Characterization of Arabidopsis SUMO conjugation as orchestrator of nuclear bodies and transcription
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Citation for published version (APA):
Mazur, M. J. (2016). Characterization of Arabidopsis SUMO conjugation as orchestrator of nuclear bodies and transcription

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Chapter 1

General introduction
Plants as sessile organisms, in order to survive, must be able to interpret and respond quickly to the signals from their changing environment. Recently, post-translational modifications are rising as key molecular mechanisms to finely control the plant responses to biotic and abiotic stresses. Therefore, the characterization of post-translational regulatory networks is essential for deeper comprehension of the molecular mechanisms governing plant adaptation to environment. In this thesis we focus our studies on post-translational modification called Small ubiquitin-like modifier (SUMO). SUMO modification regulates protein functions by modulating protein-DNA interactions, protein-protein interactions, subcellular protein localization and protein activity (Flotho and Melchior, 2013). In Arabidopsis several studies have demonstrated that SUMO regulates, among others, processes like development, cell cycle regulation, DNA and RNA homeostasis, and responses to biotic and abiotic stresses (Miller et al., 2010, 2013; Castro et al., 2012; Park and Yun, 2013).

**SUMO conjugation cycle**

SUMO is a small polypeptide. The Arabidopsis genome encodes eight SUMO paralogues, but only four of them have been shown to be expressed (Kurepa et al., 2003). Of those, SUMO1 and SUMO2 are closely related showing 89% protein sequence identity. SUMO paralogues have distinct functions in the cell as they have different expression profiles (van den Burg et al., 2010) and different affinities for the SUMO activation (E1) and conjugation (E2) enzymes (Tomanov et al., 2014; Colby, 2006; Elrouby et al., 2013). A study on the evolution of the SUMO gene family in plants showed that SUMO1 and SUMO2 represent the archetype SUMO, as they are the closest homologue of the yeast and human SUMO genes (Hammoudi et al., 2016). Biochemically, the attachment of SUMO to targets largely resembles that of ubiquitin conjugation (Fig. 1A). It involves the formation of an isopeptide bond between the side chain of a lysine residue in target proteins and the C-terminal carboxyl group of the di-glycine motif of mature SUMO. Experimental studies have shown that the lysine residues, which are SUMO-modified, reside in approximately 50% of the cases in the consensus motif ψKxE or in an inverted consensus motif E/DxKψ, where ψ is a hydrophobic amino acid and x any amino acid residue (Sampson et al., 2001; Rodriguez et al., 2001; Matic et al., 2010; Hendriks et al., 2015; Tammsalu et al., 2014a).

In Arabidopsis, conjugation of SUMO on substrates involves two consecutive steps catalyzed by the SUMO E1 Activating Enzyme (a dimer of SAE1 and SAE2) and the SUMO E2 Conjugating Enzyme (SCE1). Sumoylation is essential in Arabidopsis, as null mutations in the *SAE2* and *SCE1* genes are embryo-lethal (Saracco et al., 2007). The catalytically site of the SUMO E2 enzyme can directly recognize SUMO substrates when
they contain the consensus motif; in these cases SUMO is directly transferred to the acceptor Lys in the consensus motif (Yunus and Lima, 2006; Bernier-Villamor et al., 2002). In many cases SUMO conjugation is facilitated by SUMO E3 ligases such as SIZ1 (SAP and Miz domain 1) and HPY2 (High Ploidy 2) (Ishida et al., 2009; Miura et al., 2007). While SIZ1 promotes SUMO conjugation of substrates, the SUMO E4 ligases PIAL1 and PIAL2 (Protein Inhibitor of Activated STAT 1 and 2) promote the formation of SUMO chains (Tomanov et al., 2014).

Sumoylation is reversible and SUMO is removed from modified proteins by a specific

![Diagram](image)

**Figure 1.** Biochemistry of the covalent and non-covalent interactions of SUMO with other proteins. (A) The conjugation and deconjugation cycle of SUMO with the enzymes indicated for SUMO precursor maturation and (de)conjugation: ULPs, E1a/E1b (SAE1/SAE2) dimer, E2 (SCE1), and E3. (B) Non-covalent protein-protein interactions are enforced by SUMO attachment on substrates, which allows novel SUMO-SIM interactions in protein complexes. These SUMO-SIM interactions often occur in multi-SUMO decorated protein complexes where they act as 'SUMO glue'.

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class of cysteine proteases, called Ubiquitin-like proteases (ULPs) or SUMO proteases. The SUMO proteases not only deconjugate SUMO paralogues from their targets by hydrolyzing the isopeptide bond, but they are also responsible for the maturation of the precursor of SUMO. During maturation a C-terminal peptide chain is removed from the precursor to expose a di-glycine motif. To this date, several Arabidopsis SUMO proteases have been studied in molecular detail, such as EARLY IN SHORT DAYS 4 (ESD4), ULP1a (also named ESD4-like SUMO protease 1 or ELS1), ULP1c (OVERLY TOLERANT TO SALT STRESS 2 or OTS2) and ULP1d (OTS1) (Colby, 2006; Murtas et al., 2003; Hermkes et al., 2011; Miura et al., 2007; Kurepa et al., 2003; Novatchkova et al., 2012; Conti et al., 2009). Others like ULP1b, ULP2a and ULP2b remain to be functionally characterized. The characterized Arabidopsis SUMO proteases all appear to have distinct localization patterns in the cell and have, therefore, been proposed to act in a substrate-specific manner (Conti et al., 2008; Murtas et al., 2003).

Non-covalent interactions with the SUMO-modified proteins enforce (existing) protein-protein interactions

Upon conjugation, SUMO appears to enforce protein-protein interactions by interacting non-covalently with other proteins or distant sites on the modified protein (Fig. 1B) (Jentsch and Psakhye, 2013). The non-covalent interactions involve hydrophobic interactions between SUMO and a short hydrophobic peptide called the SUMO-interaction motif (SIM)(reviewed by Flotho and Melchior, 2013). The SIM itself consists of a four-residue hydrophobic sequence [V/I/L]-[V/I/L]-x-[V/I/L] or [V/I/L]-x-[V/I/L]-[V/I/L] (where x is any amino acid), that is either preceded or succeeded by an acidic stretch composed of Asp, Glu and/or phosphorylated Ser and Thr residues (Hecker et al., 2006; Kerscher, 2007). These non-covalent interactions are paralogue-specific and can influence the recruitment of the SUMO E2 enzyme to its targets. In this way, these SUMO-SIM interactions determine the paralogue-specific modification of certain targets (Hecker et al., 2006; Meulmeester et al., 2008; Ghisletti et al., 2007).

An emerging idea is that SUMO acts as molecular glue that strengthens protein–protein interactions via SUMO-SIM interactions (Fig. 1B) (Bergink and Jentsch, 2009). This notion extends to large protein complexes, where multi-SUMO-SIM interactions occur within the protein complex with many of the proteins in the complex being SUMO modified (Jentsch and Psakhye, 2013). For example, a recent screen confirmed that dozens of mammalian proteins interact with multi-SUMO platforms and that these interactions are mediated by multi-SIM modules (Aguilar-Martinez et al., 2015). In a different approach, an affinity resin was used that contains a tetramer repeat of the SIM2-SIM3 peptide found in the SUMO-targeted ubiquitin E3 ligase RNF4, which recognizes poly-SUMO chains and marks
them for degradation (Da Silva-Ferrada et al., 2013). This resin, called SUMO-trap or SUMO binding entity (SUBE), was successfully used to purify and identify many SIM-interacting sumoylated proteins (Lang et al., 2015; Da Silva-Ferrada et al., 2013).

Although the non-covalent SUMO-SIM interactions are well studied in mammalian cells, only in a few cases has the role of such SUMO-SIM interactions been studied in Arabidopsis. For example, GID1, a gibberellin (GA) binding receptor, has a predicted SIM that is able to recruit SUMO (Nelis et al., 2015). Importantly, DELLAs proteins, which are transcriptional repressors downstream of GA perception, are sumoylated (Nelis et al., 2015; Conti et al., 2014) These studies showed that the GID1-DELLAs interaction is SUMO dependent. Interestingly, a yeast two-hybrid study retrieved several Arabidopsis proteins that contain a SIM (Elrouby et al., 2013). These findings indicate that the SUMO-SIM interactions are also biological relevant in Arabidopsis.

Outline of the thesis

While many studies have addressed the role of SUMO in mammalian cells, the role of sumoylation in plants is still relatively unexplored. Identification of additional SUMO substrates and SIM-containing proteins, especially among proteins involved in transcriptional regulation, is needed to comprehend how SUMO modification controls gene regulation in plants in response to biotic and abiotic stresses.

This thesis focuses on identifying novel interactors of the Arabidopsis SUMO machinery and SUMO conjugation targets. In chapter 2 we review the existing catalog of in vivo and in vitro Arabidopsis SUMO targets, and compare that with the existing SUMO target lists for mammalians and yeast cells. We describe processes that are possibly regulated by SUMO and underscore the involvement of SUMO in transcriptional regulation. We also review novel technical advancements to study sumoylation.

Next, in chapter 3 we characterize the Arabidopsis SUMO1-SCE1-SIZ1 complex. We show the existence of a functional SIM-binding cleft on SUMO1 and identify binding sites on SCE1, that are essential for SCE1 to interact with SUMO and SIZ1. We show that the SUMO1-SCE1-SIZ1 complex localizes to nuclear foci, hereafter called nuclear bodies (NBs). Importantly, this localization to NBs requires the presence of mature SUMO and catalytically active SCE1. These SUMO-NBs co-localize with a master regulator of the photomorphogenic response, COP1. Previously, COP1 has been suggested to be a functional homolog of the human protein PML. As PML also localizes to nuclear bodies and the formation of PML-NBs requires SUMOylation of PML itself, in chapter 3 we discuss whether Arabidopsis COP1 is a functional homolog of human PML.
Chapter 1

To identify new SUMO targets and/or interactors, we have performed a set of yeast two-hybrid (Y2H) and three-hybrid (Y3H) screens. To this end we used the SUMO1, -2 and-3 proteins and the SUMO (de)conjugation enzymes as baits. In this way we tried to obtain a comprehensive map of the SUMO machinery interactome. In chapter 2 we concluded that many transcriptional regulators are SUMO substrates. Consequently, in chapter 4 we study the interactions of the SUMO machinery proteins with Arabidopsis transcription factors (TFs) using a cDNA library that contains about 1,200 ORFs of Arabidopsis TFs. Combined, these screens yielded 76 interactors representing different TF families. The most represented family is the TCP family. Several lines of evidence are provided that suggest that these TCPs are direct substrates for SCE1 sumoylation. The TCP-SCE1 complexes often localize to subnuclear foci. Importantly, these TCP-SCE1 nuclear foci differ from the SUMO1-SCE1-SIZ1 NBs discussed in chapter 3. In chapter 5 we describe an Y2H screen with an arrayed cDNA library of 13,000 Arabidopsis ORFs to identify interactors other than TFs. We used high stringency conditions for the interaction tests to only identify high confidence interactors. The Y2H and Y3H screens provided 20 novel SUMO interactors. For further studies we selected four candidates – RIN13, a homolog of RIN13 (hRIN13), MORC1 and GPDHc –, as they were previously linked to plant immunity. The data suggest that RIN13, hRIN13 and GPDHc are SIM-driven interactors, while MORC1 requires SIZ1 for its sumoylation.

Finally, in chapter 6, all the findings presented in the experimental chapters of this PhD dissertation are discussed, and future perspectives on the various topics are depicted.
References


Chapter 1


