

Post-translational maturation of IDA, a peptide signal controlling floral organ abscission in *Arabidopsis*

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ABSTRACT

The abscission of sepals, petals and stamens in *Arabidopsis* flowers is controlled by a peptide signal called IDA (Inflorescence Deficient in Abscission). IDA belongs to the large group of small post-translationally modified signaling peptides that are synthesized as larger precursors and require proteolytic processing and specific side chain modifications for signal biogenesis. Using tissue-specific expression of proteinase inhibitors as a novel approach for loss-of-function analysis, we recently identified the peptidases responsible for IDA maturation within the large family of subtilisin-like proteinases (subtilases; SBTs). Further biochemical and physiological assays identified three SBTs (AtSBT5.2, AtSBT4.12, AtSBT4.13) that cleave the IDA precursor to generate the N-terminus of the mature peptide. The C-terminal processing enzyme(s) remain(s) to be identified. While proline hydroxylation was suggested as additional post-translational modification required for IDA maturation, hydroxylated and non-hydroxylated IDA peptides were found to be equally active in bioassays for abscission.

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Peptides are emerging as key signals for the coordination of plant growth, development, and interactions with the environment. Since the discovery of systemin as the first plant peptide with hormone-like activity in 1991,¹ many more signaling peptides have been characterized in recent years,² and there are more than 1000 genes predicted to code for secreted peptides in *Arabidopsis thaliana*.^{3,4} Most of the known signaling peptides are derived from larger precursor proteins that lack biological activity as a pre-protein, pro-protein, or pre-pro-protein.² Precursor processing and post-translational modifications are thus required for the formation of the bioactive peptides.^{5,6} This is also the case for IDA (Inflorescence Deficient in Abscission) a peptide precursor controlling the abscission of floral organs and other cell separation processes in *Arabidopsis*.^{7–9}

IDA belongs to the large group of small post-translationally modified signaling peptides that are synthesized from pre-pro-proteins of 70 to 110 amino acids (Fig. 1A).⁵ As a first obligatory step of maturation, its N-terminal signal peptide is cleaved co-translationally upon entry into the secretory pathway.⁷ The resulting precursor needs to be further processed in order to release the active peptide that is contained within a conserved 20-amino acid proline-rich region, the extended PIP (EPIP)

motif (Fig. 1B).¹⁰ We recently identified the peptidases responsible for IDA maturation within the large family of mostly extracellular subtilisin-like proteinases (subtilases; SBTs).

There are 56 SBTs in *Arabidopsis*, some of which contribute to non-specific protein turnover, while others are processing enzymes for the cleavage of selected substrates at highly specific sites.¹¹ Such a precursor-processing function has clearly been shown for AtSBT6.1 (alias AtS1P) that is responsible for the release of Golgi-localized transcription factors and for the processing of secreted pectin methyl esterases.^{12,13,14} SBT6.1 also cleaves the precursors of RALF and GOLVEN1 peptide growth factors at conserved S1P cleavage sites, but this does not result in the mature signaling peptides.^{15–17} Any potential pro-protein processing function of other SBT family members appears to be obscured by functional redundancy, as most of the single-gene loss-of-function mutants lack obvious phenotypes. In a novel approach, addressing functional redundancy at the level of enzyme activity rather than gene expression, we found that at least three functionally redundant SBTs are responsible for the maturation of IDA in the abscission zones of *Arabidopsis* flower organs.¹⁸

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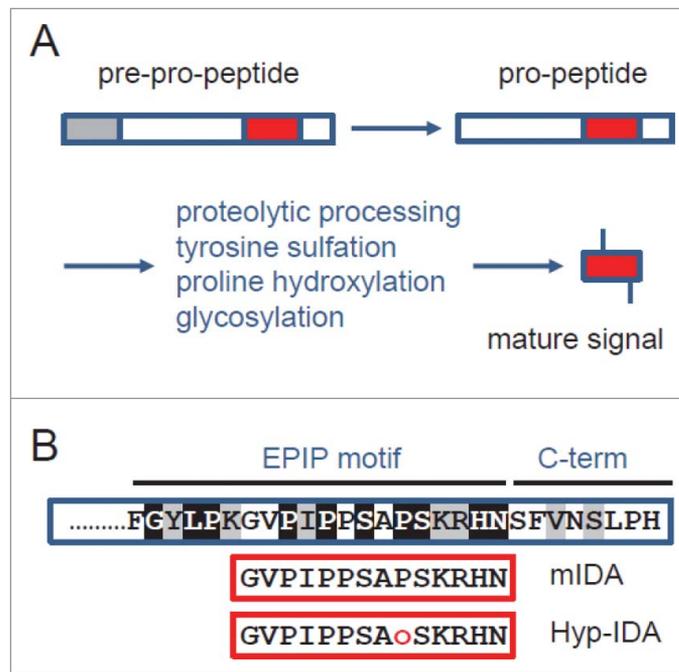


Figure 1. Biogenesis of small post-translationally modified signaling peptides and maturation of IDA. (A) Schematic view of the post-translational events leading from the pre-pro-peptide to the mature signaling peptide. (B) Primary structure of the IDA precursor and the mature IDA peptide. The C-terminus of the IDA precursor is shown comprising the conserved 20-amino-acid EPIP motif and a C-terminal extension. Black and gray shading is used to indicate different levels of amino acid conservation with IDA-like homologs in Arabidopsis. The 14-mer mIDA and Hyp-IDA with hydroxyproline replacing the proline residue in position 9 are shown below.

Activity-based loss-of-function analysis by tissue-specific expression of proteinase inhibitors relies on specific inhibitors of the enzymes of interest.¹⁹ The Extracellular Proteinase Inhibitors (EPI) 1a and EPI10 from *Phytophthora infestans* have been described as specific inhibitors of SBTs with no activity against other serine proteinases.^{20,21} Consistent with the proposed specificity to subtilisin-like proteinases, the modelled protease/inhibitor complex revealed a close fit for EPI1a in the substrate binding channel of subtilisin (Fig. 2A). EPI1a and EPI10 were codon-optimized for expression in plants, and equipped with an N-terminal signal peptide for targeting to the secretory pathway.¹⁸ Transgenic plants expressing EPI1a or EPI10 in abscission zones under control of the *IDA* promoter retained their flower organs, indicating that SBT activity is indeed required for floral organ abscission. Further biochemical and physiological assays identified three SBTs (AtSBT5.2, AtSBT4.12, AtSBT4.13) that cleave the IDA precursor to generate the N-terminus of the mature peptide. The requirement of SBT-mediated N-terminal processing for signal biogenesis was confirmed in genetic complementation experiments.¹⁸

In this addendum, we would like to address some still open questions related to the biogenesis of IDA. Schardon et al. showed that IDA maturation relies on SBT-mediated cleavage of the Lys/Gly bond within the EPIP motif,¹⁸ thus generating Gly7 as the N-terminus of

the mature peptide (Fig. 1B). However, the protease(s) that mark the C-terminus of mature IDA (mIDA) are still elusive. Crystal structure analysis of the peptide/receptor complex and bioassays for receptor activation previously identified Asn20 as the C-terminus of the bioactive IDA peptide.^{22,23} Indeed, the Gly⁷-Asn²⁰ peptide was found to be most active in bioassays for floral organ abscission (Fig. 2B)¹⁸ and we conclude that this 14-mer constitutes the endogenous abscission signal. Extraction from abscission zones and structural characterization of the native peptide will be required to confirm its identity. The C-terminal Asn residue is conserved in several other peptide families including the CLE, RGF, and PEP families, and it was repeatedly shown to be important for receptor binding.²⁴ In case of CLE19, the C-terminal Asn is generated by the carboxypeptidase SOL1 (SUPPRESSOR OF LLP1 1),²⁵ and a similar mechanism may be considered for C-terminal maturation of IDA.

Proteolytic processing is not the only post-translational modification during passage through the secretory pathway. Sulfation of tyrosines by tyrosylprotein sulfotransferase, proline hydroxylation by 2-oxoglutarate-dependent dioxygenases, and O-glycosylation of hydroxyproline (Hyp) by arabinosyltransferases take place in the ER and/or Golgi.^{26–28} These modifications may all be relevant for peptide activity,²⁴ as they affect peptide secretion, peptide stability, or

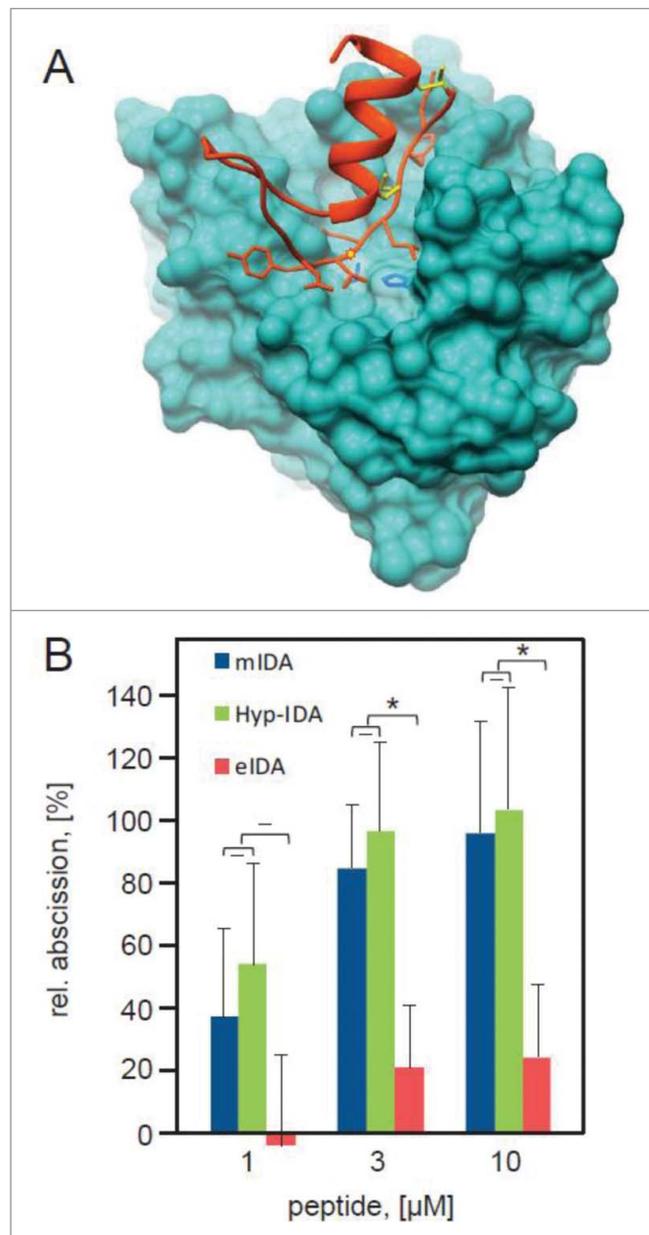


Figure 2. Binding of EPI inhibitors and activity of IDA peptides. (A) Structural model of the EPI1a/subtilisin A complex. The model was calculated using the SWISS-Model Workspace in the automated mode at <https://swissmodel.expasy.org>.³⁰ The crystal structure of subtilisin A in complex with greglin (PDB code 4gi3) was used as the template.³¹ The EPI1a homology model was calculated in ProMod3 based on the target/template alignment with greglin (0.37 sequence similarity). Predicted local similarity to the target was 0.6 or higher for each aligned residue. QMEAN and GMQE quality scores were 0.32 and 0.6, respectively. Subtilisin A is shown in cyan, with side chains of active site Ser and His residues highlighted in blue. EPI1a is shown in red including the side chains of the active site loop that are accommodated by respective substrate binding pockets of the enzyme. Six predicted backbone hydrogen bonds further stabilize enzyme/inhibitor interaction. The yellow asterisk marks the scissile bond in the active site loop. Cysteine residues engaged in disulfide bonds that maintain inhibitor structure and binding after cleavage by the protease are shown in yellow. (B) Bioassay for IDA peptide activity. Transgenic lines expressing the EPI10 inhibitor in abscission zones were treated with the 14-mer IDA peptide (mIDA), mIDA hydroxylated at Pro in position 9 (Hyp-IDA), and an extended IDA peptide with 9 additional amino acids at the N-terminus (eIDA) at the indicated concentrations. Synthetic peptides were obtained from PepMic (Suzhou, China) at > 95% purity. Abscission-inducing activity was analyzed as described previously.¹⁸ It is shown relative to water-treated controls and wild-type plants set at 0 and 100%, respectively (mean \pm SD for $n = 4$ biological replicates; asterisks indicate statistically significant differences at $p < 0.05$ (t-test; non-significant differences are indicated by -).

peptide-receptor interaction. Receptor binding and activation assays performed in *N. benthamiana* leaves after transient expression of the IDA receptor indicated that hydroxylation of Pro9 (Pro15 of the EPIP motif; Fig. 1B) is required for maximum activity of mIDA.²² Specific interactions of the hydroxyl group within a binding pocket of the IDA receptor, and the restricted size of this binding pocket as indicated by crystal structure analysis suggested that there is no further arabinosylation of this residue.²³ We therefore tested the hydroxylated mIDA derivative (Hyp-IDA; Fig. 1B) in our bioassay for floral organ abscission and compared it to the non-modified mIDA peptide. With similar dose-response curves, mIDA and Hyp-IDA were found to be equally active in this bioassay (Fig. 2B). This finding is inconsistent with previous studies that suggested a requirement for Hyp in position 9 for full activity. In order to explain this apparent discrepancy, we speculate that mIDA may become hydroxylated after being taken up by the plant. Known prolyl-4-hydroxylases cannot be involved as they are located in the ER and Golgi.²⁹ We therefore posit an extracellular activity for proline hydroxylation of cell wall-localized peptides. Such an activity remains to be identified. It becomes clear that despite recent progress, much remains to be learned about peptide signal biogenesis, particularly with respect to the enzymes involved and the sequence and subcellular compartmentalization of post-translational modification events.

Disclosure of potential conflicts of interest

The authors report no conflict of interest.

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