



# Root-knot nematodes manipulate plant cell functions during a compatible interaction

Marie-Cécile Caillaud, Géraldine Dubreuil, Michaël Quentin, Laetitia Perfus-Barbeoch, Philippe Lecomte, Janice de Almeida Engler, Pierre Abad, Marie-Noëlle Rosso, Bruno Favery\*

INRA-UNSA-CNRS, UMR1064-6192, Interactions Plantes–Microorganismes et Santé Végétale, 400 route des Chappes, F-06903 Sophia Antipolis, France

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## Summary

Sedentary endoparasitic nematodes are root parasites that interact with their hosts in a remarkable way. These obligate biotrophic pathogens establish an intimate relationship with their host plants, inducing the redifferentiation of root cells into specialized feeding cells. The successful establishment of feeding cells is essential for nematode development. Root-knot nematodes, of the genus *Meloidogyne*, have evolved strategies enabling them to induce feeding cell formation in thousands of plant species, probably by manipulating fundamental elements of plant cell development. Feeding cells enlarge and are converted into multinucleate giant cells through synchronous nuclear divisions without cell division. Fully differentiated giant cells may contain more than a hundred polyploid nuclei that may have undergone extensive endoreduplication. Hyperplasia and hypertrophy of the surrounding cells lead to the formation of the typical root gall. Giant cell formation requires extensive changes to gene expression. The induction of feeding cells remains poorly understood, but it is thought that effectors secreted by the nematode play a key role in parasitism, with potential direct effects on recipient host cells. In this review, we focus on the most recent investigations of the molecular basis of the plant–root-knot nematode interaction. Recently, microarray technology has been used to study the plant response to *Meloidogyne* spp. infection. Such a genome-wide expression profiling provides a global view of transcriptional changes, especially for genes involved in cell wall, transport processes and plant defense responses during giant cell formation. The identification of nematode-responsive plant genes constitutes a major step toward understanding how root-knot

*Abbreviations:* dpi, day post-inoculation; dsRNA, double-strand RNA; J2, infective second stage juveniles; ROS, reactive oxygen species; RKN, root-knot nematode; siRNA, short interfering RNA

\*Corresponding author. Tel.: +33 492386464; fax: +33 492386587.

E-mail address: [favery@antibes.inra.fr](mailto:favery@antibes.inra.fr) (B. Favery).

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nematodes dramatically alter root development to induce and maintain giant cells. The characterization of nematode secretions as parasitism effectors and the development of RNAi technology should improve our understanding of the molecular events and regulatory mechanisms involved in plant parasitism. Finally, *Meloidogyne* genome sequences should provide further insight into plant–root-knot nematode interactions.

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## Introduction

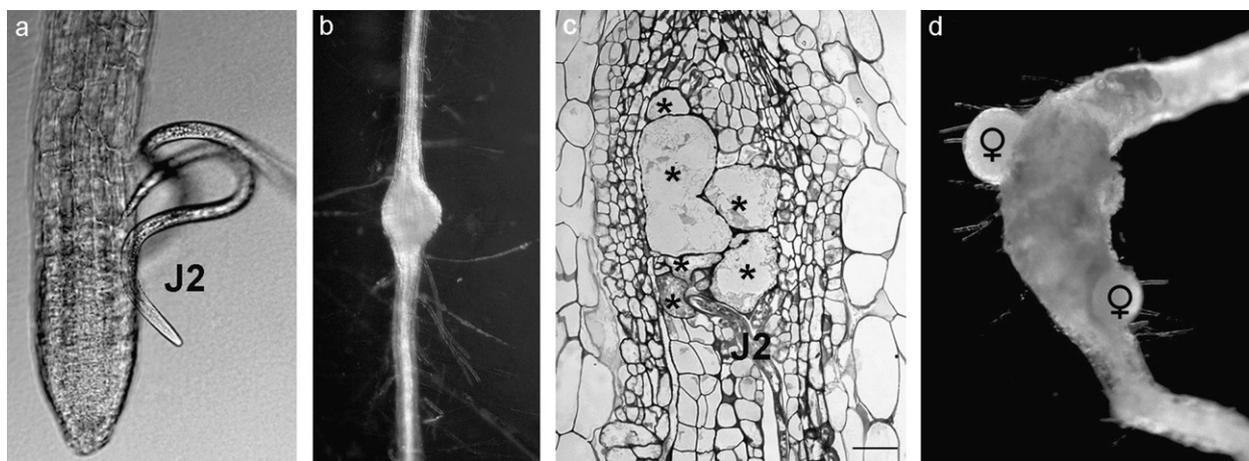
Root-knot nematodes (RKNs), *Meloidogyne* spp., are found in all temperate and tropical areas, and are among the most damaging plant pathogens worldwide (Trudgill and Blok, 2001). Their economic importance is increasing as most chemical control agents for RKNs have been prohibited for environmental and health reasons. RKNs interact with their hosts in a remarkable manner. These obligate biotrophic pathogens have evolved strategies for infesting thousands of plant species in a similar manner, probably by manipulating fundamental elements of plant cell development. They establish and maintain permanent feeding cells, the giant cells, constituting the exclusive source of nutrients for the developing nematode. Hyperplasia and hypertrophy of the surrounding cells lead to the formation of the typical root gall, the primary visible symptom of infection (Figure 1). Like other plant parasitic nematodes, RKNs have a stylet, a hollow retractable needle connected to the esophagus and three unicellular esophageal glands. This structure is used to pierce and penetrate plant cell walls, to release esophageal secretions into the host tissue and to take up nutrients from the giant

cells. Plant nutrient and water uptake are substantially reduced by the resulting damage to the root system, and infested plants are therefore weak and give low yields.

This review focuses on recent investigations of the molecular basis of the plant–RKN interaction. The identification of nematode-responsive plant genes and the characterization of RKN secretions as parasitism effectors constitute a major step toward understanding how RKN dramatically alter root development to induce and maintain giant cells to their own benefit. Knowledge of the compatible plant–RKN interaction should allow the development of target-specific strategies to limit crop damage by these pathogens.

## Root-knot nematode life cycle and giant cell formation

During a compatible interaction, the infective second-stage juveniles (J2; 400  $\mu\text{m}$  long and 15  $\mu\text{m}$  wide) penetrate just behind the root tip (Figure 1a) and migrate between cells. They initially move toward the apex of the root and then turn around



**Figure 1.** Parasitic cycle of the obligate biotrophic root-knot nematode *M. incognita* on *A. thaliana*. Infective second stage juveniles (J2) penetrate just behind the root tip (a) to induce feeding cells in the vascular cylinder. The visible symptom of root-knot nematode infection is the formation of typical root gall (b). Gall section 7 dpi shows multinucleate giant cells (\*) (c). The pear-shaped female (♀) will produce eggs (d). Bar = 10  $\mu\text{m}$ .

to invade the vascular cylinder. Each J2 then induces the dedifferentiation of five to seven parenchymatic root cells into multinucleate and hypertrophied feeding cells (Figure 1c). These “giant cells” function as specialized sinks, supplying nutrients to the nematode until reproduction. With the onset of feeding, the nematode becomes sedentary, going through three molts before becoming a mature adult. Most RKNs reproduce by parthenogenesis (Castagnone-Sereno, 2006). Males migrate out of the plant and play no role in reproduction. After the development of the pear-shaped female (Figure 1d), eggs are released on the root surface in a protective, gelatinous matrix. Embryogenesis within the egg is followed by the first molt, leading to the second juvenile stage.

Nematode growth and reproduction depend on the successful establishment and maintenance of specialized feeding sites within the root. The first sign of giant cell induction is cell cycle activation leading to the formation of vascular binucleate cells (de Almeida Engler et al., 1999). Selected cells become multinucleate and enlarge considerably through additional synchronous nuclear divisions in the absence of cell division (Jones and Payne, 1978). Giant cells expand by isotropic growth and may reach a final size about 400 times that of root vascular cells. In *Arabidopsis thaliana*, cytological observations have shown that, 7 days post-inoculation (dpi), expanding giant cells are multinucleate with a dense cytoplasm. A week later (14 dpi), DNA synthesis significantly decreases, but gall and giant cells continue to expand. By 21 dpi, a mature gall containing fully expanded and differentiated giant cells is formed. Hypertrophied mature giant cells contain more than a hundred polyploid nuclei, which may have undergone extensive endoreduplication (Wiggers et al., 1990). Giant cells have a very dense cytoplasm containing numerous mitochondria, plastids, ribosomes, a well-developed Golgi apparatus and smooth endoplasmic reticulum, generally organized in swirls (Jones and Payne, 1978). The vacuole disappears and gives rise to many small vacuoles. To enhance solute uptake from the vascular system, cell wall ingrowths develop in contact with the xylem. Thus, mature giant cells are metabolically active and act as transfer cells for the feeding nematode.

### Key plant functions manipulated during giant cell development

The transformation of root cells into hypertrophied feeding structures with a unique morphology

and function requires extensive changes to gene expression in infected root cells. These pathogens have evolved an ability to recruit these plant genes to manipulate host functions to their own benefit. The identification of nematode-responsive plant genes remains a major challenge and should greatly improve our understanding of the way in which nematodes dramatically alter root development to produce and maintain giant cells. Plant responses during a compatible nematode interaction have been analyzed molecularly essentially in *A. thaliana*, *Solanum lycopersicum*, *Medicago* spp. and *Nicotiana tabacum*. Approaches based on (i) differential gene expression between healthy and infected root regions, such as cDNA subtraction or differential display, (ii) analyses of known candidate genes by promoter GUS fusion, *in situ* hybridization or RT-PCR and (iii) promoter trap strategies, have resulted in the characterization of a few tens of plant genes, mostly upregulated in response to RKN infection (reviewed by Gheysen and Fenoll, 2002; Williamson and Gleason, 2003; de Almeida Engler et al., 2005). These nematode-responsive plant genes have been assigned to categories relating to plant developmental pathways and a role in giant cell development. These studies have highlighted changes to certain fundamental aspects of plant development, such as defense and hormone response, cell wall, cell cycle and cytoskeleton organization, following nematode infection.

The distribution of microtubules and microfilaments in these nematode-feeding cells has recently attracted considerable attention. RKN can induce long-term changes in the organization of the cytoskeleton in giant cells (de Almeida Engler et al., 2004). A functional mitotic apparatus containing multiple large spindles and arrested phragmoplasts is present throughout the multiple mitotic events observed in giant cells. During giant cell expansion, the actin cytoskeleton displays a particular organization, with large numbers of unusual, randomly oriented actin bundles and cables (de Almeida Engler et al., 2004). The first plant candidate genes implicated in giant cell actin cytoskeleton reorganization have been characterized (Favery et al., 2004; Jammes et al., 2005). Formins are actin-nucleating proteins that stimulate the *de novo* polymerization of actin filaments. Three formin genes, *AtFH1*, *AtFH6* and *AtFH10*, are specifically induced in giant cells. *AtFH6* is uniformly distributed throughout the plasma membrane and, as such, may regulate giant cell isotropic growth by controlling the assembly of actin cables. Actin cables are probably required to guide the vesicle trafficking needed for extensive

plasma membrane and cell wall biogenesis. Chemical treatments blocking cytoskeleton dynamics result in the arrest of nematode development (de Almeida Engler et al., 2004). Thus, the manipulation of the plant cytoskeleton is a key step in giant cell formation and successful completion of the RKN life cycle. However, only one plant gene has yet been really demonstrated, by gene knockout, to be essential for giant cell formation – *rpe*, encoding a key enzyme in the pentose phosphate pathway (Favery et al., 1998).

The recent development of plant microarray technology has made it possible to generate large-scale information about patterns of plant gene expression during giant cell formation. Genome-wide expression profiling, using gene-specific CATMA or Affymetrix genechips, has been used to study the response of *A. thaliana* to *Meloidogyne incognita* infection (Jammes et al., 2005; Hammes et al., 2005). In addition, a tomato microarray containing 12,500 cDNAs has been used to profile the response of tomato to *Meloidogyne javanica* infection (Bar-Or et al., 2005). Jammes et al. (2005) and Bar-Or et al. (2005) minimized the contamination and dilution of giant cell-enriched infected tissue by comparing dissected galls from infected roots with uninfected dissected root fragments over time. These studies identified a large number of new genes regulated in response to RKN infection and demonstrated the temporal regulation of a fraction of these genes. The proportion (5–15%) of genes displaying differential expression reflects the complexity of nematode-feeding site ontogenesis. In total, 3373 *A. thaliana* genes were found to be differentially expressed between uninfected and giant cell-enriched root tissues, with similar proportions of genes up- and downregulated (Jammes et al., 2005). Thus, microarray experiments have shown that gene downregulation may also be essential for correct gall formation. As might be expected, not all genes from a given pathway are similarly induced or repressed. Moreover, most plant genes in a given family display different patterns of regulation in compatible interaction, possibly accounting for the conflicting results obtained in previous studies. Plant aquaporin genes, for example, may be upregulated, downregulated or unaffected by nematode infection. As 65% of all *A. thaliana* genes belong to gene families, this highlights the importance of the specificity of the nucleic acids spotted on the microarray. Gene expression profiling of the plant response to RKN has thus provided a global view of transcriptional changes, especially for genes involved in cell wall, transport processes and plant defense responses during giant cell formation.

A characteristic feature of giant cells is their outstanding isotropic growth. Such cell expansion requires extensive and coordinated cell wall remodeling. Wall extension involves a loosening of the cellulose/cross-linking glycan network, and this is achieved through endo- $\beta$ -D-glucanases, expansins and xyloglucan endotransglycosylases. This process is associated with subtle changes in the embedding pectin network. Goellner et al. (2001) have demonstrated that cell wall-modifying enzymes of plant origin, such as endo- $\beta$ -D-glucanases, may be implicated in feeding cell formation. *AtCEL1* has been shown to be upregulated in developing giant cells (Mitchum et al., 2004). All members of the class A and class B expansin superfamily regulated upon *Meloidogyne* infestation have been shown to be activated in *A. thaliana* (Jammes et al., 2005). *LeEXPA5*, encoding a tomato expansin, is strongly expressed upon RKN infestation, and the silencing of this gene affects root-knot development and the ability of the nematode to complete its life cycle (Gal et al., 2006). In addition, all regulated *A. thaliana* pectate lyases, and most of the polygalacturonases and pectinesterases of this plant are also activated in response to *M. incognita* infestation (Jammes et al., 2005). The concomitant deposition of newly synthesized wall material is associated with this loosening process and, throughout feeding site development, further modifications to the cell wall result in wall thickening and the development of wall ingrowths. Several additional genes encoding cell wall proteins (e.g. hydrolases, structural proteins) have been identified as potentially induced or repressed upon infestation (Jammes et al., 2005).

Giant cells are metabolically hyperactive and form a nutrient sink for the nematode. There is evidence that suggests that transport across the plasma membrane plays an important role in feeding cells. However, few genes encoding membrane transport proteins have been demonstrated to be differentially expressed (reviewed by Gheyssen and Fenoll, 2002). A transcriptome analysis of 635 transporter genes identified 50 genes up- or downregulated in whole RKN-infected roots (Hammes et al., 2005). The results obtained with dissected galls may be different, but several key transporters seem to be instrumental in feeding site development. Jammes et al. (2005) analyzed the regulation of 25 aquaporins. These proteins are known to be involved in water transport, growth control and osmoregulation. Seven aquaporin genes were downregulated, including the *AtTIP1.1* and *AtPIP1.5* genes and genes encoding tonoplast and plasma membrane intrinsic proteins. Two PIPs and one Nod26-like intrinsic protein were found

to be specifically activated in root knots. Hammes et al. (2005) confirmed by quantitative RT-PCR and promoter GUS fusion that *AtPIP2.5* was specifically upregulated in galls. Peptide transport seems to display overall downregulation, whereas an upregulation of amino acid transporters is observed after *M. incognita* inoculation (Jammes et al., 2005; Hammes et al., 2005). Two genes encoding amino acid transporters, *AtAAP6* and *AtCAT6*, have been shown to be expressed at higher levels in the root-knot, including giant cells (Hammes et al., 2005, 2006). The induction, in two independent microarray studies, of *AUX1* and *AtAUX4/LAX3*, encoding putative auxin transporters, is consistent with a role for plant hormones in the establishment of successful RKN infestation. The early, localized and transient activation of synthetic auxin-responsive promoter element DR5 points to a local increase of auxin in feeding sites (Karczmarek et al., 2004). The upregulation of a cytokinin-responsive *ARR5* (*Arabidopsis* response regulator) promoter during early stages of plant–RKN interactions suggests that a spike of cytokinin is also required during giant cell initiation (Lohar et al., 2004). However, the regulation of only a few components of the auxin, abscisic acid, gibberellin and cytokinin pathways has been demonstrated in giant cells. Only one hormone mutant, the tomato *diageotropica*, has been reported to alter RKN parasitism (Richardson and Price, 1984).

RKNs cause little damage to the roots during the infestation process as they migrate between the cells. However, wound or defense responses are detected from initial J2 penetration onwards. In general, the initial reaction and associated pattern of gene regulation are similar in susceptible and resistant plants. In compatible tomato–RKN interaction, the generation of reactive oxygen species (ROS) has been observed at the time of nematode invasion (12 h post-inoculation) but was not detectable cytologically 2 dpi, at the time of giant cell induction (Melillo et al., 2006). Global analysis has shown that the successful establishment of RKN infestation is associated with the suppression of plant defense responses (Jammes et al., 2005). Thus, in *A. thaliana*, 70% of the nematode-regulated genes involved in defense pathways were repressed locally. The suppression of plant defenses includes effects on resistance genes and resistance-associated genes (*PAD4*, *NHL3*), jasmonic acid/ethylene-dependent pathway-associated genes (*EIN3*, *ERF1*, *PR4*) and potential antimicrobial genes. Interestingly, no local change is observed in the expression of genes known to be involved in the salicylic acid pathway

(*ICS1*, *SID1*, *NPR1*, *WHY1*, *PR5*). In galls, 17 of the 21 *WRKY* genes identified are downregulated, whereas the accumulation of *WRKY* transcripts appears to be a general characteristic of plant defenses in response to pathogens. The importance of the suppression of host defenses during plant–pathogen interactions has also been highlighted in recent studies (Abramovitch and Martin, 2004). Both successful pathogens and symbiotic microbes have evolved specialized strategies for suppressing plant defense responses and generating susceptibility in host plants. Studies of plant defenses in the context of giant cell development would certainly provide useful information about the interplay between defense responses and plant development, about which little is currently known (Whalen, 2005).

Genome-wide characterization of the genes specifically regulated during giant cell development is a first step toward understanding compatible plant–RKN interactions. Genes directly implicated in giant cell ontogenesis have yet to be identified and characterized with knockout mutants, biochemical data and expression analysis at the cellular level. It is also necessary to distinguish between formation of giant cells and associated gall tissues. Laser capture microdissection is a recently developed powerful tool for targeting giant cells *sensu stricto* at early stages of infection (Ramsay et al., 2004, 2006).

Comparisons of data from plant–nematode interactions with those from other compatible pathosystems, particularly those inducing feeding structures, should make it possible to distinguish between the nematode-specific and common plant pathways manipulated by other pathogens in the successful establishment of disease. These analyses should also include the response to mutualistic microbes. Several studies have stressed the existence of common features in the developmental programs of gall formation and nodule development (reviewed by Bird, 2004). One of the first marker genes induced in response to *Sinorhizobium meliloti* Nod factors, *MtENOD11*, is expressed during the arbuscular mycorrhizal association, and in tissues surrounding the developing giant cells during RKN infestations (Boisson-Dernier et al., 2005). Weerasinghe et al. (2005) showed that RKNs trigger a cytoskeletal response identical to that induced by Nod factors, leading to root-hair waviness and branching in legumes, mediated by a signal functional at distance. In addition, studies in *Lotus japonicus* mutants have shown that SYMRK, NFR1 and NFR5 are probably involved in the perception of nematode-derived signals (Weerasinghe et al., 2005).

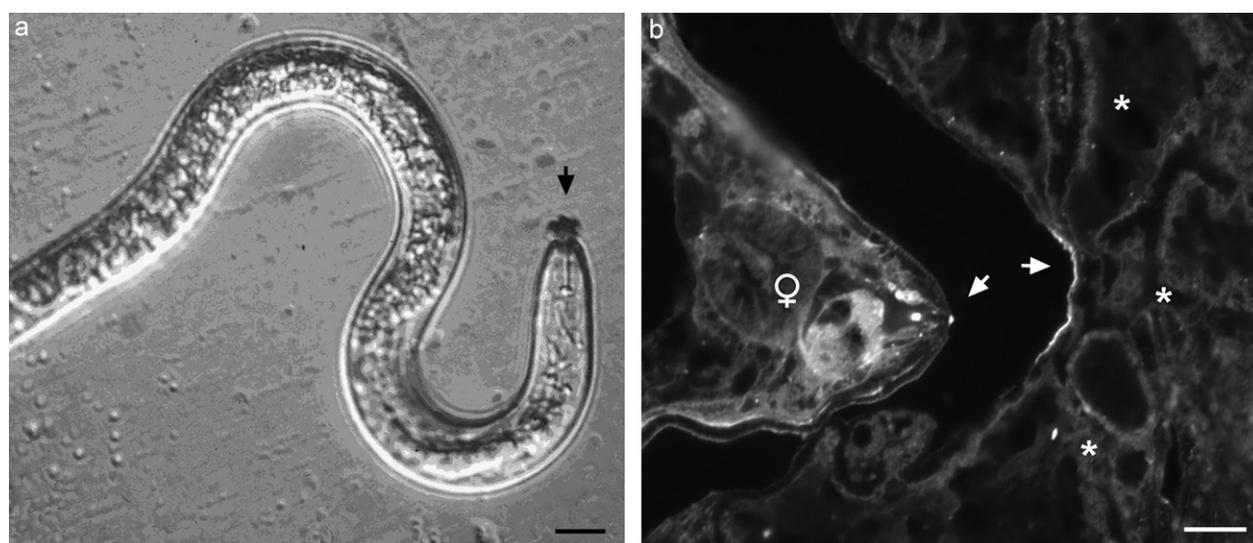
## Nematode parasitism genes

Nematode secretions are considered to be the effectors of parasitism and the signaling molecules at the plant–nematode interface (Neveu et al., 2003; reviewed by Jasmer et al., 2003; Davis et al., 2004). Secretions injected through the stylet (Figure 2a) are thought to play a key role in parasitism, but secretions from the chemosensory amphids (Semblat et al., 2001), cuticle or body orifices may also be involved in parasitism. Stylet secretions are thought to be involved in plant cell wall degradation during root invasion, nematode protection against plant defense responses and the induction of giant cells. Proteome and transcriptome analyses of esophageal secretions suggest that at least 60 different proteins are potentially secreted by the stylet during parasitism (Jaubert et al., 2002; Huang et al., 2003). The multiplicity of stylet-secreted proteins and the predicted functions of the secreted molecules already identified indicate that several cellular processes are targeted by the nematode for successful manipulation of the host response.

RKNs invade the root tissues by producing cell wall-degrading enzymes similar to some plant pathogenic bacteria and fungi. Several RKN enzymes with  $\beta$ -1,4-endoglucanase, xylanase or pectate lyase activity have been shown to be involved in plant cell wall degradation during parasitism and have been characterized (reviewed by Vanholme et al., 2004; Huang et al., 2005; Ledger et al., 2006; Mitreva-Dautova et al., 2006). Both the gland cell-specific expression of the genes concerned and

the presence of predicted secretion signal peptides suggest that these proteins are secreted by RKN. Some of these enzymes have already been demonstrated to be secreted in plants. The similarity between RKN and bacterial genes suggests that some of these genes may have been acquired by horizontal gene transfer from bacteria (Scholl and Bird, 2005; Ledger et al., 2006).

Once established in root tissue, the endophytic RKN stages must deal with plant defenses. RKNs secrete proteins with scavenger activities that protect the parasite from the damaging effects of ROS (Molinari and Miacola, 1997). At later stages, some RKN effectors probably dampen plant defenses sufficiently, as described above, to allow successful interaction. An active role in the suppression of plant defenses has been proposed for an esophageal gland-specific chorismate mutase (Doyle and Lambert, 2003). Plant chorismate mutases are key enzymes in the shikimic acid pathway directing the synthesis of cellular aromatic amino acids and several secondary metabolites, including phytohormones and plant defense compounds. If secreted into the plant cell cytoplasm, the nematode chorismate mutase would reduce the synthesis of flavonoids, salicylic acid, phytoalexins and auxin. A second RKN effector that could modulate plant defense is a calreticulin that accumulates in the walls of giant cells (Figure 2b). Calreticulin was the first molecule shown to be secreted via the nematode stylet into the feeding site (Jaubert et al., 2005). Calreticulins secreted by animal eukaryotic parasites have been identified as key modulators of host immune defenses (Kasper



**Figure 2.** RKN stylet secretions. Stylet secretions (arrow) of a second stage *M. incognita* juvenile visualized after Coomassie staining (a). The RKN calreticulin protein (arrows) detected *in planta* at the tip of the stylet of a female (♀) and along the cell wall of adjacent giant cells (\*) (Jaubert et al., 2005) (b). Bars = 10  $\mu$ m.

et al., 2001; Suchitra and Joshi, 2005). However, the function of RKN calreticulin in the modulation of plant responses remains unclear and it is thought that this molecule may also be involved in calcium signaling and cell cycle regulation (Borisjuk et al., 1998; Ghiran et al., 2003) during giant cell formation.

Even if the accumulation of the secreted calreticulin has been observed at the giant cell wall (Jaubert et al., 2005), nematode effectors are thought to be injected into the cytoplasm and diffuse into the enlarged giant cells. The identification of effectors known to act intracellularly (Jaubert et al., 2004) and the recent demonstration of physical interactions between an RKN secretion and plant transcription factors (Huang et al., 2006a) support the activity of RKN secretions in the plant cell cytoplasm. Determining whether stylet secretions are injected in the cytoplasm and their role in the induction of giant cell formation constitutes a major challenge because of the low amount of such secretions *in planta*.

First RKN effector candidates involved in the induction of giant cells are emerging. RKNs have been shown to produce auxin conjugates and cytokinins that may interfere with the hormone balance of the plant cell (de Meutter et al., 2003, 2005), but it remains unclear whether these nematode products play a role *in planta*. Interestingly, a 14-3-3 isoform  $\zeta$  produced in the esophageal glands of infective juveniles has been identified in purified stylet secretions (Jaubert et al., 2004). In eukaryotic cells, 14-3-3 proteins play a crucial role in modulating the signaling events involved in responses to environmental stimuli, progress through the cell cycle and programmed cell death (Takahara et al., 2000). In plants, a 14-3-3 protein regulates the plasma membrane H<sup>+</sup>-ATPase in response to fungal infection and a similar role has been suggested during nodule development (Wienkoop and Saalbach, 2003). It has also been suggested that secreted nematode peptide signaling molecules are involved in feeding cell formation. A 13-amino acid peptide, 16D10, secreted from the esophageal glands of parasitic J2, has been shown to stimulate root growth and the generation of extensive lateral roots in tobacco hairy roots. 16D10 has been shown to interact *in planta* with two putative plant SCARECROW-like transcription factors (Huang et al., 2006a). The role of 16D10 and the identified transcription factors in plant cell development remain to be determined, but this work provided the first characterization of plant targets for a nematode effector.

Finally, a few putative parasitism genes encode proteins with nuclear localization signals (Huang

et al., 2003), suggesting that some nematode effectors may be targeted to the nucleus of the host cell, like antigens from the animal parasitic nematode *Trichinella spiralis* detected in the nucleus of animal muscle cells (Yao and Jasmer, 2001). However, many other effectors have been identified for which no predicted function could be attributed on the basis of sequence similarity. These “pioneer” genes highlight the current lack of knowledge on effectors from eukaryotic plant pathogens (Jones and Dangl, 2006). The development of RNA interference (RNAi) in RKN species has been a huge breakthrough in gene functional analysis for this obligate parasite refractory to transgenesis.

RNAi is the suppression of gene expression induced by nucleotide sequence-specific double-strand RNA (dsRNA) and short interfering RNA (siRNA) leading to degradation of the homologous transcripts. Rosso et al. (2005) have described a method for inducing dsRNA uptake after stimulation of the feeding activity by soaking *M. incognita* infective juveniles with neurostimulant chemicals. RNAi has since been used in *Meloidogyne* spp. A number of genes involved in RKN development and expressed in different tissues and cell types have been targeted, including those encoding chitin synthase (Fanelli et al., 2005), dual oxidase (Bakhietia et al., 2005) and an intestinal cathepsin L cysteine protease (Neveu et al., 2003; Shingles et al., 2007). Studies of the knockdown of two parasitism genes expressed in the subventral esophageal glands of the nematode – *Mi-pg-1* (encoding polygalacturonase) and *Mi-crt* (encoding calreticulin) – showed that the duration of silencing probably depends on the levels and turnover of the targeted transcripts (Rosso et al., 2005). The use of RNAi techniques based on soaking for functional analysis would therefore be limited to genes involved in development or in the early steps of parasitism, as targeted transcripts are detected again 4 days after soaking (Bakhietia et al., 2005; Fanelli et al., 2005; Rosso et al., 2005).

The production of dsRNA in plants can be used to ensure the continuous ingestion of dsRNA homologous to the targeted transcript by the feeding stages of the nematode. As a result, it should knockdown functions involved in later stages of parasitism. RNAi silencing of two genes essential for RKN development in transgenic tobacco – a splicing factor and an integrase – was demonstrated by Yadav et al. (2006). In the few females that developed on transgenic plants, no transcript of these two genes was detected. However, no evidence was provided for the presence of siRNA in the transgenic plants. Huang et al. (2006b)

reported the development of transgenic *A. thaliana* plants expressing dsRNA homologous to 16D10 transcripts described above. They showed that the dsRNA was produced in the transgenic plants and processed to generate 21 bp siRNA. The infection of these transgenic plants with all four RKN species resulted in smaller numbers of galls and eggs than usual (Huang et al., 2006b). Although the detection of siRNA in nematodes has not yet been reported, RNAi represents the most effective tool for determining gene function in RKNs (Gheysen and Vanholme, 2007).

## Combining plant and RKN genomics

While some free-living nematode genomes have recently been fully sequenced (*Caenorhabditis elegans*, *C. briggsae*), plant parasitic nematode genome sequencing projects are underway (*M. hapla*, *M. incognita*; D. Bird, P. Abad, personal communication). These data will generate new opportunities for comparative genomics of nematodes belonging to different functional and ecological traits. Knowledge of the full genome sequences of the pathogens and the host plant *A. thaliana* will undoubtedly generate new opportunities for studying the interaction between RKNs and plants.

Comparative genomics of plant and animal parasites and free-living nematodes will lead to the identification of pathogen-specific genes and provide insight into the evolutionary history of parasitism within the phylum Nematoda (Parkinson et al., 2004; Mitreva et al., 2005). The analysis of the variability of parasitism genes in field populations should make it possible to estimate the selective pressure acting on these genes. Positive selection has been already demonstrated for parasitism genes in other plant pathogens (Moury et al., 2002; Weber and Koebnik, 2006). RKN genes encoding secreted proteins under such positive selection are likely to be highly useful for studies of interactions with target proteins in the host plant cell, and will provide new insight into the molecular basis of plant–nematode interactions.

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