Identification and validation of *Arabidopsis thaliana* SUMO5-interacting proteins

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SUMO and SUMOylation: In eukaryotes, posttranslational modification by the addition of Small Ubiquitin-like Modifier (SUMO) alters the activity of many substrate proteins. First, this regulatory mechanism was described in yeast and mammals, and afterwards in plants where it is implicated in various cellular processes such as chromatin modifications, RNA transport, transcriptional regulation, cell development, interaction with pathogens… Protein SUMOylation is an enzymatic process with several well characterized players: SUMO protease, SUMO-activating (E1) and -conjugating enzymes (E2), ligase (E3) and deSUMOylating isopeptidase (Figure 1) [1]. In *Arabidopsis*, eight SUMO paralogs have been identified but only four showed time- and condition-dependent expression pattern and, interestingly, the disruption of this pathway is lethal, highlighting the significance of SUMOylation in plant development [2]. Despite this, little is known about SUMO targets in plants. The proteomics studies are mainly performed in *Arabidopsis* with the focus on *At*SUMO1, -2, and -3 [1] leaving the most distinct *At*SUMO5 apart. Here, we centered on in identification of potential SUMO5-interacting proteins using yeast 2-hybrid approach combined with *in planta* validation by bi-molecular fluorescence complementation (BiFC) and flow cytometry approach (FACS).

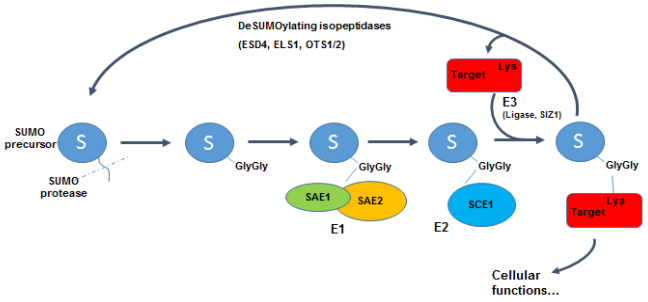
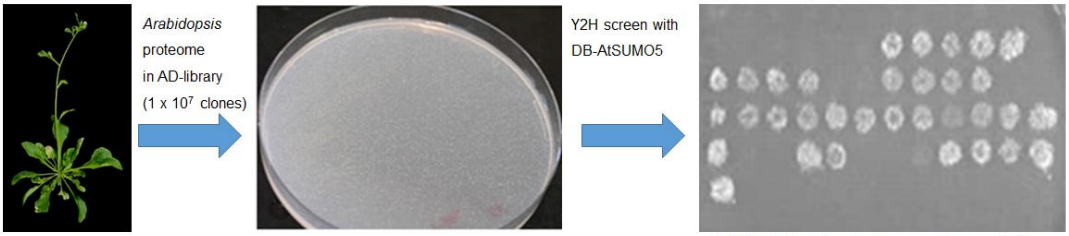


Figure 1. Players in SUMO conjugation and deconjugation system:

- protease- SUMO processing leaving GlyGly at C-terminal
- SUMO-activating enzyme (SAE, subunits 1 and 2; E1)
- SUMO-conjugating enzyme (SCE1, E2)
- SUMO-ligase (E3)
- DeSUMOylating isopeptidase

Identification of *At*SUMO5-interacting proteins: The Yeast-2-Hybrid system offers an experimental approach to investigate protein-protein interactions *in vivo*. We conducted a yeast two-hybrid screen against a cDNA library from whole *Arabidopsis* plants using the Invitrogen Gal4-based Y2H system with *At*SUMO5 as bait to identify potential targets (Figure 2). Candidate DNA sequencing revealed 212 potential SUMO5-interacting proteins which were further subjected to *in planta* validation.

Figure 2. Y2H screening for SUMO5 partners



AtSUMO5 interacting proteins growing on selective drop-out medium (-Leu – Trp – His)

Validation *in planta* of *At*SUMO5 substrates by BiFC and FACS:
Our validation is based on capturing the bi-molecular complementation of YFP between SUMO5-YN fusion protein and SUMO5-YC partner with FACS *in planta* using transfected protoplast (Figure 3). *In planta* protein-protein confirmation is potentially more reliable in regards of minimizing unspecific behavior in heterologous systems. In Figure 4, an example for validation of SUMO interacting/ non-interacting protein by BiFC/FACS is presented.

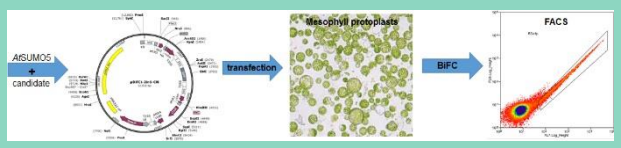


Figure 3. Schematic representation of in planta validation

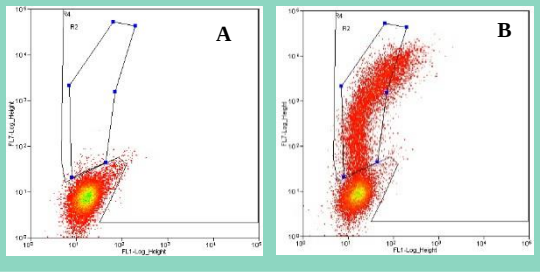


Figure 4. Cytometric plots of testing for BiFC between: (A) SUMO-YN and PRK-YC (phosphoribulokinase, a protein which does not interact with SUMO) as negative control. (B) SUMO-YN and Cab-YC (chlorophyll a/b binding protein), a protein identified in our Y2H as SUMO substrate.

Conclusion: This is the first report of identification of *Arabidopsis* SUMO5 partners using Y2H combined with *in planta* BiFC/FACS validation.

References:
[1] Elrouby N. 2015. Plant Physiol 169: 1006-17
[2] Elrouby et al. 2013. PNAS 49: 19956-19961
[3] Berendzen K et al. 2012. Plant Methods 8: 25
[4] Grefen et. Al. 2012. Biotechnique 5: 311- 314