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Research article

The *AAP* gene family for amino acid permeases contributes to development of the cyst nematode *Heterodera schachtii* in roots of Arabidopsis☆

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ABSTRACT

The beet cyst nematode *Heterodera schachtii* is able to infect Arabidopsis plants and induce feeding sites in the root. These syncytia are the only source of nutrients for the nematodes throughout their life and are a nutrient sink for the host plant. We have studied here the role of amino acid transporters for nematode development. Arabidopsis contains a large number of different amino acid transporters in several gene families but those of the *AAP* family were found to be especially expressed in syncytia. Arabidopsis contains 8 *AAP* genes and they were all strongly expressed in syncytia with the exception of *AAP5* and *AAP7*, which were slightly downregulated. We used promoter::GUS lines and *in situ* RT-PCR to confirm the expression of several *AAP* genes and *LHT1*, a lysine- and histidine-specific amino acid transporter, in syncytia. The strong expression of *AAP* genes in syncytia indicated that these transporters are important for the transport of amino acids into syncytia and we used T-DNA mutants for several *AAP* genes to test for their influence on nematode development. We found that mutants of *AAP1*, *AAP2*, and *AAP8* significantly reduced the number of female nematodes developing on these plants. Our study showed that amino acid transport into syncytia is important for the development of the nematodes.

1. Introduction

Nematodes are a large group of animals with different life styles, including free-living bacterial feeders such as the model nematode *Caenorhabditis elegans* as well as a variety of pathogens of plants and animals. Obligate biotrophic plant parasitic nematodes attack mainly the roots of many plant species and cause severe damage to their host plants, either directly or as vectors of plant viruses. It has been estimated that the worldwide crop losses due to nematode damage amount to more than $100 billion per year [1]. Some of the most economically important species belong to the family Heteroderidae and induce the formation of specialised feeding sites which are their sole source of nutrients throughout their life. Root-knot nematodes of the genus *Meloidogyne* induce a feeding structure which is composed of several giant cells [2] while cyst nematodes (genera Heterodera and Globodera) induce a feeding structure which is a syncytium. Cyst nematodes enter the plant roots as second stage juveniles (J2) and select a single root cell to induce a syncytium, which then expands by incorporation of up to several hundred neighbouring cells by local cell wall dissolution. The adult male cyst nematodes leave the roots to mate with females. After fertilization, the female cyst nematode continues to feed from the syncytium until the egg development is completed. The dead body, which is then called a cyst, protects several hundred eggs until infective J2 hatch in favourable conditions. Cysts can

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survive in the soil for many years which makes the cyst nematodes difficult to control in agriculture. The sugar beet cyst nematode *Heterodera schachtii* completes its life cycle on Arabidopsis roots *in vitro* within six weeks [3] and this interaction has been established as a model system. Arabidopsis can be cultured on artificial media under sterile conditions in Petri dishes and the translucent roots facilitate the study of the development of the nematodes inside the root.

The drastic changes in cell morphology of syncytial elements [4,5] as compared with normal root cells imply an underlying global change in gene expression and a variety of methods were used to identify genes that are specifically induced in syncytia or in giant cells (reviewed by Gheysen and Fenoll [6]). We have recently analysed the transcriptome of syncytia induced by *H. schachtii* in roots of Arabidopsis at 5 and 15 days post-inoculation (dpi) [7] and found that one of the strongly induced genes in syncytia coded for the amino acid permease AAP8.

Amino acids represent one of the essential long-distance transport forms for the distribution of organic nitrogen in plants. This allocation of amino acids is mediated by both xylem and phloem [8]. In the xylem, the transport occurs unidirectionally upwards, whereas in the phloem the translocation is bidirectional and the nutrients flow from source to sink tissues. On their way from the sites of uptake and biosynthesis in roots or leaves, amino acids have to be transported across several membranes to enter the long-distance pathways or to reach sink tissues. Amino acid translocation thus requires proteins which control the transport across these membranes.

By functional analysis and sequence homology, a large number of potential amino acid transporters from different gene families were found in the Arabidopsis genome [9,10]. The most important groups are amino acid permeases (AAP), cationic amino acid transporters (CAT), and lysine/histidine transporters (LHT), which all mediate proton-dependent import of amino acids into the cell [11–18]. Prior to a proton coupled import, amino acids have to be exported into the apoplast. This is especially required where no intracellular connections, such as plasmodesmata, are present. An apoplastic pathway exists, for example, in roots, where passive diffusion between epidermis and cortex cells ends at the casparian strip of the endodermis [19] and solutes must enter the symplast. Finally, the nutrients need to be exported out of the symplast into the tracheary elements, as these are dead by maturity and therefore belong to the apoplast. So far, only few plant proteins have been published that mediate a bidirectional transport, and, hence, also an efflux of amino acids. *At*BAT1 [20] was shown to mediate the efflux of glutamate and lysine, but also the influx of alanine and arginine. Another Arabidopsis membrane protein, SIAR1 [21] has been shown to play an important role in organic nitrogen allocation and particularly in amino acid homeostasis in developing siliques.

Physiological functions have been proposed for several amino acid transporters. The import of amino acids into the filial part of the seeds is most likely mediated by members of the AAP family. The expression of the high affinity transporter AAP1 was detected in embryos and is responsible for the import of amino acids into the filial tissue [11,13,22–24]. Besides AAP1, other secondary active amino acid importers were identified to be involved in the amino acid uptake into developing seeds such as AAP2 and AAP8 [25,26].

Microsporogenesis represents a major sink for nitrogen [18]. The situation in stamen resembles the one in developing seeds as the filaments contain a strand of vascular tissue which ends at the connective. Thus, the delivery of nutrients to the anthers must occur via an unloading process and a subsequent transfer across apoplastic barriers towards the developing pollen grains. The uptake of neutral and acidic amino acids into tapetum cells is dependent on LHT2 [18,27]. Amino acid uptake is also essential in symbiotic interactions with mycorrhizal fungi and rhizobia [8] and in plant–pathogen interactions. Amino acid transporters are, for instance, specifically expressed in haustoria which are produced by biotrophic fungal pathogens for the uptake of nutrients from plant cells [28].

Amino acids supply is also important in the pathogenic interaction between nematodes and plant roots. Syncytia and also giant cells, feeding sites induced by several genera of sedentary plant pathogenic nematodes, have a high metabolic activity and are a severe sink for the plant since the nutrients that are taken up by the nematode have to be continuously restored. Amino acids and other nutrients must either be taken up from the apoplast with the help of specific transport proteins or provided symplastically through plasmodesmata. It was originally thought that syncytia are symplastically isolated [29] but, recently, evidence has been reported that there is a direct connection between syncytia and the phloem [30]. Still, the apoplastic pathway seems to play an important role for nutrient uptake into syncytia since several genes for sugar transporters are induced in syncytia and are important for nematode development [31]. Besides sugars, syncytia also have to take up amino acids as a nitrogen source to cope with the constant loss due to nematode feeding. Indeed, the level of 14 amino acids was higher in syncytia as compared with uninfected roots and with root tissues surrounding the syncytium [32]. Correspondingly, our recent transcriptome analysis has revealed that several AAP amino acid transporter genes are strongly upregulated in syncytia [7]. A similar situation has been found in giant cells induced by the root-knot nematode *Meloidogyne incognita* in Arabidopsis roots. A microarray analysis of root sections containing root knots showed that the expression of many amino acid transporters was significantly altered as compared to control root sections [33,34].

Here we report our expression analysis of AAP-type amino acid transporter genes in syncytia. In addition, we included the *LHT1* gene which is also expressed in syncytia and roots at a high level. To test the importance of these genes for the development of the nematodes we used the available knock-out mutants.

2. Results

We recently performed a transcriptome analysis of syncytia induced by *H. schachtii* in Arabidopsis roots [7]. *AAP8* was one of the genes that was found to be strongly upregulated in syncytia as compared to control root sections and this was confirmed by real-time RT-PCR and *in situ* RT-PCR. Here we have extended that work and have in addition studied the expression of several other AAP-type amino acid transporter genes together with *LHT1*. As shown in Table 1, 6 of the 8 *AAP* genes are expressed at high levels in syncytia and most of them are upregulated as compared to control root sections. Only *AAP5* and *AAP7* of the 8 *AAP* genes are slightly downregulated. Of the other 44 amino acid transporter related genes, only *LHT1* showed a very strong expression in roots and in syncytia and was therefore included in this study. However, it was approximately three folds downregulated as compared to control root sections. Among the other amino acid transporter related genes, only rather few were expressed at a higher level in syncytia than in control root sections (Table 1) which is also evident from Fig. S1. Comparing expression in 5 and 15 dpi syncytia, only *AAP8* showed a significantly different expression, being approximately expressed 10 folds higher in 15 dpi syncytia (Fig S2). These data indicate that the AAP-type gene family might be especially important for the amino acid uptake into syncytia. We have therefore in addition studied their expression using promoter::GUS lines and *in situ* RT-PCR.