ELSEVIER

Signaling between nematodes and plants

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After hatching in the soil, root-knot nematodes must locate and penetrate a root, migrate into the vascular cylinder, and establish a permanent feeding site. Presumably, these events are accompanied by extensive signaling between the nematode parasite and the host. Hence, much emphasis has been placed on identifying proteins that are secreted by the nematode during the migratory phase. Further progress in understanding the signaling events has been made recently by studying the host response. Striking parallels can be drawn between the nematode–plant interaction and plant symbioses with other microorganisms, and evidence is emerging to suggest that nematodes acquired components of their parasitic armory from those microbes.

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Abbreviations

ARR5	Arabidopsis *RESPONSE REGULATOR5*
CLV1	*CLAVATA1*
EST	expressed sequence tag
GC	giant cells
HGT	horizontal gene transfer
KNOX	*KNOTTED1*
PHAN	*PHANTASTICA*
RKN	root-knot nematode(s)
RNAi	RNA interference

Introduction

It has been just one year since a comprehensive review on nematode–plant interactions was published in *Current Opinion in Plant Biology* [1••], and this article remains essential reading. However, findings since then have contributed new insights, and in this review, I present a different view of the nematode–plant interaction that emphasizes signaling, parallels with other plant–microbe interactions (particularly plant symbioses with rhizobial bacteria) and the evolution of parasitism. I also alert the readers to unpublished data that may invite a further review in another year's time.

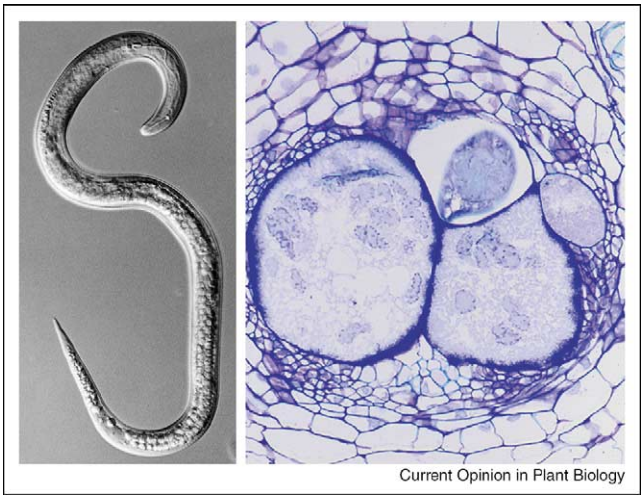
Parasitic nematodes represent the largest source of essentially uncontrollable biotic stress experienced by plants, causing as much as $US 100 billion in annual losses of crops worldwide [2]. They are cosmopolitan pests that impact all crops to some degree. Different species have evolved different parasitic modes and, collectively, nematodes exploit all parts of the plant. Feeding strategies range from simple herbivory to the formation of permanent and stereotypical feeding sites in host tissues. The majority of crop damage is caused by the tylenchid nematodes, particularly the approximately 60 species of the genus *Meloidogyne* (the root-knot nematodes [RKN]), although the cyst nematodes are especially significant pests for some crops (e.g. *Heterodera glycines* on soybean; *Globodera* spp. on potato).

Aside from economic concerns, it is the ability of certain nematodes to seemingly hijack host development to make structures such as giant cells (GC) that makes them interesting to botanists. Hatched RKN larvae (Figure 1a) invade the root in the zone of elongation, and then migrate through the apoplast, ultimately reaching the developing vascular cylinder where the formation of GC is induced (Figure 1b). RKN are believed to feed exclusively from GC. Despite decades of work, two fundamental questions remain: what is the nature of the primary signal that is responsible for the induction of GC, and is there a specific signal for GC maintenance? A major impediment for attempts to answer these questions has been the absence of a genetic system for RKN. However, the demonstration of gene knockdown by double-stranded RNA interference (RNAi) in cyst nematodes suggests that there is potential to remedy this situation [3•]. RNAi has been validated in other parasitic nematodes [4], although optimism must be tempered by results from *Caenorhabditis elegans* that indicate that this phenomenon is conditioned by many genes [5], with certain tissues in wildtype worms (including neurons) being recalcitrant to RNAi. Nevertheless, recent findings suggest that we are on the verge of understanding the primary events of GC formation. Previous conjecture that understanding the basis of the host–pathogen interaction will lead to new insights into normal plant development [6] is beginning to be proven true.

What are giant cells?

An anonymous Myb that was isolated by subtractive enrichment of tomato GC transcripts [7] has been identified as an ortholog of *PHANTASTICA* (*PHAN*) [8] that is required for meristem maintenance [9••], suggesting that GC might be a type of induced meristem. This idea was strengthened by the discovery that other 'meristem

Figure 1



(a) Newly hatched RKN larva. **(b)** Transverse section through a RKN feeding site revealing the presence of two prominent giant cells.

genes', including *KNOTTED1* (*KNOX*), also are expressed in GC [10]. Spatial mapping of *PHAN* and *KNOX* transcripts in the roots of the legume *Medicago truncatula* showed that other induced meristem cells in addition to GC, specifically those that give rise to lateral roots and rhizobia-induced nodules, also express these genes. Conversely, the nodule-regulation genes *ccs52* and *EARLY NODULATION40* (*ENOD40*) were also found to be expressed in young GC [11]. Comparison of the expression of 192 genes in established mature nodules and established GC revealed few significant transcriptional similarities [12], which is not surprising given that the anatomy and function of these cells are very different.

Genetic evidence points to a role for *KNOX* in the regulation of hormonal response pathways [9••], and there are indications that auxin plays a role in the induction and maintenance of feeding sites by cyst nematodes [13]. Cytokinins have been implicated in the formation of nitrogen-fixing nodules [14•], and the *ENOD40* gene from *Medicago sativa* is induced after exogenous application of cytokinin [15]. Activation of the cell cycle appears to be necessary for GC formation [16], and it is generally assumed that cytokinins influence the cell cycle at the $G_1 \rightarrow S$-phase transition to initiate division in non-cycling cells [17]. However, use of the cytokinin-responsive Arabidopsis *RESPONSE REGULATOR5* (*ARR5*) gene promoter (driving a β-glucuronidase [*GUS*] reporter) as a tool to spatially and temporally map the cytokinin response in *Lotus japonicus* yielded some unexpected findings [18••]. First, as assayed in this system, the cytokinin response is not strictly coupled to cell division *per se*. *ARR5* expression is undetectable in the dividing initial cells during the early stages of lateral root formation, and only becomes apparent once the primordium has organized into a definable meristem. In contrast, the cytokinin response is evident from the earliest stages of the development of nitrogen-fixing nodule primordia, and declines as the nodule emerges from the root. No cytokinin response was detectable during root penetration and migration by RKN, nor was *ARR5* expression seen in mature GC [18••]. Consistent with these findings, down-regulation of cytokinin levels *in planta* by transgenic expression of cytokinin oxidase genes produces roots that have significantly more lateral roots but fewer nodules than the roots of wildtype plants. Strikingly, the number of feeding sites that were induced by RKN also was reduced in these transgenic plants, even though established GC do not appear to mount a cytokinin response. One interpretation is that a spike of cytokinin is required at the onset of GC initiation. Experiments using cell-cycle inhibitors [19] revealed an initial transient requirement for cell cycle activation during GC formation. The action of the tomato locus *Mi* in conferring resistance to RKN also is temporally restricted to this period and exogenous application of cytokinin suppresses *Mi*-mediated resistance [20].

RKN have long been known to produce biologically active cytokinins [21] and this has been re-confirmed recently [22•], but whether nematode-produced cytokinin plays a role *in planta* remains unknown. The production of cytokinins directly by RKN is an appealing hypothesis, but other models are possible. Receptors such as *CLAVATA1* (*CLV1*), which plays an important role in maintaining meristem size [9••], are also expressed in the meristem. Interestingly, both rhizobia [23] and RKN [24••] hyperinfect plants that are homozygous for *hypernodulation and aberrant root formation1-1* (*har1-1*), a *CLV1* allele in *L. japonicus*. Similarly, beet cyst nematodes hyperinfect *Arabidopsis* plants that carry a mutation in the equivalent gene *clv-1* (John Jones, pers. comm.). What makes these results especially intriguing is the computational discovery of a potential CLV3-like peptide in a cyst nematode expressed sequence tag (EST) dataset [25••]; CLV3 is the presumed ligand for the CLV1 receptor [9••]. Like the possible role for nematode-produced cytokinin, any role for nematode-encoded CLV3-like peptides awaits formal confirmation. It also will be important to distinguish primary signaling between the nematode and host from secondary host responses. Indeed, in the afore-mentioned experiments using the *ARR5* promoter, a cytokinin response was observed in curled/deformed root hairs shortly after interaction with rhizobia [18••], which presumably was a secondary response. Although it is not yet known whether nematodes induce a cytokinin response in root hairs, preliminary experiments suggest that RKN produce a diffusible signal that can elicit root hair deformation and branching (DMcK Bird, unpublished). Analysis of *L. japonicus* plants that carry mutations in root-hair receptors [26•,27••] has suggested that the RKN signal has two components, one that acts through known receptor kinases and one that is independent of these kinases (DMcK Bird,

unpublished). The physical characterization of these signaling molecules will probably provide significant insight into the RKN–plant interaction.

RKN gene discovery

Beginning more than a decade ago, a large number of genes have been identified from RKN and related cyst nematode genera. Initially, genes were isolated on the basis of specific biological criteria, such as the presence of their protein product in nematode secretions. Secretions have been a favorite target for study by plant and animal parasitologists alike because they largely define the physical interface between host and parasite [28–30]. The use of antibodies raised against nematode-stylet secretions [31] to screen expression cDNA libraries proved to be a remarkably powerful approach that identified numerous genes [32–37]. Two general pictures have emerged from these studies.

First, during their migration through the root, plant-parasitic nematodes secrete a wide range of enzymes (including cellulases, chitinases and extensins) that are specifically targeted to degrade or modify host tissues, as well as other secretion products whose precise role less obvious [38–41]. Recently, attempts have been made to catalog the entire proteome secreted from root-knot [42] and cyst nematodes [43,44], dubbed the 'parasitome', by constructing specific sub-libraries from nematode pharyngeal glands [45]. It is hoped that characterization of the parasitome will shed new light on the nematode–plant interaction.

Second, it has been hypothesized that certain RKN genes were acquired from bacteria via horizontal gene transfer (HGT) [33,38,46–48]. Although this hypothesis is controversial [49], the results of a computational sampling that was undertaken blindly (i.e. without pre-conceived notions of gene function) support the HGT argument [50••]. In addition to identifying genes already postulated to have been acquired by HGT, several new candidate genes that have apparent roles in parasitic function were revealed by such analysis [51•], including several that are closely related to rhizobial genes. The most striking of these genes is that encoding NodL [50••,52••], an enzyme previously believed to be unique to rhizobia that is a component of the biosynthetic pathway for Nod factors (the family of signaling molecules responsible for root-hair deformation). Whether or not the enzymatic function of RKN NodL is the same as that in rhizobia is not known, but an attempt to rescue a rhizobial *NodL* null by transgenic expression of the nematode gene was unsuccessful (DMcK Bird, unpublished). Nevertheless, the preliminary evidence that nematode signaling at the root surface is influenced by mutation of the proposed Nod-factor receptor(s) [26•,27••] is consistent with an RKN interacting with plant via the Nod-factor pathway.

A significant number of tylenchid nematode genes have been identified as ESTs [53–55] and can be conveniently queried at www.nematode.net. The current datasets are summarized in Table 1, and work on adding sequences from several additional species (including *Meloidogyne konanensis* and *Meloidogyne exigua*) and developmental stages (including males) is in progress. A preliminary analysis of genes from species across the RKN genus to identify orthologs suggests that the current EST set defines at least 4000 distinct genes, which correspond to an estimated 20% of the RKN genome (DMcK Bird, unpublished). Groups of orthologs that are present in multiple species are termed 'clusters of orthologous groups' (COGs) [56]. These groups are identified on the basis of a reiterative series of reciprocal pairwise comparisons for each gene in each species, leading to a network that defines the COG. Some genes are likely to be highly conserved across the genus, whereas others might be very diverged or even species-specific. The *Meloidogyne artiellia* polyglutamate synthase gene seems to be an example of such a species-specific gene [48]. The *NodL* gene appears to be generally present in RKN species but is yet to be detected in other tylenchid nematodes (DMcK Bird, unpublished).

Table 1

Tylenchid nematode gene sequences (mainly ESTs) from all sources available in GenBank as of March 11, 2004. Species with fewer than 100 entries are not listed.

Nematode species	Number of entries
Globodera pallida	1880
Globodera rostochiensis	6057
Heterodera glycines	24 449
Heterodera schachtii	2837
Meloidogyne arenaria	5064
Meloidogyne chitwoodi	12 254
Meloidogyne hapla	17 079
Meloidogyne incognita	16 605
Meloidogyne paranaensis	2514
Pratylenchus penetrans	1940
Zeldia punctata	395

In light of the hypothesis some RKN genes may have been acquired from bacteria, it is intriguing to speculate that HGT events may have been key steps in the evolution of the diverse tylenchid species [51•]. I hope that an improved understanding the sequence diversity across the *Meloidogyne* genus and between tylenchid genera will answer many important questions about the nematode–plant interaction that bear directly on practical agriculture. For example, why does the genus *Meloidogyne* have such a broad host range whereas the host ranges of other tylenchid nematodes are very restricted? What determines the actual host range of a particular *Meloidogyne* species? The biology that underlies these important differences must ultimately be encoded in the genomes of the particular nematode species. It is reasonable to suspect that the presence of genes or alleles that are

unique to particular species of RKN contribute to the biological differences between the species. It is also likely that qualitative and quantitative differences in gene expression may play a significant role. Understanding which nematode genes become active or inactive and when will help to identify regulatory networks and biochemical pathways that are necessary for the establishment of the parasitic interaction, and will shed new light on the resistance response.

Conclusions

The adoption of the model legumes *M. truncatula* and *L. japonicus* as hosts for nematodes [11,12,18••,24••] has allowed comparisons to be made between the mutualistic symbioses that are mediated by rhizobia and arbuscular mycorrhizal fungi and parasitic nematode–plant interactions. Discerning differences and similarities between these associations is proving to be a productive strategy in extending our understanding of both of these interactions, and further, is providing new insights into fundamental plant processes.

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