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Role of the Arabidopsis leucine aminopeptidase 2

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Proteolysis-related genes have diverse functions across taxa and have long been considered as key players for intracellular protein turnover. Growing evidence indicates the biological significance of peptidases in degradation, maturation and modulation of bioactive peptides/proteins. By screening T-DNA tagged lines and functional analysis approaches we unraveled the Arabidopsis leucine aminopeptidase (AtLAP2) function in amino acid turnover. Transcriptomics and metabolomics profiling data suggested involvement of AtLAP2 in specific metabolic pathways. Loss-of-function of AtLAP2 resulted in early-leaf senescent and stress-sensitive phenotypes. Our work indicates an important in planta role for AtLAP2 contributing to a further understanding of the proteases having several implications in higher plants.

Key words: leucine aminopeptidase, Arabidopsis, stress response, plant growth, senescence

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Leucine aminopeptidase (LAP) is ubiquitously found in all living organisms. LAPs are members of M1 or M17 families[1-3] that cleave the leucine (Leu) residue from *N*-terminal of proteins or peptides. Substantial activities of LAPs may also be evident on other amino acids. Functional diversification of a LAP family across taxa has been reported. In mammals, LAPs contribute in generating antigenic peptides and processing of bioactive peptide hormones.[4] In addition, LAP along with other peptidases is important in the degradation of crystalline protein in the eye after oxidative stress.[5] Most recently, the role of ERAP1 in innate immunity by processing particular substrate(s) was also suggested.[6] In prokaryotes, LAPs have a role in proteolysis, potential virulence,[7] breakdown of hemoglobin in infected red blood cells,[8] and replication.[9]

In higher plants, the LAP family has been extensively studied in tomato but to a lesser extent from other plant species. Given our interest in understanding biological roles of LAPs in higher plants, we recently performed functional and expression analyses of LAP2 in a model dicot plant genome model, *Arabidopsis thaliana*. We demonstrated Arabidopsis LAP2 (AtLAP2) is an enzymatically active aminopeptidase, which is responsible for the cleavage of Leu, Met (methionine), and Phe (phenylalanine) from *N*-terminal peptides, and plays important roles in various cellular processes in planta.[10] In this addendum, we elaborate our discussion on a distinct biological function of AtLAP2, expression pattern, compared with well-characterized plant LAPs.

AtLAP2 is Expressed in all Organs but Unresponsive to Mechanical Wounding

To date, the genome sequences of several model plants have been completed. Information of numerous Expression Sequenced Tag (EST) libraries is now available. Analysis of the LAPs among the sequenced genomes using KEGG GENES database (www.genome.jp/kegg/genes.html) has shown a multiplicity of LAPs

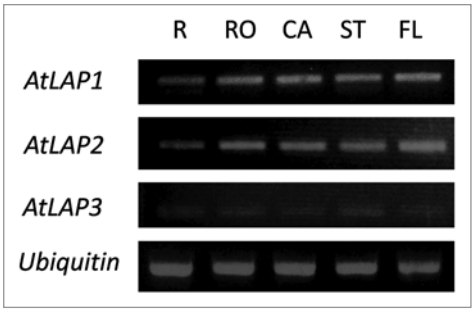

Figure 1. Expression of the *AtLAP* genes in Arabidopsis organs. The expression level of a ubiquitin gene was used as an internal control. Two-week-old roots (R) were used. Mature (rosette, RO and cauline, CA) leaves including stems (ST) were obtained from 6-week-old plants. Flowers (FL) were from 8-week-old plants.

(e.g., *Lycopersicum esculentum, A. thaliana, A. lyrata, Oryza sativa* and *Sorghum bicolor*). The redundancy of LAPs might reflect various requirements, overlapping functions and/or different localization in plants. Extensive studies of the tomato LAP (LeLAP) revealed two classes (LeLAP-A, acidic *p*I and LeLAP-N, neutral *p*I) exhibiting distinct biochemical characteristics.[11] Immunoblotting analysis revealed the presence of two additional LeLAP-like proteins.[12] LeLAP-A modulates plant defense against pathogen and is expressed in response to various stimuli.[13,14] Suppression of LeLAP-A impaired the wound response.[14] By contrast, LeLAP-N is not known to respond to environmental stresses.[11] In the Arabidopsis genome, three putative *LAP* genes have been identified. We recently presented a new functional study of the AtLAP2 and showed that AtLAP2 loss-of-function leads to early leaf senescence and rendered plant more sensitive to applied stresses.[10]

AtLAP2 was expressed in all organs (R, root; RO, rosette; CA, cauline; ST, stem; FL, flower) examined. Expression pattern of *AtLAP1* and *AtLAP2* was very similar whereas the *AtLAP3* was expressed at very low levels (**Fig. 1**). Spatial expression of promotor-AtLAP2 showed high levels of expression in apices, vascular tissue and quiescent center.[10] Unlike the LeLAP-A, AtLAP2 was not induced by mechanical wounding (**Fig. 2**) and applied environmental stimuli (data not shown). Isoelectric point analysis of the predicted AtLAP proteins revealed that AtLAP1, 2, 3 have theoretical *p*Is of 5.56, 6.62 and 6.26, respectively. Thus, the putative AtLAP2 and AtLAP3 are likely neutral LAPs while AtLAP1 is likely to be an acidic LAP.

Exploring Natural Substrate(s) for Plant LAPs: Tougher than Expected!

LAPs are aminopeptidases that usually cleave Leu most preferentially among synthetic substrates. Indeed in vitro analysis demonstrated that all plant LAPs exhibit a broad specificity toward Leu-, Met-, Phe-, Arg (arginine)-, Pro (proline)-, Ile (isoleucine)-, Val (valine)-, Ala (alanine)-MCAs.[10,15-17] Although LAP can cleave many peptides in vitro, the biological relevant target should be determined not only by substrate specificity but also by the natural substrates. In mammals, substrate specificity of LAPs toward natural substrates is usually rather broad. For instance, Laeverin, a novel bestatin-sensitive LAP, was able to cleave the *N*-terminal amino acid of several natural peptides such as angiotensin III, kisspeptin-10 and endokinin C.[18] Another LAP, P-LAP/IRAP cleaves oxytocin, vasopressin and angiotensin III efficiently.[19] However, identification of physiological substrate in vivo is still rare.[20] Information of plant natural substrate peptide is also scarce. To date, very few examples show possible natural peptides for plant aminopeptidases. Cortes et al. (2006) reported aminopeptidase activity from pollen *Parietaria judaica* was able to degrade two neuropeptides, namely substrate P and VIP angiotensin.[21] In 2011, it was reported that CLE-3, a well known plant active peptide, was possibly degraded by serine protease and carboxypeptidase.[22] Thus, identification of the natural substrates for LAP and/or other aminopeptidases would be a very interesting topic for future research.

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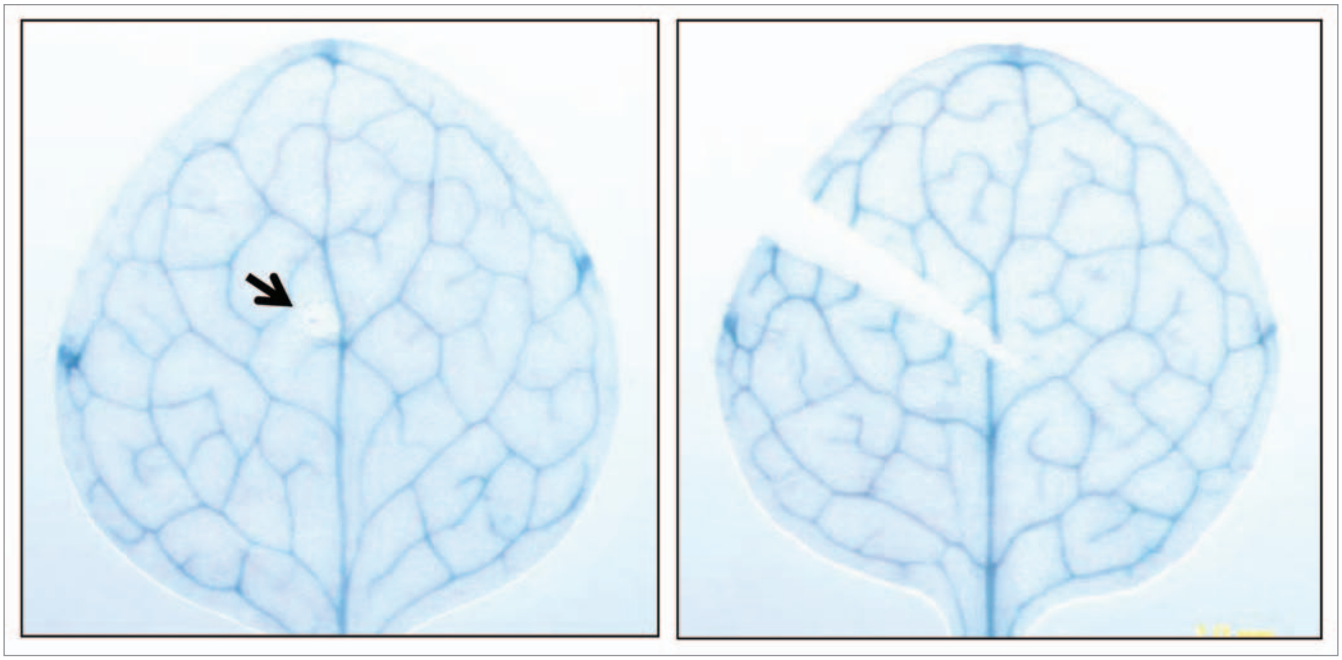

Figure 2. AtLAP2 promoter activity as determined by histochemical GUS staining in respective reporter gene lines was not induced upon mechanical wounding by piercing with a needle (left) and cutting off the leaf with scissors (right).

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