# Functional analysis of a host microtubule-associated protein acting in nematode feeding cell formation

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# Functional analysis of a host Microtubule-Associated Protein acting in nematode feeding cell formation

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Among plant pathogens, sedentary endoparasitic nematodes interact with their hosts in a quite unique and intriguing way. During a compatible interaction, root-knot nematodes *Meloidogyne* spp. establish and maintain permanent multinucleate and hypertrophied feeding cells in the plant. Nematodes induce the root cell redifferentiation by repeated karyokinesis without cell division. The giant cells are essential for the growth and reproduction of this obligate biotrophic pathogen. Hyperplasia and hypertrophy of the surrounding cells lead to the formation of a typical root gall.
Genes involved in diverse processes, such as cell cycle activation, cell wall modification, hormone and defense responses have been identified as involved in giant cell formation (Gheysen and Fenoll, 2002; de Almeida-Engler et al., 2005; Jammes et al., 2005; Caillaud et al., 2007). However, only one gene function has been identified in knockout studies as essential for giant cell formation that of the *rpe* gene, which encodes a key enzyme in the pentose phosphate pathway (Favery et al., 1998). Cytoskeletal rearrangements in giant cells have recently been identified as a key plant component in compatible plant-nematode interaction (de Almeida-Engler et al., 2004). In addition, a candidate plant protein involved in the actin cytoskeleton organization has been identified (Favery et al., 2004). This plant-parasite interaction therefore constitutes an interesting model system for studies of the regulation of microtubules (MTs) cytoskeleton organization during plant development.
We investigated the molecular mechanisms underlying giant cell formation. Using a promoter trap strategy in *Arabidopsis*, we identified *AtMAP65-3* gene to be expressed at early stage of nematode cells formation. This gene encodes a Microtubule-Associated Protein (MAP), AtMAP65-3, from the MAP65 family (Müller et al., 2004). MAP65s are thought to organized MT arrays integrating individual MTs into complex arrays. Detailed functional analysis *in planta* highlighted the role of AtMAP65-3 as an essential host susceptibility factor in the plant-nematode interaction.

## Results and Discussion

### AtMAP65-3 was induced in the early stage of nematode giant cells ontogenesis

We used a promoter trap strategy to isolate genes involved in the formation of giant cells induced by *Meloidogyne incognita*. We have identified a line, DYC283, displaying early GUS

activity in galls. Molecular and genetic analysis of DYC283 line showed that this line carried a single T-DNA integrated into the *AtMAP65-3* gene. This gene has 11 exons and encodes a 707-amino acid protein, AtMAP65-3, from the MAP65 family (Müller et al., 2004). In the DYC283 line, the T-DNA was inserted into the fifth exon, placing the GUS gene ATG in frame with the *AtMAP65-3* gene and resulting in a functional gene fusion. We confirmed the AtMAP65-3 expression pattern deduced from experiments with the GUS reporter gene, by RT-Q-PCR analysis and using the *AtMAP65-3* promoter ($Pro_{MAP65\text{-}3}$) GFP fusion. During plant development, *AtMAP65-3* was expressed in all tissues enriched in dividing cells (e.g. the root and shoot meristems, young leaves and flower buds). In galls, we showed that *AtMAP65-3* was expressed early after initiation of the feeding site, and this expression rapidly weakened before the development of fully mature giant cells. Similar expression patterns associated to root knot nematode infestation have been reported previously for cell cycle marker genes (de Almeida-Engler et al., 1999).

**In the absence of AtMAP65-3, giant cells were induced but do not differentiate fully**

We investigated the role of AtMAP65-3 in giant cells ontogenesis by carrying out detailed functional analysis *in planta.* To analyse the effect of *map65-3* loss-of-function, we isolated homozygous dyc283 T-DNA insertion plants. Phenotypic analysis of the *map65-3* mutant showed that AtMAP65-3 played a key role in root, embryo and shoot development. The cellular organization of the *Arabidopsis map65-3* mutant was studied on sections through different tissues. Polynucleate and hypertrophied cells, harboring cell wall stubs, could be observed in all organs of the map65-3 mutant plants. This mutant phenotype suggests an important role of AtMAP65-3 in cytokinesis and in organizing mitotic MTs arrays.

We examined the response of the T-DNA *map65-3* mutant to the nematode *M. incognita*. We described a defect in nematode giant cells formation. In the absence of functional AtMAP65-3, giant cells started to develop but did not complete their differentiation and finally decayed. These giant cell defects impaired the maturation of the infecting nematodes, which are dependent on the nutrients supplied by fully developed giant cells. Thus, AtMAP65-3 is an essential host susceptibility gene, playing a key role in plant-nematode interactions, as shown by the requirement of this protein for giant cell development.

**AtMAP65-3 colocalized with the "mini cell plate" in developing giant cells**

We investigated the subcellular distribution of AtMAP65-3 *in planta*, using functional GFP-AtMAP65-3 fusions under the control of the native promoter. The colocalization of MTs and AtMAP65-3 demonstrated the presence of AtMAP65-3 in all MT arrays during mitosis and early cytokinesis (i.e. both spindle and phragmoplast). At the end of the mitosis, AtMAP65-3 colocalized with the nascent cell plate. As AtMAP65-3 was present in dividing cells, we suggest that AtMAP65-3 is involved in giant cell mitotic MT array organization. *In vivo* confocal and electron microscopy analyses of the first giant cell nuclear division showed that the newly formed cell plate initially lined up between the two daughter nuclei, but did not develop further. In developing giant cells, the AtMAP65-3 signal was associated with "mini-cell plate" separating daughter nuclei. These "mini-cell plates" represent a novel kind of cell plate involved in giant cell formation.

## Conclusion

We demonstrated that AtMAP65-3 is an essential host susceptibility factor in plant-nematode interaction. In the absence of AtMAP65-3, giant cells were induced, but failed to complete their differentiation. Thus, the manipulation of the plant cytoskeleton is a main step in giant cell formation and successful completion of the nematode life cycle. It is generally assumed

that giant cells resulted from repeated nuclear divisions without cytokinesis. We reported that cytokinesis is initiated in giant cells resulting in “mini cell plate” formation. We hypothesized that “mini cell plates” form a physical barrier separating the daughter nuclei which is essential for successive rounds of karyokinesis. AtMAP65-3 is crucial for proper rounds of karyokinesis, which were required for large multinucleate cell ontogenesis.

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