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The way out and in: phloem loading and unloading of amino acids

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Amino acids represent the major transport form of reduced nitrogen in plants. Long-distance transport of amino acids occurs in the xylem and the phloem. However, the phloem is the main transport route for bulk flow of the organic nitrogen from source leaves to sink tissues. Phloem loading in leaves of most annual plant species follows an apoplasmic transport path and requires the coordinated activity of transport protein mediating cellular export or import of amino acids. Phloem unloading of amino acids is generally a symplasmic process but apoplasmic transport is additionally required for efficient post-phloem nitrogen transport. In this review we summarize the current data on the physiology of amino acid phloem loading and unloading, and the molecular players involved. We discuss the implications of amino acid transporters in nitrogen signaling and highlight the necessity to investigate the coordination of symplasmic and apoplasmic transport processes.

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Introduction

Growing vegetative and reproductive sink organs such as roots and seeds rely on the supply of large amounts of nitrogen (N) for their development, metabolism and, in case of seeds, storage protein accumulation. N delivery to heterotrophic sinks generally occurs as amino acids in the phloem. The amino acids may derive from uptake from the rhizosphere, result from reduction of nitrate or ammonium in roots or be synthesized in leaves after nitrate translocation in the xylem [1]. In addition, N remobilization from transient storage pools or protein hydrolysis contributes to the amount of amino acids present in the long-distance transport path. Root-to-shoot movement of the N solutes occurs in the xylem. Once in the leaf, xylem-derived and/or leaf-produced amino acids are loaded into the phloem and transported in the sieve tube to sinks using a hydrostatic pressure difference between source and sink [2]. Upon arrival in sink organs, phloem unloading and subsequent import into sink cells conclude the journey of amino acids. This review discusses the role of amino acid transporters in phloem loading and unloading, and their importance for source and sink function.

Transporter function in phloem loading of amino acids

Amino acid transport from source leaves to sinks generally starts in the minor vein phloem and is assumed to follow an apoplasmic phloem loading mechanism in Arabidopsis and most crop plants [3,4]. First, amino acids are released into the leaf apoplasm, presumably *via* facilitated diffusion, followed by active, proton-coupled import into the sieve element-companion cell (SE-CC) complex of the phloem. This pathway requires the function of plasma membrane-localized amino acid transporters [5]. The bidirectional Arabidopsis SiAR1/UMAMIT18 transporter (Siliques Are Red1/Usually Multiple Acids Move In and Out Transporters; [6••]) has been proposed to facilitate export of glutamine from the leaf cells, while import of amino acids into the phloem is mediated by AtAAP8 (Amino Acid Permease 8 [7]). However, UMAMIT18 and AAP8 are obviously not the only transporters functioning in phloem loading, as source-to-sink N translocation or seed development still persist in their mutants, although to a lower extent. In addition, generally all proteinogenic amino acids are found in the phloem, but their levels and composition change constantly as for example leaf metabolism and phloem function fluctuate depending on the time of the day, diurnal rhythm, developmental stage of leaves or environmental conditions [1,8,9]. Therefore, one can assume that, besides AtSiAR1/AtUMAMIT18 and AtAAP8, other export and import systems with varying substrate specificities and affinities are required to accommodate phloem loading throughout the plant's daily and life cycle. For efflux systems, this may include further proteins of the UMAMIT family or unidentified exporters [6••,10••]. Based on expression and localization studies, additional phloem loaders most probably involve other AAPs [1,11], cationic amino acid transporters such as CAT6 (cationic amino acid transporters; [12]) and ProTs (proline and compatible solute transporters; [13]). For example during senescence,

when N remobilization is high and leaves become a strong N source, several of these putative loaders are induced [9,14], yet their physiological function in phloem loading remains to be explored.

Transporter function in phloem unloading of amino acids

Phloem unloading in sink organs generally describes movement of assimilates from the sieve tube into neighboring cells followed by post-phloem transport to the destination or terminal sink cells (Figure 2). Phloem unloading of carbon (C) and N photoassimilates is assumed to occur by a symplasmic mechanism with variations dependent on the plant species, sink tissues and developmental stage [3]. For example, in Arabidopsis, phloem unloading happens from terminal sieve elements of roots and seed funiculi [10••,15••,16,17•]. In contrast, in grain legumes the vasculature is extended throughout the seed coat [18,19] and unloading takes place along their entire lengths rather than being restricted to the phloem pole [18]. Generally, sieve elements of the different phloem unloading zones lack companion cells. Instead, they contain numerous plasmodesmata and are symplasmically connected in roots to phloem pole pericycle cells (Figure 2a), and in the funiculus and seed coat to parenchyma cells (Figure 2b) [15••,17•]. Blockage of the plasmodesmata has been shown, at least in roots, to inhibit phloem unloading [15••]. This, together with the fact that so far no amino acid exporters have been observed in the phloem unloading domains, supports that N assimilates leave the sieve elements *via* the symplasmic path, driven by a downhill concentration gradient. It is important to note that UmamiTs have been localized to the root vasculature, with UmamiT14 and UmamiT18 being present in the root pericycle [6••,20]. However, expression of UmamiTs has not been found in the protophloem, where bulk unloading occurs [15••], but in root cells surrounding the mature metaphloem and translocating protophloem [20]. Therefore, these UmamiTs may function in roots in N cycling between the xylem and phloem, and in amino acid supply of lateral sinks instead of in phloem unloading (Figure 1; see below).

Transporter function in post-phloem transport of amino acids

Post-phloem transport of amino acids in roots and developing leaves is generally *via* the symplasm although amino acid exporters [10••] and importers [21,22,23•] are expressed in these sink tissues. The transporters might enable leakage and retrieval of apoplasmic amino acids to balance turgor and/or an osmotic gradient, but they may also facilitate apoplasmic movement, thereby affecting concentration gradients between symplasm and apoplasm, and amino acid flux rates in sinks. Post-phloem transport of amino acids in reproductive organs may involve symplasmic and apoplasmic transport pathways dependent on the plant species, developmental stage as well as the maternal or filial tissues [10••,17•,24,25]. For example in Arabidopsis, ovules are symplasmically isolated requiring not yet identified amino acid transporters for ovule nutrition and development [17•]. Following fertilization, secondary plasmodesmata are formed in developing seeds to link the terminal SEs to seed coat cells located in close proximity to the unloading zone, which are also symplasmically connected [17•]. However, despite the symplasmic continuum, amino acid transporters are found in this zone [6••,10••] suggesting an additional apoplasmic route for post-phloem N movement. Clearly, the apoplasmic path is essential, as mutants for *UmamiT11* and *UmamiT14*, which are expressed in the described unloading domain, accumulate free amino acids in fruits and produce smaller seeds [10••].

The seed coat or testa surrounds the endosperm and the developing embryo, and these tissues are symplasmically isolated from each other [16,26]. In addition, within the testa of Arabidopsis plasmodesmata are lacking between the outer and inner integuments [16,27]. Obviously, a series of export and import processes are required to efficiently move amino acids towards the seed apoplasm for final uptake by the embryo. A few Arabidopsis transporters have been shown to be essential in amino acid allocation to the embryo for proper seed development and storage protein accumulation, and these include UmamiTs, CAT6, AAP8 and AAP1 [7,10••,23•,28,29]. In contrast, in legume seed coats the symplasmic domain continues from the sieve elements to those testa cells that facilitate export of amino acids into the seed apoplasmic space for embryo N uptake [5,25].

Do amino acid transporters exert regulatory control over nitrogen and carbon acquisition, metabolism and source-to-sink partitioning?

Recent studies using Arabidopsis and pea amino acid transporter knock-out and overexpressor lines, respectively, support that the primary function of phloem- and seed-localized amino acid transporters is in controlling N allocation to, and uptake into, sinks which in turn affects sink development, seed protein levels, and yield [7,10••,12,26,30••]. However, their regulatory role is undoubtedly more complex as changes in leaf-to-sink N allocation also influence N root uptake, root-to-shoot transport as well as N assimilation and remobilization. In addition, photosynthesis, C assimilation and C source-to-sink transport are co-regulated [7,26]. For instance, in *AAP1* over-expressing pea plants with increased amino acid phloem and seed loading more C is fixed to accommodate increased N uptake and assimilation, and to support improved biomass and seed production [26].

Clearly, plants are highly plastic and adapt source and sink physiology to changes in N partitioning or local N levels [6••,7,10••,12,23•,26,29,30••,31,32]; but if amino