Plant–nematode interactions

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Root-knot nematodes and cyst nematodes are obligate, biotrophic pathogens of numerous plant species. These organisms cause dramatic changes in the morphology and physiology of their hosts. The molecular characterization of induced plant genes has provided insight into the plant processes that are usurped by nematodes as they establish their specialized feeding cells. Recently, several gene products have been identified that are secreted by the nematode during parasitism. The corresponding genes have strong similarity to microbial genes or to genes that are found in nematodes that parasitize animals. New information on host resistance genes and nematode virulence genes provides additional insight into this complex interaction.

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Abbreviations

AtSUC2	Arabidopsis thaliana *SUCROSE TRANSPORTER2*
EST	expressed sequence tag
map-1	*Meloidogyne avirulence protein-1*
***R* gene**	resistance gene
RNAi	RNA interference

Introduction

Nematodes, the tiny roundworms that make up the phylum Nematoda, are among the most abundant creatures on earth [1]. Most nematodes are free-living and sustain themselves by consuming bacteria or other microscopic organisms. Other species are parasites of plants or animals. Plant-parasitic nematodes can devastate a wide range of crop plants, causing billions of dollars in agricultural losses each year [2]. All plant parasitic nematodes are obligate parasites, feeding exclusively on the cytoplasm of living plant cells. The most economically important groups of nematodes are the sedentary endoparasites, which include the genera *Heterodera* and *Globodera* (cyst nematodes) and *Meloidogyne* (root-knot nematodes). Both the cyst and root-knot nematodes have complex interactions with their host, but there are characteristic differences in their parasitic cycles (Figure 1).

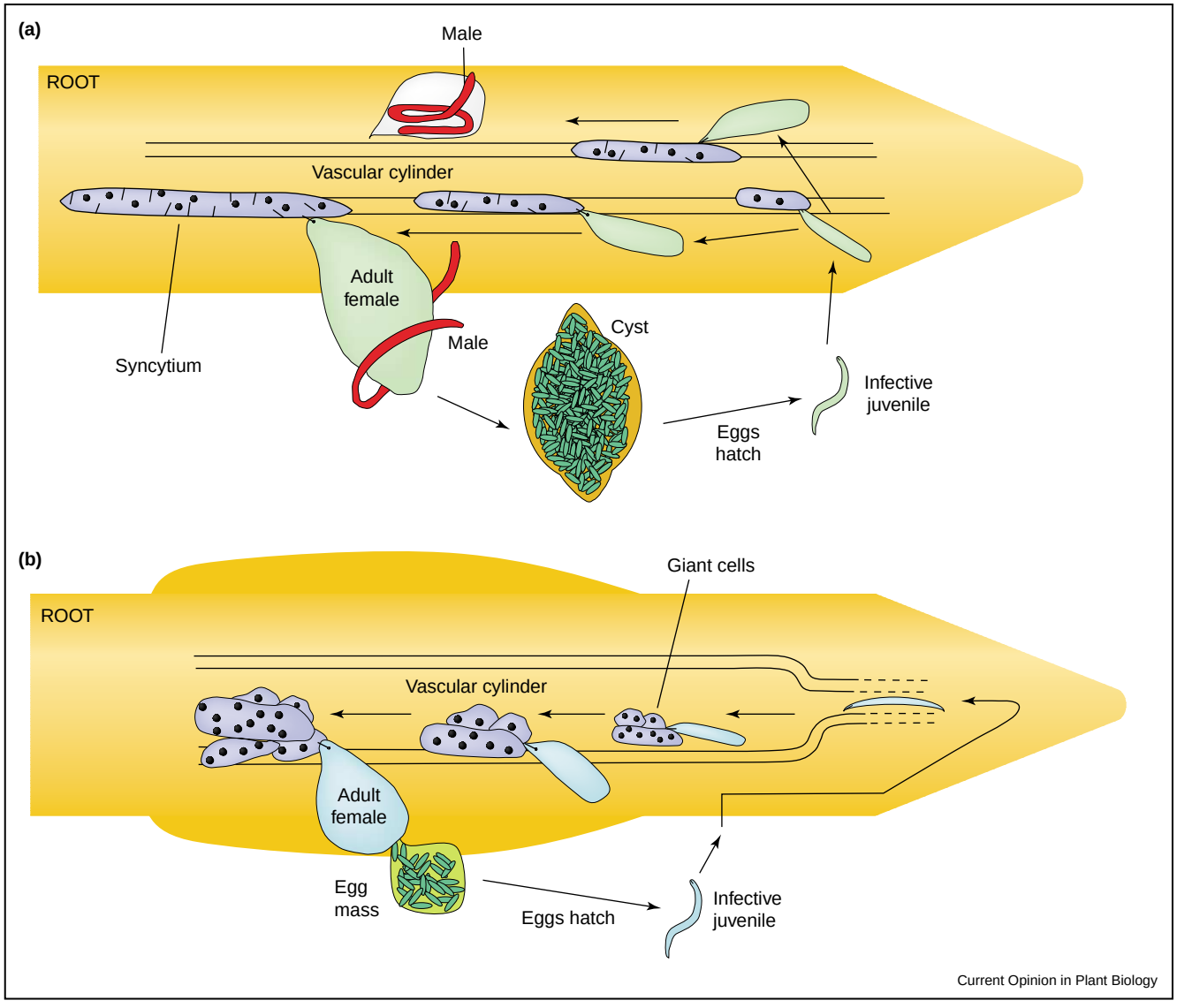
Cyst nematodes enter roots and move to the vascular cylinder, piercing cell walls with their stylets and disrupting cells as they go [3]. Upon reaching the vascular cylinder, they establish a feeding site, apparently by injecting stylet secretions. The formation of a feeding site is characterized by the breakdown of the cell walls between the initial feeding site cell and its neighboring cells, resulting in the development of a multinucleate syncytium [4]. Cyst nematodes undergo three molts inside the root before becoming adults. They generally reproduce sexually, and once fertilized, the female becomes full of eggs. After the female dies, its body becomes a protective cyst for the eggs.

In contrast to the cyst nematode, the juvenile of the root-knot nematode moves intercellularly after penetrating the root, migrating down the plant cortex towards the root tip. The juveniles then enter the base of the vascular cylinder and migrate up the root [5]. They establish a permanent feeding site in the differentiation zone of the root by inducing nuclear division without cytokinesis in host cells. This process gives rise to large, multinucleate cells, termed giant cells. The plant cells around the feeding site divide and swell, causing the formation of galls or 'root knots' [6]. The nematodes ingest the cytoplasm of the plant-derived giant cells through their stylets and, after three molts, develop into pear-shaped, egg-laying females. Both giant cells and syncytia serve as metabolic sinks that funnel plant resources to the parasitic nematode.

Plant genes induced during a compatible plant–nematode interaction

Comparisons of host transcription patterns using a variety of techniques have indicated that nematode infection initiates complex changes in plant gene expression [7]. Genes that are induced in defense responses against other pathogens are also upregulated after inoculation with root-knot or cyst nematodes [8,9,10•]. However, a large number of the genes that are induced by infection are likely to contribute to establishing the parasitic interaction [7,10•]. For example, extensive changes in cell-wall architecture occur during the development of giant cells and syncytia. It is not surprising, therefore, that nematode infection upregulates genes that encode host cell-wall-degrading enzymes. Host endoglucanase and polygalacturonase genes are upregulated after infection with root-knot or cyst nematodes [11••,12–14]. The expression patterns of endoglucanase genes are consistent with their having a role in syncytium formation and giant cell development [11••,12]. A putative pectin acetylesterase gene homolog is upregulated in *Arabidopsis* in both syncytia and

Figure 1



Life cycles of **(a)** cyst nematodes and **(b)** root-knot nematodes.

pre-giant cells, but not in mature giant cells, suggesting its role in the formation of feeding cells [15•].

Genes that function in metabolic pathways, cell-cycle progression and water transport are among those whose expression is increased in and around feeding cells [7,16]. Analysis of mutants and reporter-gene constructs indicates that auxin-response genes are induced in the susceptible response to cyst nematodes, and could account for some of the changes in gene expression [17]. Ethylene is also increased and appears to be a positive regulator of susceptibility to cyst nematodes [18]. The *Arabidopsis* sucrose transporter gene *AtSUC2*, which is normally expressed in companion cells, is highly expressed in syncytia [19••]. The AtSUC2 protein may have a role in forming or maintaining the metabolic sink activity of syncytia. However, the *AtSUC2* gene is not expressed in giant cells, which also are strong nutrient sinks.

Root-knot nematodes and endosymbiotic rhizobia induce similar structures within the host root, and this observation has stimulated comparative studies of gene induction during the two processes. Orthologs of PHAN and KNOX, transcription regulators that are required for the formation and maintenance of meristems, are co-localized in the feeding sites of root-knot nematodes and in *Rhizobium*-induced nodules [20•]. The early nodulation gene *ENOD40* and the cell-cycle gene *CCS52a* are

also upregulated upon nematode infection [20•,21•]. However, similarities between nodule and gall formation may be limited. Favery *et al.* [21•] analyzed 192 nodule genes from a *Medicago truncatula* expressed sequence tag (EST) library. Only two of these genes, nodulin 26 and cyclin D3, were upregulated upon nematode infection whereas 38 genes were upregulated in nodules.

Several genes are downregulated after nematode infection [7,10•]. Many of these are involved in pathogen defense responses, suggesting that the nematode actively suppresses the host defense response [7]. For example, a transcription factor of the ethylene-responsive element binding protein (EREBP) family that regulates defense gene expression is downregulated after infection of *Arabidopsis* with the sugar beet cyst nematode and after infection of susceptible soybean with soybean cyst nematode [22]. Interestingly, the expression of the soybean gene increases after infection of the resistant cultivar Hartwig [23]. Another EREBP family member is upregulated after *Arabidopsis* infection, however, perhaps reflecting the complex functions of this transcription factor family in plant–nematode interactions [10•].

Role of nematode secretions in parasitism

Plant-parasitic nematodes secrete substances through their stylet, a hollow, protrusible spear at the anterior of the worm. These secretions emanate from the nematode's two subventral and one dorsal esophageal gland cells, and appear to play a crucial role in infection and in the formation of host feeding cells. Because of their secretory activities, the subventral glands are thought to be important for the early stages of parasitism and the dorsal gland for the development and maintenance of feeding sites [24]. In the past few years, several proteins have been identified in nematode secretions and, in some cases, their roles in parasitism have been determined (Table 1).

The first gene that corresponds to a protein secreted from a cyst nematode esophageal gland to be characterized encodes a β-1,4-endoglucanase or cellulase [25]. Homologous genes have been identified in root-knot nematodes and in other cyst nematode species [26,27•]. Immunolocalization in tobacco roots infected by the tobacco cyst nematode localized the nematode-encoded cellulase along the migratory path but not in the syncytium, suggesting that it has a role in the infection process [12]. Genes encoding other cell-wall-degrading enzymes, including pectate lyase and polygalacturonase, have also been identified in plant-parasitic nematodes. In several cases, their transcripts have been localized to the subventral gland [28–30]. The encoded enzymes are likely to function in softening the cell wall and to facilitate nematode movement through the root.

A particularly intriguing gene family that is expressed in root-knot nematode esophageal glands encodes chorismate mutase [31]. Chorismate is a precursor in the biosynthesis of aromatic amino acids, and chorismate-derived compounds include the auxin indole-3-acetic acid (IAA) and the defense-related compound salicylic acid. Transgenic expression of the nematode chorismate mutase gene *MjCM-1* in roots suppresses lateral root formation and the development of the vascular system [32••]. The altered phenotype can be rescued by exo-

Table 1

Some gene products that are secreted from the esophageal glands of plant-parasitic nematodes.

Gene product	Species in which identified	Organisms with close homologs	Possible function	Reference(s)
β-1,4 endoglucanase (cellulase)	*G. rostochiensis* *Globodera tabacum* *Heterodera glycines* *Heterodera schachtii* *Meloidogyne incognita*	Bacteria	Cell-wall degradation	[12,25,26,58–60]
Pectate lyase	*Meloidogyne javanica* *G. rostochiensis* *H. glycines*	Bacteria and fungi	Cell-wall degradation	[28–30]
Polygalacturonase	*M. incognita*	Bacteria	Cell-wall degradation	[61]
Chorismate mutase	*H. glycines* *M. javanica* *G. rostochiensis*	Bacteria	Alter auxin balance, feeding cell formation	[32••,33•,34]
Thioredoxin peroxidase	*G. rostochiensis*	Animal parasitic nematodes	Breakdown of H_2O_2, protect against host defenses	[40]
Venom allergen-like protein	*M. incognita* *H. glycines*	Animal parasitic nematodes, *C. elegans*	Early parasitism?	[62,63]
Calreticulin	*M. incognita*	Animal parasitic nematodes	Early parasitism?	[38•]

genous application of IAA, suggesting that the expression of MjCM-1 reduces auxin levels [32••]. Homologs of chorismate mutase have also been identified in cyst nematode species [33•,34]. Interestingly, the chorismate mutase genes of the soybean cyst nematode have polymorphisms that correlate with virulence on resistant soybean cultivars [33•].

Genes that encode cell-wall-degrading enzymes and chorismate mutase are not found in *Caenorhabditis elegans* or most other animals, and are most similar to genes encoded by microorganisms (Table 1). The possibility that these genes correspond to contaminating cDNAs has been excluded by several criteria. Most of the cDNAs carry a 5′ *trans*-spliced leader sequence, a characteristic of many nematode transcripts. Southern blots, *in situ* hybridization and the presence of introns and polyA tails all support a nematode origin for these genes. The microbial gene similarity has led to speculation that these genes were transferred into plant-parasitic nematodes by horizontal gene transfer from soil microbes [24,35].

Direct investigation of salivary secretions has been limited by the difficulty of acquiring sufficient starting material. Several substances, including root diffusate, 5-methoxy-N,N-dimethyltryptamine oxalate and resorcinol, can stimulate secretion from the stylets of cyst or root-knot nematode juveniles [36,37,38•]. Analysis of these secretions has identified cellulases, superoxide dismutase and several proteases [39,40]. Antibodies produced against stylet secretions identified a gene that encodes a thioredoxin peroxidase from a cDNA library [40]. This enzyme has been found in several animal-parasitic nematode species and is thought to suppress host defense. It is found on the surface of invasive potato cyst nematode juveniles, consistent with a role in repressing reactive-oxygen-mediated host defense.

Other gene products in the secretions of plant-parasitic nematodes are most similar to proteins secreted by animal-parasitic nematodes. Clones identified from cDNA that was derived from microaspirated esophageal gland cytoplasm encode venom allergen-like proteins, resembling those secreted by animal-parasitic nematodes [41]. Analysis of resorcinol-induced secreted proteins from root-knot nematodes by two-dimensional gels and microsequencing identified calreticulin, a calcium-binding protein that is secreted by animal-parasitic nematodes [38•]. The presence of these proteins in secretions of both plant and animal parasites may indicate that they have a general role in parasitism.

The recent characterization of small molecules in nematode secretions has been limited. A low-molecular-weight peptide that is secreted by cyst nematodes has been shown to stimulate the proliferation of both leaf protoplasts and human peripheral blood mononuclear cells [37]. Other small peptides and non-peptide molecules in the stylet secretions may also have roles in parasitism.

Genome-wide approaches with plant-parasitic nematodes

Broad investigations of the genomes and gene products of plant-parasitic nematodes are also underway. More than 200 000 nematode ESTs from 28 nematode species, excluding *C. elegans* but including 19 animal- and seven plant-parasitic nematode species, have been produced [42]. Existing EST collections from plant-parasitic nematodes are mostly derived from eggs and infective juveniles, but future projects will likely expand to include the parasitic stages [27•,34,42]. DNA-sequence analyses of these ESTs have shown that they contain most of the genes previously identified in stylet secretions, indicating that random EST analysis may be an efficient approach for identifying both genes that are involved in parasitism and possible targets for nematode control. The genome sequence of *C. elegans* provides a valuable resource for studies of plant-parasitic nematodes because 66% of *Meloidogyne incognita* EST clusters have a *C. elegans* homolog [42,43].

Some of the most interesting nematode genes that have roles in plant parasitism are likely to be those with homology to microbial genes or that have no homologs in current databases. At present, genetic analysis is not a viable approach for determining the role of these genes but other techniques have promise. *In situ* hybridization has been applied extensively to study the tissue specificity and developmental expression of nematode genes [29,44]. Microarray analyses, differential-display methods, and real-time PCR using transcripts from pre-parasitic and parasitic nematodes are providing additional information on the roles of specific genes in nematode development and parasitism [29,45,46]. The expression of candidate parasitism genes in plants is another approach to characterizing function; this approach has already provided strong support for a role for chorismate mutase in parasitism [32••]. Blocking gene expression by RNA interference (RNAi) has been widely used in *C. elegans*, but transferring the technology to plant-parasitic nematodes has been challenging because of their thick cuticles, obligate parasitic feeding and lack of selection for transformation. However, the silencing of specific genes with double-stranded RNA has been recently demonstrated in cyst nematodes and should be a powerful tool for examining plant–nematode interactions [47••].

Host resistance genes and nematode virulence

Numerous genes that confer resistance against plant-parasitic nematodes have been described, and several of these have now been cloned [6,48]. The best-studied of these genes is the tomato gene *Mi*, which confers resistance against three species of root-knot nematode. *Mi* also confers resistance to some isolates of the potato

aphid *Macrosiphum euphorbiae* and to the white fly *Bemisia tabaci* [49,50]. The encoded protein contains a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region, protein motifs that are found in numerous plant resistance genes (*R* genes) against a variety of pathogens [51]. Other recently cloned nematode *R* genes, *Gpa2* and *Hero*, also belong to the NBS-LRR family [52,53•]. However, nematode resistance gene sequences do not cluster together. The sequence of *Gpa2*, for example, is much more similar to that of the virus resistance gene *Rx* than to those of other nematode resistance genes [52].

Avirulence genes — that is, single pathogen genes that are required for *R*-gene-mediated resistance — have been identified in bacteria, viruses and fungi [51]. To date, no avirulence genes have been conclusively isolated from nematodes, although there has been progress in this area. There is genetic evidence for avirulence genes in *Globodera rostochiensis* that correspond to the resistance gene *H1* [54]. Genetic analyses of inbred strains of soybean cyst nematodes have identified dominant and recessive determinants of parasitism on different soybean lines [55].

The root-knot species against which *Mi* is effective does not reproduce sexually, making Mendelian analysis of its avirulence and pathogenicity genes impossible. Nearly isogenic strains of root-knot nematodes that differ in virulence in the presence of *Mi* have been used to investigate pathogenicity [56,57]. Differential-marker analysis identified a polymorphic band that was present in avirulent strains but absent from closely related virulent strains of *M. incognita*. The corresponding gene, *Meloidogyne avirulence protein-1* (*map-1*), was cloned and found to encode a protein that localized to nematode amphidial secretions [57]. Secretions from the virulent and avirulent nematodes were not compared, however, and functional analysis of *map-1* has not yet been carried out. A transcript that is present in avirulent but lacking in virulent *Meloidogyne javanica* has also been identified (CA Gleason, VM Williamson, unpublished data). However, this gene does not resemble *map-1*, suggesting that there may be more than one gene that can mediate nematode recognition in tomato plants that have the *Mi* gene.

Conclusions

Despite the problems of working with obligate parasitic nematodes, much insight has been gained into the complex interactions between these organisms and their hosts. In a susceptible response, the nematode uses its esophageal gland secretions to harness expression of the plant's own genes and to establish feeding structures. The homology of genes encoding several of these secretions to bacterial genes suggests that horizontal gene transfer may have been a key in the development of nematode parasitism. Genes that are shared with animal parasites are also induced in plant-parasitic nematodes and may have a role in evading host defense.

Substantial agricultural losses are caused by nematodes each year throughout the world. Currently, measures to control plant-parasitic nematodes are limited and include the use of agrochemicals such as methyl bromide or planting crops that have natural resistance. The availability of chemical pesticides is decreasing and host resistance is limited. Nematode populations that are virulent on resistant plants continue to emerge [56]. The identification of nematode genes that are involved in parasitism and other nematode-specific processes, as well as the utilization of nematode-inducible plant genes, will be valuable resources for creating new forms of durable plant resistance [47••]. Newly established tools in plant-parasitic nematode biology, such as RNAi, microarrays, and nematode genome projects, should help to expedite the process of gene discovery.

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