First publ. in: Biology of Plant-Microbe Interactions. Vol. 4. / ed. by Igor Tikhonovich et al. - St. Paul, Minn., 2004, pp. 177-179

42. Rust haustoria as sink in plant tissues or – how to survive in leaves

Struck C.[1], Voegele R.T.[1], Hahn M.[2] and Mendgen K.[1]
[1]University of Konstanz, Department of Biology, Phytopathology, Konstanz, Germany.
[2]University of Kaiserslautern, Department of Biology, Phytopathology, Kaiserslautern, Germany

Abstract

The plant pathogenic rust fungi differentiate haustoria within cells of their host plants. These structures are assumed to be special adaptations for the nutrition of these biotrophic parasites with highest metabolic activity. Here we present genes and corresponding proteins involved in nutrition of *Uromyces fabae*. In addition, we have identified haustorium-specific genes that encode proteins with putative secretion signals. One of these gene products is transferred from the haustorium into the infected plant cell nucleus and might be involved in signalling phenomena between host and parasite.

Introduction

Fungal haustoria are formed within living plant cells by three taxonomically diverse groups, such as oomycetes (downy mildews), ascomycetes (powdery mildews) and basidiomycetes (rust fungi). Since the discovery of haustoria as special fungal structures they are intensively studied by cytological techniques [5]. During the last 10 years the isolation and characterization of haustorium-specific genes and proteins have made it possible to analyse haustorial functions at biochemical and molecular levels.

Results and Discussion

Differential screening of a haustorium-specific cDNA library of the rust fungus *Uromyces fabae* resulted in the isolation of a large number of genes, showing preferential expression in parasitically growing hyphae and haustoria [3; Hahn et al., unpublished]. Several of these genes were further analysed to get more insights into functions of haustoria.

Success of biotrophic parasitic fungi depends much on their ability to utilize efficiently the plant metabolites [7]. A central task of haustoria and parasitic mycelium is therefore the nutrient uptake. In the intercellular space of the host plant, nitrogen metabolites, such as nitrate, ammonia and amino acids, are present only at very low concentrations as compared to the symplastic host compartments which are certainly better sources for nitrogen [8]. We have cloned three genes encoding putative amino acid transporters (*AAT1*, *AAT2* alias *PIG2*, *AAT3*). As revealed by Northern hybridization and RT-PCR, two of these transporter genes (*AAT1*, *AAT3*) are expressed in fungal infection structures including germ tubes and intercellular hyphae, and further upregulated in haustoria [9; Struck et al., submitted]. In contrast, mRNA of the *AAT2* transcript was not detectable at f the early rust growth stages that are formed in the absence of the plant. However, high levels of *AAT2* mRNA were detected in haustoria. With antibodies raised against synthetic AAT2 peptides, the *AAT2*-encoded protein was localized exclusively to plasma membranes of haustorial bodies [4]. Thus, amino acid transporters of *U. fabae* are developmentally regulated in different ways.

Functional analyses performed by heterologous expression in yeast cells and *Xenopus* oocytes revealed two amino acid transporters (AAT1p and AAT3p) a broad spectrum of amino acids

as substrates. However, these transporters show different specificities. Whereas AAT1p preferentially translocates histidine and lysine [9], AAT3p shows highest transport activities with the sulfur-containing amino acids cysteine and methionine as well as with leucine [Struck et al., submitted]. Interestingly, AAT1p shows no transport of cysteine and only intermediate transport of methionine and leucine. On the other hand, AAT3p shows no transport of lysine and has only weak affinity for histidine. Thus, it appears that these amino acid transporters complement each other. So far, we have been unable to detect transport activity of AAT2p. Analyses of the amino acid concentrations in the apoplast of bean plants revealed asparagine, alanine, glutamine and glutamate as the predominant free amino acids, whereas other amino acids were present at low concentrations (0.02-0.16 mM) [Struck et al., submitted].

Amino acid concentrations in uredospores of *U. fabae* are about 1.8 mM for glutamine and 0.6 mM for alanine whereas the concentrations of other amino acids are much lower [Struck and Lohaus, unpublished]. Therefore, rust fungi likely need further uptake systems for N-metabolites to manage the production of the vast numbers of uredospores during sporulation. Recently we have identified an *Uromyces* gene (*AMT1*) with high similarities to Basidiomycete ammonium transporters. *AMT1* is not expressed in ungerminated uredospores but during all phases of the fungus development. Heterologous expression of *AMT1* in *Xenopus* oocytes with subsequent electrophysiological measurements confirmed proton-dependent ammonium transport activity [Struck et al., unpublished].

Currently, we are interested in the regulation of the primary nitrogen metabolism in rust fungi and especially a potential influence of the host plants. On the other hand, we are interested in the question whether the nutrient supply of the host plant has any influence on pathogenesis. A comparison of the expression analysis of several rust genes encoding enzymes of the nitrogen metabolism together with the identified transporters revealed different patterns between infected plants under nitrogen starvation and plants that are not under nitrogen-limiting conditions. Whereas the transcript levels of the amino acid transporters and the ammonium transporter did not show changes during the first two weeks after inoculation, the expression of the *Uromyces* glutamine synthetase is lower in host plants under nitrogen starvation already five days after inoculation [Struck et al., unpublished]. Furthermore, in these plants fungal development is restricted. Thus, nutritional limitation affects fungal pathogenesis.

Another important factor in nutrition is the carbohydrate supply. Saccharides are present in abundance in all plants representing a rich nutrient source for attacking pathogens. Recently we isolated a PMF-driven transporter, HXT1p, from the broad bean rust *Uromyces fabae* which is exclusively localized in the haustorial plasma membrane [13]. The specificity of this transporter for the monosaccharides D-glucose and D-fructose raised the question as to how the pathogen mobilizes these hexoses to be taken up and metabolized by the fungus. We have identified a gene, *INV1*, with homology to fungal and plant invertases [12]. The predominant localization of the gene product in the extrahaustorial matrix suggests that invertase and hexose transporter work hand in hand to supply the fungus with sucrose-derived monosaccharides. Heterologous expression of *INV1* and the biochemical characterization of the enzyme are in progress [Voegele et al., unpublished]. Expression of *INV1* in early infection structures and in intercellular hyphae indicates a possible role of the enzyme in the regulation of source – sink proportion. Carbon partitioning in higher plants is mainly controlled by the activity of invertases [10]. Expression of the fungal invertase in the bulk apoplast might condition the infected plant organ for the transition from source to sink tissue. This hypothesis is corroborated by our findings that infection with *Uromyces fabae* affects the expression pattern of certain plant invertases [Voegele et al., manuscript in preparation].

We are interested in the metabolic fate of the plant-derived sugars. Glucose taken up by HXT1p presumably is channeled into glycolysis. All enzymes of the glycolytic pathway except for phosphofructokinase could be identified in an EST-sequence database obtained from haustorial cDNAs [Hahn et al., unpublished]. We are currently analyzing a clone encoding glucokinase/hexokinase.

Fructose might take another pathway in addition to glycolysis. During a screen for metabolite changes during infection we found that a C6-polyol rose by more than 100-fold in rust-infected leaf tissue during infection. This polyol was identified as D-mannitol. The mannitol is of fungal origin since its production could be correlated with the increase of a fungal major alcohol dehydrogenase which was identified previously as an *in planta* induced rust gene. MAD1p was found to be a classical NADP-dependent mannitol dehydrogenase. The results of *MAD1* transcript analysis and immunolocalization of MAD1p led us to propose that MAD1p acts in a dual manner: as a fructose reductase in the haustorial lumen and as a mannitol dehydrogenase in spores [Voegele et al., MS]. Mannitol was detected in spores at high levels, this polyol can therefore be viewed as a primary carbohydrate storage metabolite, entering right at the top of glycolysis. In addition, we have found that mannitol is released from the mycelium into the apoplast [11]. There is growing evidence from several pathosystems [2, 6] that mannitol can be used by pathogen as an antioxidant to survive host defense responses involving reactive oxygen species. MAD1p would therefore directly link nutrient acquisition and suppression of host defense responses in rust fungi.

Another line of action the fungus might take to circumvent or suppress host defense responses is an active influence on regulatory processes in the host. In order to achieve this one would expect effectors being transferred from the fungus into the plant cell. Such a transfer has so far only been shown for bacterial effector proteins [1]. Within our EST-sequencing project [Hahn et al., unpublished] a large number of contigs did not show significant hits in publicly accessible databases. Several of these genes coded for proteins with potential signal sequences and were therefore selected as candidates for components enrolled in host pathogen interaction. Recently we were able to show that one of these rust transferred proteins, RTP1p, can be detected in the nucleus of infected host cells [Hempel, Haerter, Hahn, Kemen, Voegele, and Mendgen, unpublished]. This would be the first example for a fungal effector being translocated into the targeted host cell.

References

1. Buttner D. and Bonas U. (2002) EMBO J. 21: 5313-5322.
2. Chaturvedi V. et al. (1996) J. Immunol. 156: 3836-3840.
3. Hahn M. and Mendgen K. (1997) Mol. Plant-Microbe Interact. 10: 427-437.
4. Hahn M. et al. (1997) Mol. Plant-Microbe Interact. 10: 438-445.
5. Harder D.E. and Chong J. (1991) In: Electron Microscopy of Plant Pathogens. Eds. Kurt Mendgen and Dietrich-E. Lesemann Springer, Berlin, p. 235-250.
6. Jennings D.B. et al. (2002) Plant J. 32: 41-49.
7. Solomon P.S. et al. (2003) Mol. Plant Pathol. 4: 203-210.
8. Snoeijers S.S. et al. (2000) Eur. J. Plant Pathol. 106: 493-506.
9. Struck C. et al. (2002) Mol. Plant Pathol. 3: 23-30.
10. Sturm A. (1999) Plant Physiol. 121: 1-8.
11. Voegele R.T. and Mendgen K. (2003) New Phytol. 159: 93-100.
12. Voegele R.T. et al. (2001) ISMPMI, Conference Proceedings, p. 445.
13. Voegele R.T. et al. (2001) Proc. Natl. Acad. Sci. USA 98: 8133-8138.