although critical kinetic parameters of sumoylation and de-sumoylation may differ from the Gly-Gly terminal SUMO isoforms.

SUMO activation

A protein complex homologous to E1 of the ubiquitin conjugation pathway activates SUMO at the carboxyl-terminal Gly residue (Fig. 1). SUMO-activating enzyme

(SAE) consists of two proteins, one with similarity to the amino-terminal half, one to the carboxyl-terminal half of ubiquitin-activating enzyme. *Arabidopsis thaliana* contains two genes for the smaller SAE subunit, SAE1a (At4g24940) and SAE1b (At5g50580, which appears also as At5g50680 in the *Arabidopsis* genome data base, a possible annotation artefact). SAE1a and SAE1b are contained in segments that are duplicated between chromosomes 4 and 5 (http://www.tigr.org/tdb/e2k1/ath1/ath1.shtml). The larger subunit of SAE, SAE2, is represented by a single-copy gene in the *Arabidopsis* genome (At2g21470).

The available structural data for the activating enzyme of RUB1, another protein modifier, suggest a mechanism for activation that probably holds true for all protein modifiers including SUMO (Walden et al. 2003; see Fig. 1). The enzymatic steps of SUMO activation are linkage of SUMO's carboxyl-terminal Gly with ATP to form an acyl-adenylate (AMP–SUMO), and subsequent conversion of the adenylate into a thioester by linkage to a Cys residue in the enzyme. Both subunits of SAE contain an adenylation domain (formed by the two boxes designated MoeB/ThiF and MoeB in Table 1; for further explanations, see legend to Table 1). The catalytic Cys residue of SAE is located adjacent to the carboxyl-terminal end of the first MoeB box of SAE2. Another sequence with specific functional assignment lies next to the second MoeB box of SAE2 and adopts a ubiquitin-like fold (Table 1). It is supposedly involved in recruitment of SUMO-conjugating enzyme.

SUMO conjugation

After activation, SUMO is transferred from SAE to the SUMO-conjugating enzyme (SCE). Catalyzed by SCE, SUMO is finally linked to substrates (Fig. 1). While the SUMO–SCE linkage occurs via thioester to an active-site Cys residue, substrates are linked to SUMO via an isopeptide bond between the ϵ-amino group of an internal Lys residue, and the activated SUMO carboxyl terminus.

Arabidopsis thaliana has one pseudogene, and one active gene for SUMO-conjugating enzyme (SCE1 or SCE1a; At3g57870; see Table 1). The enzyme is called Ubc9 in baker's yeast *Saccharomyces cerevisiae* because of its similarity to ubiquitin-conjugating enzymes, and hus5 in fission yeast *Schizosaccharomyces pombe*. The presence of only one gene in *Arabidopsis* is interesting in light of the fact that there are eight distinct SUMO proteins. The situation therefore differs from ubiquitin conjugation, where there is one single type of modifier, but many different types of conjugating enzyme (for reviews, see Bachmair et al. 2001; Smalle and Vierstra 2004).

In animals, and by inference probably also in plants, another difference between the enzymology of ubiquitylation and sumoylation is that in vitro, and possibly also in vivo, many SUMO conjugation reactions proceed without the assistance of protein ligases. Protein ligases are defined as proteins that bind a substrate and a conjugating enzyme, to catalyze transfer of the modifier to an ϵ-amino group of a lysine residue in the substrate (see Fig. 1). In line with the in vitro data, animal SCE has been found to bind certain substrates in yeast two-hybrid assays and in other interaction tests. A consensus sequence for SUMO addition in animals and fungi has been proposed (ΨKXE/D, where Ψ is a hydrophobic aliphatic residue, X can be any residue, and K, E and D correspond to the standard one-letter symbols for amino acids; K is the attachment site for SUMO). In addition to the consensus, other properties of the substrate protein sequence appear necessary. For example, X-ray structure data of an SCE–substrate complex indicate that, in order to specifically attract sumoylation, this consensus sequence has to be positioned in a large and accessible loop (Bernier-Villamor et al. 2002).

Apart from sumoylation at consensus sites, more and more examples are found where sumoylated Lys residues are not positioned in a canonical consensus sequence. These sumoylation events are prime candidates for in vivo dependence on SUMO ligases. So far, three distinct types of SUMO ligase have been identified in animals or fungi. The SIZ group (prototype members are SIZ1 and NFI1/SIZ2 of budding yeast, and the PIAS family of animals) is similar to the major class of ubiquitin ligases in that it uses a RING-like domain for binding of the SCE–SUMO complex (Johnson and Gupta 2001; Kahyo et al. 2001). *Arabidopsis* homologs to this class are listed in Table 1. The second type, RanBP2 (Ran-binding protein 2; Pichler et al. 2002), is probably restricted to animals, because its prominent substrate RanGAP1 is apparently not sumoylated in fungi, and a similar situation may hold in plants. In particular, the SUMO acceptor domain is lacking in plant RanGAP (Rose and Meier 2001). The third type of SUMO ligase presently characterized is a member of the Polycomb family, Pc2 (Kagey et al. 2003). It is difficult to identify candidate ligases of this type in *Arabidopsis*, because a precise definition of the subdomain(s) involved in sumoylation is not yet available. Similarity of *Arabidopsis* proteins to domains common to all Polycomb members, however, may be insufficient to define functional homologs of Pc2, because most Polycomb proteins have no known SUMO ligase activity.

De-sumoylation

The active center of proteases cleaving at the SUMO carboxyl terminus has similarity to certain viral cysteine proteases (Li and Hochstrasser 1999; for a general survey of proteases, see Barrett et al. 2004, and http://merops.sanger.ac.uk; SUMO-specific proteases were assigned to the clan CE in the latter references). The prototype enzymes are Ulp1 and Ulp2 from baker's yeast (Li and Hochstrasser 1999, 2000). Animal enzymes

were called SENPs (Sentrin proteases); some of the *Arabidopsis* homologs were called AtULPs (Kurepa et al. 2003). Not all members of the SENP group are specific for SUMO. For instance, SENP8 was found to cleave at the carboxyl terminus of the small protein modifier NEDD8 (Mendoza et al. 2003; NEDD8 is called RUB1 in most organisms including *Arabidopsis*; Rao-Naik et al. 1998). In plants, the enzyme specificity is even more difficult to evaluate since *Arabidopsis* has at least 67 genes with similarity to the SUMO-specific protease domain (search with the PFAM domain PF02902, E-value < 0.21). Thus, there has been a huge expansion in this class of proteases, and it is unlikely that all of them are specific for SUMO. One of these protease genes, however, *ESD4* (*early in short days 4*; At4g15880), has been functionally characterized to encode a SUMO protease (Murtas et al. 2003). Table 1 lists seven more likely candidates, and groups five genes with yeast SUMO proteases Ulp1 or Ulp2. AtULP1c and AtULP1d (Kurepa et al. 2003) are close homologs, located in segmentally duplicated regions of chromosome 1. Subcellular localization has been identified as a critical aspect of SUMO protease function (Huang and Dasso 2002; Li and Hochstrasser 2003). The recent finding that the plant SUMO protease ESD4 specifically localizes to the nuclear periphery suggests a similar situation in plants (Murtas et al. 2003).

SUMO-domain-containing multi-domain proteins

In addition to ubiquitin-like proteins of type 1 (UBL1) such as SUMO and Rub1, which function as protein modifiers, a second group of ubiquitin-like proteins has recently been defined. UBL proteins, type 2, contain one or more UBL domains in a larger protein. Examples are elongin B, RAD23, and Bag1. While these latter proteins are neither processed at the carboxyl end of their UBL domain nor conjugated to target proteins, representatives were shown to interact with components of the ubiquitin conjugation/protein degradation pathway (for review, see Buchberger 2002).

Apparently, there is a class of SUMO-domain-containing multi-domain proteins similar to UBL2. Table 1 shows a representative from *Arabidopsis* (At1g68185) that has similarity to SUMO in the carboxyl-terminal part (detected with COG5227, E-value 7e-6 in the CDD search; Marchler-Bauer et al. 2003). By analogy to ubiquitin domain proteins, SUMO domain proteins may perform their function by interaction with components of the SUMO conjugation system.

Sumoylation in animals and in yeast

It can be expected that many features, and a considerable number of substrates, are conserved among animals, yeast and plants. A few major aspects are listed below:

Table 1 Listing and sequence characterization of *Arabidopsis thaliana* SUMO-related proteins and predicted proteins involved in SUMO conjugation. Proteins of a potential ortholog relationship in *Saccharomyces cerevisiae* and *A. thaliana* have been identified in a (BeT) approach as reciprocal best hits in these two proteomes (Tatusov et al. 2003). Further *A. thaliana* genome searches have been used to confirm the completeness of the set. The genomic map view has been derived from the NCBI Mapviewer (Wheeler et al. 2004). Domain architectures have been determined using Conserved Domain Database (CDD) queries (Wheeler et al. 2004). In the case of At4g24940 and At5g50580, borderline hits to the MoeZ_MoeB domain (PF05237) initiated further analysis. Fold prediction analysis (Gough and Chothia 2002) shows the presence of a MoeB-like adenylation domain in Aos1p/SAE1a/SAE1b as well as in Uba2p/SAE2 (prototype enzyme *Escherichia coli* MoeB catalyzes acyl-adenylate formation in molybdenum cofactor biosynthesis; Hochstrasser 2000). In both SAE subunits, the MoeB-like adenylation domain is interrupted by a highly helical insert shown as a line between the two boxes symbolizing the MoeB-like domain. The PFAM profile (ThiF PF00899) recognized solely the N-terminal part of the adenylation domain, where it overlaps with the fold prediction (for further reading, see Walden et al. 2003). The ubiquitin-like fold of SAE2 and homologs can be detected by Superfamily (Gough and Chothia 2002), and by FFAS03 (Rychlewski et al. 2000). The *asterisk* in the Chromosome locus column indicates that the database MIPS code assignment differs from the presented analysis. Abbreviations:*Chr*. Chromosome,*NA* not available. The *A. thaliana* sequence sets of the Arabidopsis Genome Initiative (AGI release: 20040228) used in this work were obtained from its website (http://www.arabidopsis.org/)

1. Sumoylation is probably essential for multicellular organisms. In baker's yeast, abolition of either SUMO conjugation by mutation in the conjugating enzyme (Seufert et al. 1995) or of de-conjugation (Li and Hochstrasser 1999) causes arrest at a specific stage of the cell cycle. The essential substrates have not yet been identified.
2. Most (but not all) SUMO substrates are nuclear proteins, or proteins that spend part of their life in the nucleus. A recurrent theme is that sumoylated forms of transcription factors are either more active as repressors or are no longer efficient activators (e.g., Bies et al. 2002; Ross et al. 2002; Sapetschnig et al. 2002; Girdwood et al. 2003). Frequently, changes in activity caused by sumoylation coincide with altered subnuclear distribution (e.g., Sachdev et al. 2001; Best et al. 2002; Rallabhandi et al. 2002).
3. Sumoylation can team up with ubiquitylation (e.g., Hoege et al. 2002; Huang et al. 2003), or with phosphorylation (Hietakangas et al. 2003), to regulate biological processes. Different modifications may operate on a substrate in an alternative, or in a sequential manner.
4. Many enzymes of DNA metabolism (histone deacetylases, topoisomerases, enzymes of DNA repair), and chromatin proteins including histones can be sumoylation substrates in vivo (e.g., Bachant et al. 2002; David et al. 2002; Rallabhandi et al. 2002; Shiio and Eisenman 2003).
5. Usually, only a small fraction of a SUMO substrate is in the sumoylated form at a given time point,

Yeast Homologs	Name	BAC locus	Chr. locus	GB accession (protein)	Genomic Map-view	Domain Architecture
SUMO						
SMT3p / Q12306	SUM1	F10M23	At4g26840	NP_194414		
	SUM2	MCO15	At5g55160	NP_200327		
	SUM3	MCO15	At5g55170	NP_200328		
	SUM4	K24G6	At5g48710	NP_199682		
	SUM5	F24L7	At2g32765	NP_565752		
	SUM6	K24G6	At5g48700	NP_199681		
	SUM7	MWJ3	At5g55855*	NA		
	SUM8	MWJ3	NA (Chr. 5)	NA		
	SUM9	F5I10	NA (Chr. 4)	NA		NA
SUMO-like domain fusion protein						
		T22E19	At1g68185	NP_564924		
SUMO-activating enzyme						
Aos1p / NP_015506	SAE1a	F13M23	At4g24940	NP_567712		
	SAE1b	MBA10/ MFB16	At5g50580/ At5g50680	NP_568741		
Uba2p / NP_010678	SAE2	f3k23	At2g21470	NP_179742		
SUMO-conjugating enzyme						
Ubc9p / NP_010219	SCE1a	T10K17	At3g57870	NP_191346		
	SCE1b	T1E22	NA (Chr. 5)	NA		NA
SUMO ligase candidates						
Nfi1p / Q12216	SIZ1	MUF9	At5g60410	NP_200849		
	PIASlike1	F7G19	At1g08910	NP_172366		
	PIASlike2	MBK23	At5g41580	NP_198973		
SUMO cleaving protease candidates						
Ulp1p / Q02724	ESD4	FCAALL	At4g15880	NP_567478		
	ULP1a	F17A9	At3g06910	NP_187347		
	ULP1b	F6N23	At4g00690	NP_191978		
Ulp2p / P40537	ULP2like1	T16L1	At4g33620	NP_195088		
	ULP2like2	F21M12	At1g09730	NP_172444		
	ULP1c	T10O24	At1g10570	NP_172527		
	ULP1d	T13D8	At1g60220	NP_176228		
	SENPlike1	F15L12	At5g60190	NP_200827		

Ubiquitin-like (PF00240) | Ubiquitin-like (sub-signif) | Ubiquitin-like (fold similarity)
ThiF (PF00899) | MoeB-similar (fold similarity) | UBACT (PF02134)
UQ_con (PF00179) | SAP (PF02037) | zf-MIZ (PF02891)
PHD (PF00628) | Peptidase_C48 (PF02902) | -- Protein extends further

suggesting that SUMO conjugation provides additional functionality required for non-standard tasks. Alternatively, the modification may be essential for the protein in question, and the substrate has to cycle between sumoylated and de-sumoylated forms for proper functioning (cf. Johnson 2004).

Functions of SUMO in plants

An understanding of sumoylation in plants is still in its infancy. Recent work has demonstrated a role for this process in stress response, pathogen

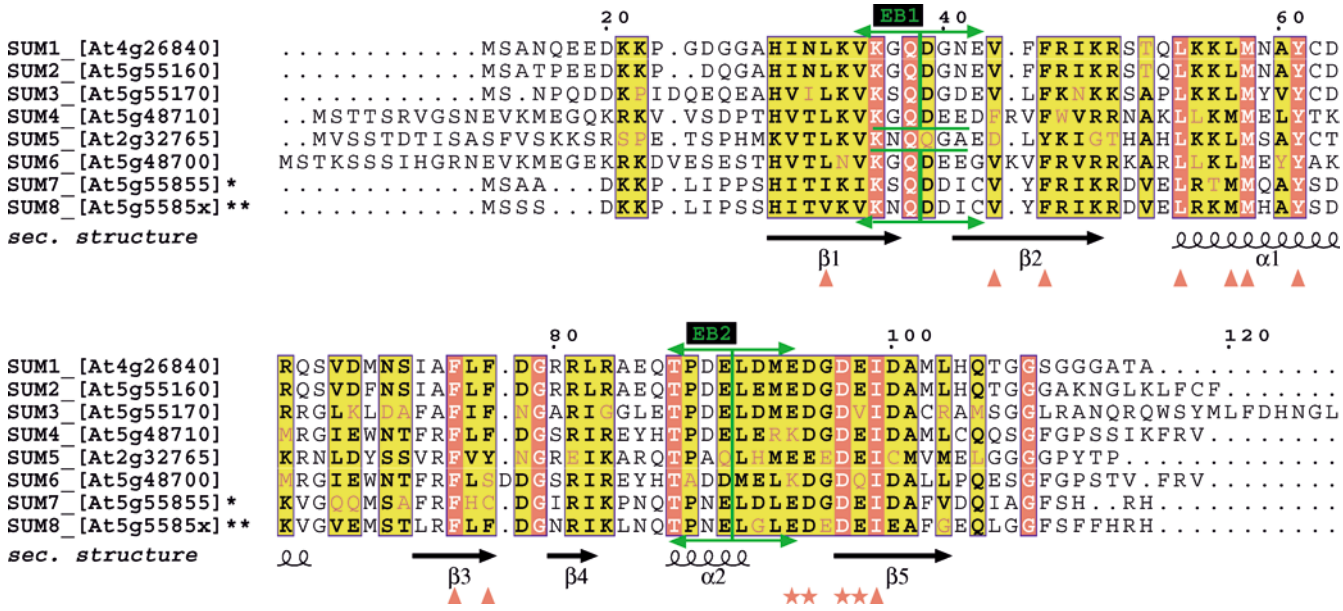

Fig. 2 Alignment of SUMO protein sequences of *Arabidopsis thaliana*. Conserved residues in the SUMO core domain have a *yellow background*; highly conserved residues have a *red background*. *EB1* and *EB2* indicate position of introns 1 and 2. *sec. structure* indicates the predicted secondary structure. *Red triangles* below the alignment indicate hydrophobic residues important for stability of the compact ubiquitin-like core of SUMO. *Asterisks* below the alignment indicate amino acid residues that form an acidic patch on the SUMO surface, a feature that distinguishes SUMO from other protein modifiers. *Dots* indicate spaces introduced to optimize alignment. As a maturation step, all SUMO proteins are predicted to be cleaved after the last conserved Gly residue at position 108. A cDNA splicing variant of SUM7 (not listed) contains the three-amino-acid insertion ELQ at position EB2. *, SUM7 differs from, but overlaps with At5g55855; **, SUM8 lies between At5g55855 and At5g55860

defense, abscisic acid (ABA) signaling, and in flower induction.

Abiotic stress response

Kurepa et al. (2003) found that the intracellular levels of SUMO conjugates rise dramatically upon heat stress, or after exposure of *Arabidopsis* cells to H_2O_2, or ethanol. A similar stress-inducibility was also found in animals (Saitoh and Hinchey 2000). Conjugate levels are restored to normal upon withdrawal of the stimulus, underscoring the reversible nature of the modification (Kurepa et al. 2003). It is not known at present whether substrates released from stress-induced sumoylation are functionally intact, or channeled into further modification pathways such as degradation.

Pathogen defense

Hanania et al. (1999) showed that a xylanase from the fungus *Trichoderma viride* binds to tomato SUMO. Upon injection into the leaf mesophyll, the xylanase induces ethylene formation and cell death in tobacco and tomato varieties. Increasing intracellular SUMO levels by sense expression, or co-injection of SUMO together with the xylanase, down-regulated ethylene production and cell death, whereas a decrease in SUMO levels by an antisense construct stimulated both ethylene production and cell death in tomato. An open question regarding these findings is whether the xylanase can enter plant cells to directly interact with the SUMO conjugation system or its substrates. If this is the case, the xylanase may target sumoylated proteins with a role in plant defense. Alternatively, manipulation of sumoylation leads to general changes in defense reactions, and the reported affinity of the xylanase to SUMO is unrelated to intracellular events.

Issues of *in planta* localization are easier to interpret in another case: a class of type-III effector proteins (i.e., proteins injected into plant cells by the bacterial type-III secretion system) of pathogenic bacteria have similarity to SUMO proteases (Orth et al. 2000; Hotson et al. 2003; Roden et al. 2004). In one case, XopD, in vitro specificity of the presumptive protease for cleavage of SUMO peptide linkages was demonstrated (Hotson et al. 2003). The protease-domain-containing fragment of XopD of *Xanthomonas campestris* pv. *vesicatoria* can cleave SUMO precursor proteins. The same fragment can also de-sumoylate SUMO–RanGAP1, a prominent substrate from animal cells with an isopeptide linkage between SUMO and RanGAP1. Furthermore, in an in vitro reaction, the XopD fragment decreases the level of SUMO conjugates present in plant extracts (Hotson et al. 2003). Similarly, *in planta* expression of the effector AvrXv4 leads to a decrease in abundance of SUMO conjugates (Roden et al. 2004). It is tempting to speculate that because pathogen proteins exist that interfere with sumoylation the latter modification plays an

essential role in defense. Future work will certainly deepen the understanding of the process.

ABA signaling

Lois et al. (2003) have shown that *Arabidopsis* plants overexpressing SUM1 are less sensitive to the inhibitory effect of ABA on root growth. Inhibition of sumoylation by co-suppression of SCE1 leads to the opposite phenotype, and root growth is inhibited more severely. Furthermore, expression of stress-induced genes RD29A and AtPLC1 is stronger in SUM1- or SUM2-overexpressing plants. Interestingly, ABA plays an important role as a ''stress hormone'', suggesting a connection to stress-induced sumoylation observed by Kurepa et al. (2003).

Flower induction

Murtas et al. (2003) elucidated that an *Arabidopsis* mutant with premature flower induction, *esd4* (*early in short days 4*), has a defect in a SUMO-specific protease. The protease localizes to the inner nuclear periphery, suggesting that most of its substrates are nuclear proteins. While it was shown by Western analysis that the *esd4* mutation increases the level of sumoylation for a number of proteins, and the mutation has a number of phenotypes in addition to early flowering (Reeves et al. 2002), ESD4 has nonetheless a very specific role in de-sumoylation. In particular, mutants deficient in other SUMO proteases of *Arabidopsis* have distinct phenotypes (Yong-Fu Fu and G.C., personal communication). An open question is how SUMO conjugation is linked to the intricate system of flower induction. One possibility is that SUMO's role in chromatin structure regulation is necessary for proper flower timing. Interestingly, expression of the floral repressor FLC is exquisitely sensitive to chromatin structure (Amasino 2004) and could be affected by abnormally high sumoylation levels of chromatin structure modulators such as histone deacetylases. Consistent with this idea, FLC expression is reduced in *esd4* mutants (Reeves et al. 2002).

It can be expected that the currently concise list of SUMO functions in plants will increase considerably as mechanisms of plant development and homeostasis are further elucidated. Thus, future work holds promise to add exciting insights regarding sumoylation in plants.

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