Parasitism Genes: What They Reveal about Parasitism

Eric L. Davis(✉), R.S. Hussey, and Thomas J. Baum

Abstract Nematodes are parasites of plants and animals that have evolved diverse and often specific mechanisms to promote a given parasitic lifestyle (Baldwin et al. 2004; Jasmer et al. 2003), including modifications of developmental and reproductive potential, dissemination amongst and location of primary or alternate hosts, and survival strategies in the absence of a suitable host or favorable environment. The genetic pathways underlying these lifecycle adaptations may have parallels with or origins in nonparasitic nematode species that must also adapt to a dynamic or unstable niche. Distinct to the parasites, however, are adaptations to obtain organic nutriment while living in or on another organism. The products of such *parasitism genes* "may be manifested as morphological structures that provide access to parasitism of a particular host (e.g. a nematode stylet) or they may play critical physiological roles in the interaction of the nematode with its host" (Davis et al. 2000).

1 Introduction

The stylet (Fig. 1), a protrusible oral spear, is the primary adaptation that allows all plant-parasitic nematodes to breach the plant cell wall to access host cell nutrients, which is essential for nematode growth and reproduction (Hussey 1989). The stylet is a hardened structure of sclerotized cuticle that connects directly to the lumen of the alimentary canal in the nematode esophagus (Bird and Bird 1991), and in all but the trichodorid species, the stylet itself has a hollow lumen with an aperture that provides a continuous channel between the feeding nematode and the parasitized

E.L. Davis
Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, USA
e-mail: eric_davis@ncsu.edu

Plant Cell Monogr, doi:10.1007/7089_2008_29

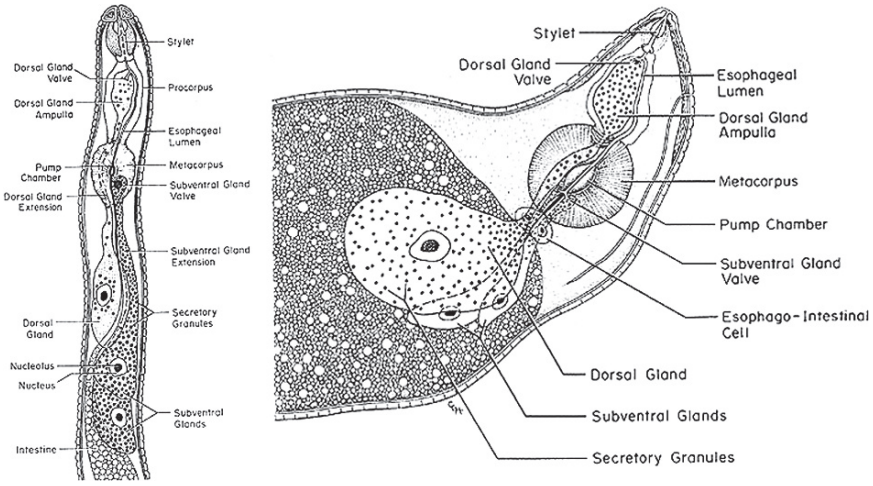

Fig. 1 Illustrations of the anterior portions of the migratory and sedentary stages of endoparasitic nematodes that contain esophageal gland secretory cells associated with the nematode stylet, a hollow oral feeding spear. (**a**) A migratory, pre-infective second-stage juvenile with the two subventral esophageal gland cells packed with secretory granules. (**b**) A swollen female from within infected roots with reduced subventral glands and an enlarged dorsal esophageal gland cell now packed with secretory granules. Reprinted from Hussey (1989) with permission from Annual Reviews

host plant cell. Successful parasitism requires feeding from a living host cell (obligate biotrophy), and for some nematode species, modifications of the host cell are required to promote a sustained feeding relationship.

Detailed investigations summarized in this volume and elsewhere indicate that molecules at the interface of the nematode and host play critical roles in the parasitic process (Hussey 1989; Davis et al. 2004, 2008; Jasmer et al. 2003; Maizels et al. 2004). Potential origins of interacting molecules from the nematode include a dynamic surface coat and natural openings such as the excretory-secretory pore, anal and reproductive openings, chemosensory organs, and the oral aperture. Since modifications of host plant cells for feeding occur at the nematode anterior, molecules secreted from the stylet and amphids emerge as the most likely organs to be directly involved in adaptations for plant parasitism. Most notably, the esophageal gland cells in tylenchid nematodes (Hussey 1989) have evolved into three relatively large secretory cells (one dorsal and two subventral) that are connected to the esophageal lumen and stylet through complex valves (Fig. 1). In the root-knot and cyst nematodes, change in the morphology, activity, and contents of the esophageal gland cells occurs throughout the course of parasitism (Davis et al. 2000, 2004). Activity within the subventral gland cells predominates in the migratory stages of these nematodes while enlargement and activity in the dorsal gland cell is dominant in the subsequent sedentary stages (Hussey 1989). The secreted protein products of parasitism genes expressed in these gland cells have been the subject of intense investigation and form a primary foundation of what we currently know about

nematode parasitism genes (Baum et al. 2006; Davis et al. 2004, 2008; Jasmer et al. 2003; Mitchum et al. 2007; Van Holme et al. 2004).

2 Parasitism Gene Discovery

Early investigations of plant-parasitic nematode secretions were biochemical in nature out of necessity and included elegant experiments that provided insights valuable to this day (Bird 1968; Hussey 1989; Veech et al. 1987). The difficulties in working with microscopic obligate parasites, especially parasitic stages from within plant tissue, remain today and provided a challenge to identify the point of origin of isolated nematode molecules in early investigations. Methods to stimulate stylet secretions from infective larvae – so-called second-stage juveniles (J2) – of the root-knot and cyst nematodes (Fig. 2) in vitro using resorcinol (McClure and von Mende 1987) and 5-methoxy DMT oxalate (Goverse et al. 1994), respectively, provided increased quantities of secretory proteins for direct analyses and antibody production. Relatively recently, secretions collected from nematode J2 stimulated in vitro were subjected to proteomic analyses, and the amino acid sequence generated from the analyses has been used to identify candidate parasitism genes (De Meutter et al. 2001; Jaubert et al. 2002). The adoption of monoclonal antibody technologies to specifically tag and isolate target nematode secretory proteins (Fig. 2) was used to identify discrete secretory proteins in plant nematodes and to monitor their differential synthesis during plant parasitism (Davis et al. 2000, 2004). An amino-terminal sequence of a cyst nematode subventral esophageal gland antigen that was affinity-purified with

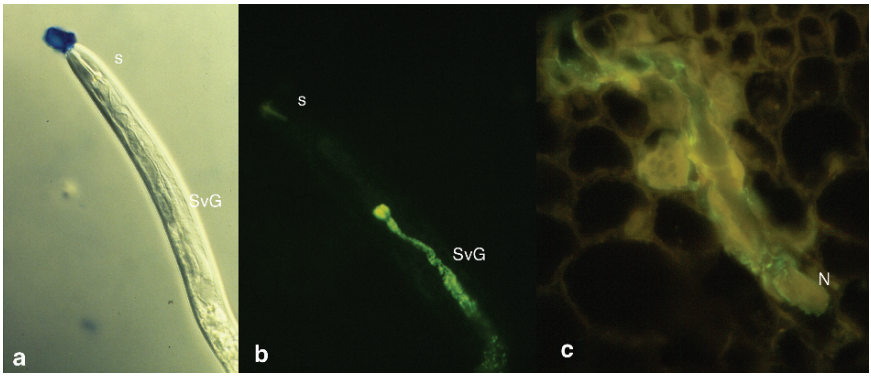

Fig. 2 Proteins produced in the nematode esophageal gland cells and secreted through the nematode stylet. (**a**) Esophageal gland proteins stained blue with Coomassie Briliant Blue are secreted from the stylet of a soybean cyst nematode (SCN) second-stage juvenile (J2) that has been incubated in the serotonin agonist, 5-methoxy-DMT oxalate. (**b**) Fluorescence immunolocalization of the antigen of monoclonal antibody MGR48 (Deboer et al. 1998) in secretory granules synthesized within the subventral esophageal gland cells of a SCN J2. (**c**) Immunolocalization of beta-1,4 endoglucanase (green fluorescence) secreted from an infective SCN J2 along its path of intracellular migration through a soybean root. Reprinted from Wang et al. (1999) with permission from APS Press

monoclonal antibody MGR48 (De Boer et al. 1996) was used to develop PCR primers to obtain the first expressed parasitism genes isolated from plant-parasitic nematodes, beta 1,4-endoglucanases (Smant et al. 1998). This discovery was able to confirm a point of origin of these cell wall-modifying enzymes as suggested in earlier investigations (Deubert and Rohde 1971). The cyst nematode cellulases were the first endoglucanase genes to be cloned from an animal and their striking similarity to bacterial Family 5 glycosyl hydrolases provided some of the first evidence of potential horizontal gene transfer from prokaryotes to eukaryotes (Smant et al. 1998; Hotopp et al. 2007; Ledger et al. 2006; Keen and Roberts 1998). A technique developed for mRNA in situ hybridization in plant nematodes (De Boer et al. 1998) and polyclonal antibodies generated to the recombinant products of the cyst nematode endoglucanase genes confirmed endoglucanase expression exclusively within the subventral esophageal glands (Smant et al. 1998). The anti-endoglucanase sera were subsequently used to confirm for the first time (Wang et al. 1999) the secretion of a nematode esophageal gland protein into plant tissues (Fig. 2).

The rapid advance of techniques in molecular biology, including methods to work with sub-microgram quantities of starting material, ushered in an era of expressed gene characterization in plant-parasitic nematodes. Complimentary DNA (cDNA) amplified from mRNA that was isolated from the dissected anterior and posterior halves of hatched, preparasitic J2 root-knot nematode, *Meloidogyne javanica*, was used to screen cDNA clones derived from the anterior halves (which contained the esophageal glands) of J2 to isolate a gene encoding a secreted chorismate mutase (CM) expressed specifically within the nematode esophageal gland cells (Lambert et al. 1999). Interestingly, the root-knot nematode CM was also most similar to genes in bacteria, and expression of *Mjcm1* in bacteria complemented a CM-deficient mutant (Lambert et al. 1999). Combined with the cyst nematode endoglucanase gene discoveries, these data encouraged early speculation that a number of plant nematode parasitism genes were derived via ancient horizontal gene transfer (Davis et al. 2000). Analyses of the genomic organization of cyst nematode endoglucanase genes have identified differences in intron size with conservation of intron position (Yan et al. 1998) and one endoglucanase gene (*Hg-eng*-5) that lacks any introns (Gao et al. 2004a). Furthermore, multiple endoglucanase genes within close genomic proximity (Yan et al. 2001) suggested the potential for "pathogenicity islands" in plant-parasitic nematodes.

The use of cDNA-AFLP to compare life stages of parasitic nematodes also has been relatively successful for the isolation of potential parasitism genes. The observation (Perry et al. 1989) that secretory granules are synthesized within the subventral esophageal gland cells of *Globodera rostochiensis* J2 within eggs upon hydration, and that subsequent exposure of the same J2 within eggs to potato root diffusate stimulated secretory granule synthesis within the dorsal gland cell (Smant et al. 1997), was exploited to compare cDNA-AFLP profiles derived from nematodes in each treatment (Qin et al. 2000). A number of differential transcript-derived fragments (TDFs) were identified among the different *G. rostochiensis* treatments, and a useful program (GenEST) was designed to cross-reference the TDFs to expressed sequences tags (ESTs) derived from cDNA libraries (Qin et al. 2001). In situ mRNA hybridization was conducted with *G. rostochiensis* clones that were differentially

expressed in cDNA-AFLP, and a number of genes expressed exclusively within the esophageal gland cells were isolated. Similar cDNA-AFLP analyses have been conducted in developmental stages of sugarbeet cyst nematodes (Tytgat et al. 2004) and among root-knot nematode near-isogenic lines (Neveu et al. 2003).

The most powerful and successful approach in identifying parasitism genes has been the direct microaspiration of the esophageal gland contents of multiple parasitic stages of *Heterodera glycines* and *Meloidogyne incognita* dissected from host roots to isolate mRNA and generate cDNA libraries that profiled esophageal gland gene expression throughout the parasitic cycle (Gao et al. 2001a, 2003; Huang et al. 2003, 2004; Wang et al. 2001). Methods of cDNA synthesis that favor inclusion of 5'-end sequence of transcripts were used to construct all gland-cell libraries. A number of cDNA selection procedures including yeast-secretion signal peptide selection (Wang et al. 2001) and subtraction against cDNA derived from nematode intestinal tissues (Gao et al. 2001a; Huang et al. 2004) have been used to identify clones within the gland cell cDNA libraries that encode secreted products that are exclusively expressed within the esophageal gland cells. Expressed sequence tag analyses have also been conducted with relatively complex gland-cell cDNA libraries that also incorporated hybridization with intestinal tissue cDNAs to gland cell macroarrays to enrich subsequent samples for unique ESTs (Gao et al. 2003; Huang et al. 2003). Putative parasitism genes were identified among gland cell cDNA clones using SignalP prediction of a putative secretion signal peptide (Nielsen et al. 1997) and confirmation of expression of the gene within the esophageal gland cells by mRNA in situ hybridization. Using these methods, more than 50 putative parasitism genes developmentally expressed in the esophageal gland cells have now been isolated in both *H. glycines* and *M. incognita*. With the exception of cell wall-modifying enzymes and a few other secreted products, relatively few common parasitism genes exist between *H. glycines* and *M. incognita* (R.S. Hussey, unpublished), and more than 70% of the parasitism gene sequences in both species have no significant database homology (i.e. so-called pioneers), indicating they may be unique to plant-parasitic nematodes.

A large-scale project designed to generate ESTs from multiple species of both plant and mammalian-parasitic nematodes had generated more than 400,000 total ESTs as of 2005 (McCarter et al. 2005; Mitreva et al. 2005a). These EST data are of tremendous significance to our understanding of nematode biology, including the potential discovery of new nematode parasitism genes. The ESTs are derived from mRNA of whole nematodes using several methods of cDNA synthesis, usually representing the life stages(s) that are most readily procured or in highest abundance. Plant-parasitic nematode ESTs (125,412) available (McCarter et al. 2005) by species include *Globodera pallida* (4,378), *Globodera rostochiensis* (5,941), *Heterodera glycines* (24,438), *Heterodera schachtii* (2,818), *Meloidogyne arenaria* (5,108), *Meloidogyne chitwoodi* (12,218), *Meloidogyne hapla* (24,452), *Meloidogyne incognita* (19,934), *Meloidogyne javanica* (7,587), *Meloidogyne paranaensis* (3,710), *Pratylenchus penetrans* (1,928), *Pratylenchus vulnus* (2,485), *Radopholus similes* (1,154), and *Xiphinema index* (9,351). The ESTs from *H. glycines* were generated from discrete, stage-specific cDNA libraries, providing a global

developmental gene expression profile (Elling et al. 2007a). Interestingly, relatively few of the predicted *H. glycines* parasitism genes discovered in gland cell-specific cDNA libraries have been identified among the whole nematode ESTs, highlighting the power of cell-specific isolation of expressed genes. Gene ontology and KEGG analyses of plant nematode EST datasets (McCarter et al. 2005), however, have characterized genes that may play roles in diverse biological functions that could include parasitism. Filters applied to analyze EST data from J2 of *M. incognita* have discovered several other genes that may be candidates of horizontal gene transfer from prokaryotes, including a gene similar to *nodL* that encodes an enzyme in the biosynthetic pathway of rhizobial Nod factor (Scholl et al. 2003). As with the gland cell libraries, the ESTs from whole J2 of *Heterodera schachtii* and *H. glycines* and parasitic stages of root-knot nematodes have been analyzed for the presence of a predicted secretion signal peptide sequence and several new parasitism gene candidates have been identified (Debreuil et al. 2007; Roze et al. 2008; Vanholme et al. 2006; A. Elling and T.J. Baum, unpublished).

Projects are in progress to generate the complete genome sequence of *M. incognita*, *H. glycines,* and *M. hapla* (Abad et al. 2008; K. Lambert, pers. comm.; Opperman et al. 2008) and will undoubtedly identify genes not represented among the available ESTs. Annotation of these genomes will be crucial to identifying genes involved in many biological processes, including parasitism. Development of physical maps and alignment with the genome will provide the roadmap to isolating genes of interest; and having genomes of multiple species available within a genus will accelerate this process and provide the opportunity for comparative genome analyses. Forward genetics are available for species capable of amphimixis, but the development of genetic maps faces many challenges. Controlled crosses are technically challenging, take months to analyze, and the obligate parasitic nature of the nematodes should render genetic mutations in parasitism genes lethal. Forward genetic analyses of plant nematodes have been relegated to identifying nematode lines that vary in virulence on resistant plant cultivars, which led to the genetic identification of virulence loci (Dong and Opperman 1997; Janssen et al. 1991). The developing genome sequence of plant nematodes should be instrumental in identifying candidate virulence genes. AFLP comparisons of isolates of *Meloidogyne* that were near-isogenic for their compatibility on tomato with resistance conditioned by the *Mi* gene have identified a secreted gene product localized to the nematode amphids that was correlated with virulence on *Mi* tomato (Semblat et al. 2001) and a transcript encoding a novel protein only in the avirulent nematode line that could be silenced to revert the line to virulent on *Mi* (Gleason et al. 2008).

3 Functional Analyses

As suggested from the difficulties of forward genetics with plant-parasitic nematodes described above, the functional analyses of putative nematode parasitism genes rely heavily on reverse genetics approaches. Another confounding factor to

the assessment of the roles of nematode genes in parasitism is the current lack of a practical and reproducible transformation system for plant-parasitic nematodes – i.e. no capability exists to introduce a gene construct into a plant nematode for expression or complementation analyses. Transformation of *Caenorhabditis elegans* with the plant nematode gene of interest can generate relevant biological information (Qin et al. 1998), but data pertaining to parasitic ability cannot be derived from this nonparasite. Undoubtedly, the ability to transform plant-parasitic nematodes is critical for widespread functional analysis of parasitism genes. For example, transgenic nematodes with parasitism genes labeled with GFP will assist in determining the destination of the parasitism proteins in host tissues.

Biochemical analyses of candidate parasitism gene products provide a definitive measure of function, and the activity of a number of nematode cell wall-modifying proteins has been assessed accordingly (Bera-Maillet et al. 2000; Gao et al. 2004a; Mitreva-Dautova et al. 2006; Popeijus et al. 2000; Qin et al. 2004). Expression of these genes in plant tissues can present an in vivo measure of function that may be extrapolated to the biological interaction. In fact, expression of any candidate nematode parasitism gene in plant tissues can yield comparable functional information, and examples of such are described in the following section. Since the products of multiple parasitism genes likely act in consort and may interact among themselves, observable plant phenotypes derived from in planta expression of individual parasitism genes may be rare and must be interpreted in this context. Expression constructs for plants often employ the CaMV 35S promoter for near constitutive expression of the transgene (Benfey and Chua 1990), but the use of alternative promoters that provide more controlled expression of the transgene should also be considered. Chemical-inducible promoters (Bohner et al. 1999; Padidam et al. 2003; Zuo et al. 2000) allow both spatial and temporal control of transgene expression provided that the nontarget effects of the inducing chemicals and appropriate controls are considered. Infiltration of plant tissues (usually leaves) with constructs of nematode genes in *Agrobacterium tumefaciens* or in viral expression systems (Chapman et al. 1992) offers a relatively rapid and high-throughput system to assess potential effects on plant tissues prior to whole plant transformation. Transgenes can be expressed and assessed in whole plants such as *Arabidopsis thaliana* and appropriate mutants, or hairy root systems may be adopted for more targeted effects of transgene expression on roots (Cho et al. 2000; Doyle and Lambert 2003; Huang et al. 2006a) given the relative ease of hairy root transformation in some plant species (e.g. soybean). Expression of nematode parasitism genes in any of the plant systems above can also provide substrate to isolate interacting molecules or complexes of host plant origin in co-immunoprecipitation assays that employ antibodies specific for nematode parasitism gene products (Huang et al. 2006a). Similarly, yeast two-hybrid assays have been used to identify an interaction between a nematode parasitism gene product and a specific domain of a host plant intracellular protein (Huang et al. 2006a).

Gene silencing using RNA interference (Fire et al. 1998) technology provides the potential for powerful analyses of parasitism gene function. The discovery that *C. elegans* can ingest double-stranded RNA (dsRNA) which then induces RNAi in

tissues distal to the gut (Timmons and Fire 1998) is particularly appealing for delivery of RNAi technology to plant-parasitic nematodes. The compounds resorcinol and octopamine, respectively, have been used to stimulate hatched J2 root-knot nematodes (Rosso et al. 2005) and cyst nematodes (Urwin et al. 2002) to ingest dsRNA in vitro as a component of "soaking" solutions and induce RNAi of the target nematode gene. RNAi of a gene encoding the major sperm protein of *H. glycines* induced by the soaking method was confirmed, and the RNAi effect was observed in subsequent generations of the nematodes after initial treatment (Urwin et al. 2002). As monitored by mRNA in situ hybridization, soaking J2 stage *M. incognita* in dsRNA of an expressed calreticulin gene induced RNAi within the nematode specimens (Rosso et al. 2005), but this RNAi soaking effect was relatively short-lived (<68 h). A decrease in infectivity of host plant roots and reduced reproductive rate were observed after RNAi soaking of J2 stage *G. rostochiensis* in dsRNA to its expressed endoglucanase gene (Chen et al. 2005) and *H. glycines* in dsRNA to a gene encoding dual oxidase (Bakhetia et al. 2005a). The RNAi soaking assays have demonstrated the ability to specifically silence a target nematode gene, including genes expressed in the esophageal gland cells (Lilley et al. 2007). The RNAi soaking technique requires relatively harsh conditions, including 2–5 mg ml^{-1} of dsRNA, to soak the J2, so potential nonspecific adverse effects on the J2 must be considered in subsequent infection assays. The technology to deliver dsRNA to nematode genes in transformed plant tissues (Wesley et al. 2001) for ingestion by feeding nematodes has the potential to provide a more natural basis for RNAi assays, including the ability to target genes that are expressed exclusively in parasitic stages of nematodes within host tissues (Gheysen and Vanholme 2007; Mitchum et al. 2007). The effects of such in planta RNAi of nematodes can be monitored at the cellular and tissue levels as well as for potential impacts on nematode development and reproduction. Vectors (Wesley et al. 2001) and promoters (Gheysen and Fenoll 2002) are available to express dsRNA directly in plant cells and nematode feeding sites, or alternatively, potential virus-induced gene silencing (Valentine et al. 2004) may be translocated to nematode feeding sites by introducing expression constructs in shoot tissues. Since the size exclusion limit (Urwin et al. 1997) of the feeding tube of sedentary endoparasites (Hussey and Mims 1991) like root-knot and cyst nematodes is less than approximately 40 kDa (~62-bp dsRNA), construct design must consider host-derived molecules of ingestible size (Bakhetia et al. 2005b; Davis et al. 2004). Double-stranded RNA of ingestible size may be produced, however, via the inherent RNAi machinery of host cells that includes a DICER enzyme to digest dsRNA into small interfering RNAs (siRNAs) of 21–23-bp dsRNA, a primary component of the gene silencing process (Novina and Sharp 2004). In this scenario, construct design may span an entire transcript and target members of a gene family, but potential nontarget effects must be considered as the population of siRNAs becomes more complex. The first published report describing successful effects of host-derived RNAi on plant infection by RKN targeted nematode-specific splicing factors and integrase genes essential to nematode cellular and developmental processes (Yadav et al. 2006) was soon followed by host-derived RNAi in soybean that targeted the nematode-specific major sperm protein of the soybean cyst

nematode (Steeves et al. 2006). These encouraging data provide support for the application of similar host-derived RNAi technology to study the function and importance of nematode parasitism genes.

The potential to bioengineer crops that provide RNAi of target nematode parasitism genes and disrupt the parasitic process represents a viable and flexible means to develop novel and durable nematode-resistant crops. Silencing of a root-knot nematode parasitism gene (*16D10*) that encodes a product that interacts with a plant transcription factor (Huang et al. 2006a) by expressing dsRNA in transgenic *Arabidopsis* resulted in transgenic plants that were highly resistant to the four common root-knot nematode species (Huang et al. 2006b). While these results validate the fundamental role of parasitism gene *16D10* in root-knot nematode parasitism of plants, they more significantly provide a "synthetic" resistance gene effective against the world's most damaging plant-parasitic nematodes whose range of resistance is not conditioned by any natural root-knot nematode resistance gene. Therefore, in planta RNAi silencing of *16D10* in root-knot nematodes could lead to the development of transgenic crops with effective broad host resistance to this agriculturally important pathogen and, equally as significant, root-knot nematode resistant crops for which natural resistance genes do not exist.

4 Meet the Candidates

The majority of candidate plant nematode parasitism genes identified to date encode polypeptides that are predicted to be secreted from the nematode esophageal gland cells into plant tissues via the stylet (Baum et al. 2006; Davis et al. 2004; Mitchum et al. 2007; Vanholme et al. 2004) (Fig. 3). As genomic, bioinformatic, and functional analyses progress, the roles of the parasitism genes encoding novel proteins (that now include the majority of candidates) will be elucidated. Our current knowledge of the putative parasitism genes that have database homologues or identifiable functional domains is summarized below.

4.1 Cell Wall-Modifying Proteins

4.1.1 Beta-1,4 Endoglucanases

Early evidence of the production and secretion of cell wall-degrading enzymes by plant-parasitic nematodes (Deubert and Rohde 1971) was confirmed by the first report of expressed beta-1,4 endoglucanase genes in cyst nematodes (Smant et al. 1998). Two members of the cyst nematode endoglucanase gene family that consists of at least six members (Gao et al. 2004a) included *eng-1* which contained a bacterial type II carbohydrate binding domain (CBD) and *eng-2* which did not. The transcripts and translated products of the cyst endoglucanase genes were produced exclusively

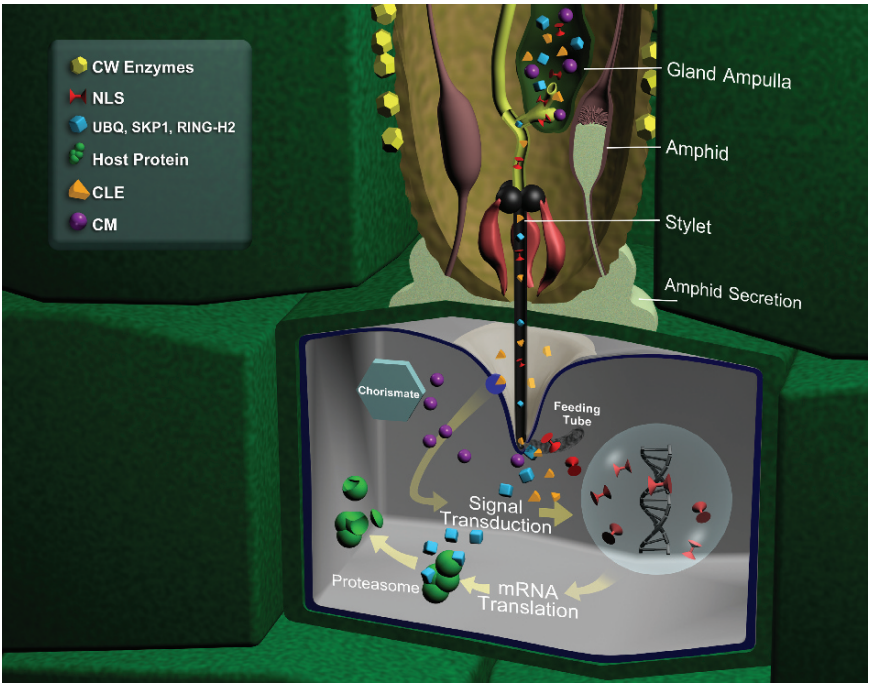

Fig. 3 A model of potential interactions of secreted products of phytonematode parasitism genes with host plant cells. Nematode esophageal gland cell secretions are released through valves within ampulla for transport out of the stylet (feeding spear) into host tissues. Cell wall (CW)-modifying proteins (endoglucanases, pectinases, hemicellulases, and expansin) may be secreted to aid the migration of infective juveniles through host plant tissues. Other nematode gland cell secretions might have multiple roles in the formation of specialized feeding cells by the nematode, including: effects on host cell metabolism by secreted chorismate mutase (CM); signaling by secreted nematode peptides such as homologs to plant CLAVATA/ESR-related (CLE) peptides; selective degradation of host proteins through the ubiquitin (UBQ)-proteosome pathway by UBQ, S-phase kinase associated protein 1 (Skp-1) and RING-H2 secreted from the nematode; and potential effects of secreted nematode proteins that contain nuclear localization signals (NLS) within the host cell nucleus. Figure designed by Bill Baverstock (North Carolina State University Creative Services). Reprinted from Davis et al. (2004) with permission from Elsevier

within the subventral glands as early as the developing J2 stage within the eggshell, and expression persisted until the early J3 stage of cyst nematodes within host roots. Endoglucanase expression was absent in subsequent stages of developing sedentary cyst nematode females, but interestingly, endoglucanase expression resumed during the development of the motile cyst nematode males that exit root tissues (De Boer et al. 1999; Goellner et al. 2000). Secretion of cyst nematode endoglucanase was detected during intracellular migration within host roots (Wang et al. 1999) but not in developing host feeding cells. However, up-regulation of plant endoglucanases was detected subsequently in nematode feeding sites as one (endogenous) component of the extensive cell wall modifications of these cells (Goellner et al. 2001). Expressed endoglucanase

genes were subsequently identified in a number of plant-parasitic nematode species (summarized in Mitchum et al. 2007; Vanholme et al. 2004), and in all cases, the nematode endoglucanase sequence had highest similarity to bacterial or reported nematode endoglucanase genes. Recombinant endoglucanases and induced nematode stylet secretions had the ability to degrade carboxymethylcellulose and a number of other cellulolytic substrates, but digestion of crystalline cellulose by nematode endoglucanases has not been confirmed (Bera-Maillet et al. 2000; Gao et al. 2004a). It is hypothesized that a combination of mechanical force of stylet thrusts and nematode cell wall-digesting enzymes promotes the breach of plant cell walls. Support for this hypothesis was recently provided from experiments in which genes for cell wall-digesting enzymes were silenced by RNAi-soaking techniques and host infectivity of the treated nematodes was reduced (Chen et al. 2005).

4.1.2 Other Hydrolytic Glucanases

In addition to beta-1,4 endoglucanases, other parasitism genes encoding cell wall-modifying proteins produced in the subventral esophageal gland cells of J2 plant-parasitic nematodes have been identified. Expressed pectinase genes encoding pectate lyase and polygalacturonase have been isolated from several nematode species (summarized in Vanholme et al. 2004), and the ability to digest pectolytic substrates was related to the ability to migrate through host root tissues. Expressed beta-1,3-endoglucanase (Kikuchi et al. 2005) and pectate lyase (Kikuchi et al. 2006) were recently reported from the pinewood nematode *Bursaphelenchus xylophilus* and hypothesized as being involved in nematode feeding from plants and co-invading fungal mycelium. The expression of an active chitinase predicted to be secreted from the subventral esophageal glands of the J2 soybean cyst nematode hatched from eggshells is also curious (Gao et al. 2002) and may reflect an adaptation to mitigate concurrent infection by organisms with chitin-containing walls. The first expressed xylanase (Mitreva-Dautova et al. 2006) and beta-galactosidase (Vanholme et al. 2006) genes of animal origin have been reported from root-knot and cyst nematodes, respectively, providing the first evidence of digestion of cell wall hemicellulose as a component of nematode migration through root tissues.

4.1.3 Expansin

The occurrence of several nematode genes encoding an expressed CBD joined with a peptide of nonendoglucnanase origin (Ding et al. 1998; Gao et al. 2004b) is curious since over-expression of a bacterial CBD gene has been reported to increase elongation of plant cells (Shpigel et al. 1998). One gene (*GR-exp1*) expressed in the subventral gland cells of the potato cyst nematode (PCN) that encoded a CBD domain was an expansin-like protein that represented the first confirmed report of such a protein outside the plant kingdom (Qin et al. 2004). Structural analyses derived from the predicted domains of one PCN expansin suggested a best fit with

the three-dimensional structure of extracellular proteins from soil *Actinobacteria* (Kudla et al. 2005). Different than the cell wall-digesting enzymes above, expansins soften cell walls by breaking noncovalent bonds between cell wall fibrils, thereby allowing a sliding of fibrils past each other (Cosgrove 2000). Expansin activity in plant cells was confirmed in proteins derived directly from PCN as well as from the expressed *GR-exp1* product (Qin et al. 2004).

4.2 Cellular Metabolism and Transport

4.2.1 Chorismate Mutase

The esophageal gland cells of both root-knot (Huang et al. 2005; Lambert et al. 1999) and cyst nematodes (Gao et al. 2003; Jones et al. 2003) express parasitism genes encoding chorismate mutase, a pivotal enzyme in the shikimate pathway that converts chorismate to prephenate (Romero et al. 1995). The activity of chorismate mutase is a key regulatory mechanism that determines the cellular balance of the aromatic amino acids phenylalanine, tyrosine, and tryptophan (Romero et al. 1995). The metabolites that have these amino acids as precursors, among which auxin, salicylic acid, and phenylpropanoid derivatives are of particular interest in plant–parasite interactions, would in theory be influenced by introduction of chorismate mutase into host cells by nematodes. Cytoplasmic expression of a root-knot nematode chorismate mutase gene in soybean hairy root cells produced tissues with an auxin-deficient phenotype that could be reversed by the application of exogenous auxin (Doyle and Lambert 2003). A model derived from these results suggests that nematode-secreted chorismate mutases will deplete the cytoplasmic chorismate pool leading to an increased export of chorismate from host cell plastids into the cytoplasm, effectively decreasing synthesis of plastid-produced chorismate-dependent metabolites like auxin or salicylic acid (Doyle and Lambert 2003). Reduction in cellular salicylic acid or phenylpropanoid production in response to the introduction of nematode chorismate mutase in host cell cytoplasm could result in a down-regulation of plant defense against the invading nematode. Consistent with a putative function in defense inactivation, chorismate mutase genes are polymorphic and apparently selected among soybean cyst nematode isolates that differ in capacity to infect soybean genotypes with different sources of resistance (Bekal et al. 2003; Lambert et al. 2005).

4.2.2 Annexin

The dorsal esophageal gland cell of *H. glycines* expresses a gene homologous to annexin that has a predicted signal peptide for secretion (Gao et al. 2003), and another annexin without signal peptide has been isolated from the amphids and other tissues of *Globodera pallida* (Fioretti et al. 2001). Immunodetection of the *G. pallida* annexin was confirmed in nematode excretory-secretory products of J2 stimulated

with 5-methoxy DMT oxalate; however, the *H. glycines* 4F01 annexin (Gao et al. 2003) was immunolocalized exclusively within the dorsal esophageal gland cell (Patel 2008). Annexin genes represent a large family coding for calcium-dependent phospholipid-binding proteins with a wide range of reported functions (Clark et al. 2001), and several known activities of annexin are related to the phenotype of the nematode feeding cells induced in hosts. Some annexins (Clark et al. 2001) are calcium-dependent membrane-binding proteins that may potentially function to regulate ion transport across (feeding) cell membranes and also act to detoxify oxygen radicals that may accumulate across cell membranes (such as those associated with hypersensitive cell death). In plant cells, annexins are associated with Golgi-mediated secretion of new cell membrane and cell wall glucans (Clark et al. 2001). Co-immunoprecipitation of plant annexin with cellulose synthase (CesA) has been reported (Hofmann et al. 2003), suggesting that annexin introduced into host plant cells by nematodes may modulate the extreme modifications of cell walls observed in nematode feeding cells. Sensitivity to osmotic stress demonstrated in the *AnnAt1* mutant of *Arabidopsis thaliana* (Lee et al. 2004) could be complemented by expression of the *H. glycines* 4F01 annexin (Patel 2008), suggesting one potential role for secreted nematode annexin in regulating the osmoticum of host feeding cells.

4.2.3 Calreticulin

Proteomic analysis isolated calreticulin in secretions induced in vitro by J2 root-knot nematode, and the gene encoding this calreticulin was expressed in the nematode's subventral esophageal gland cells (Jaubert et al. 2002). Calreticulins are calcium-binding proteins that are involved in multiple cellular processes including ER-chaperones, nuclear export, mRNA degradation, cell adhesion, and cell calcium homeostasy (Michalak et al. 2002). Although the role(s) of secreted calreticulin in plant–nematode interactions is unclear, secretion of calreticulin in host tissues by several parasites of vertebrates, including nematode parasites (Suchitra and Jochi 2005), suggests a key role for these secretions in parasitism. The secretion of root-knot nematode calreticulin has also been detected within plant tissues, including immunolocalization of secreted calreticulin outside the stylet tip along the host cell wall (Jaubert et al. 2005).

4.3 Cellular Regulation and Targeting

4.3.1 Nuclear Localized Parasitism Proteins

A significant number of the proteins encoded by parasitism genes expressed in plant nematode esophageal gland cells (Gao et al. 2003; Huang et al. 2003) contain both a predicted secretion signal peptide and a motif encoding a putative nuclear localization signal (NLS). These data present the tempting hypothesis that the

secreted products of some nematode parasitism genes become localized to the host cell nucleus. This is not without precedent since antigens of secreted products from the animal-parasitic nematode *Trichinella spiralis* have been immunolocalized to the nucleus of host muscle cells (Jasmer et al. 2003). Expression of GFP and GUS-tagged SCN parasitism proteins in plant cells has demonstrated that some of the predicted NLS domains do indeed function to import products into the plant cell nucleus (Elling et al. 2007b; Tytgat et al. 2004). DNA-binding domains are also predicted in some of the NLS-containing proteins predicted to be secreted by plant-nematodes, suggesting an extraordinary potential for regulatory control within the nucleus of host feeding cells if confirmed.

4.3.2 Ranbpm

Expressed genes encoding secretory proteins with high similarity to proteins that bind to the small G-protein Ran, so-called RanBPMs (Ran-Binding Protein in the Microtubule organizing center), were identified in the dorsal esophageal gland cell of cyst nematodes (Blanchard et al. 2005; Qin et al. 2000). If secreted into plant feeding cells, RanBPM from nematodes could hypothetically interact with the microtubule network involved in spindle formation during host cell mitosis and subsequently affect host cell cycle (Wilde et al. 2001). Altered regulation of host feeding cell cycle is a primary manifestation of plant parasitism by sedentary endoparasitic nematodes (Goverse et al. 2000), but the regulatory foundation of these effects is unknown. Confirmation of secretion of RanBPM from nematodes and functional verification of nematode RanBPM in plant cells will be important steps to understand the potential role of secreted RanBPM in plant–nematode interactions.

4.3.3 Ubiquitination/Proteasome Functions

Several parasitism genes expressed in cyst nematode esophageal gland cells encode secreted isotypes of cytoplasmic proteins involved in the ubiquitination pathway, namely ubiquitin itself, along with proteins (i.e. RING-Zn-Finger-like and Skp1-like proteins) similar to those found in the host E3 ubiquitin protein ligase complex (Gao et al. 2003). Targeted and timed protein degradation (Estelle 2001) may provide a powerful and unique means for regulation of host cell phenotype by nematodes, including potential effects within the host cell nucleus and cytoplasm. Secreted proteasome proteins from cyst nematodes could be involved in polyubiquitination of target host cell proteins for degradation to modulate cellular defense and cell signaling, to influence host cell cycle, or simply to provide a substrate for nutrient uptake by the nematode. Precedent for a potential role in modulating host defense is demonstrated by the activity of a domain of *Pseudomonas syringae* AvrPtoB that functions as a mimic of host plant E3 ubiquitin ligase (Janjusevic et al. 2006). The ubiquitin extension proteins that are predicted to be secreted from cyst nematodes contain a unique nonribosomal extension peptide that is distinct from those of plants

(Gao et al. 2003; Tytgat et al. 2004). The potential exists that ubiquitin functions as a chaperone of this unique extension peptide signal of nematode origin within host cells. Though no functional data on secreted proteasome members from nematodes exists, these putative parasitism gene products are prime candidates for protein interaction studies with host cell components.

4.3.4 The 14-3-3 Protein Family

Two isoforms of a protein identified as a member of the 14-3-3 family were isolated from stylet secretions induced in vitro from J2 root-knot nematode (Jaubert et al. 2004). The expressed genes encoding each 14-3-3 were cloned, and in situ hybridization analysis indicated that one isoform (14-3-3a) was expressed in genital primordia and the other isoform (14-3-3b) was expressed within the dorsal esophageal gland of root-knot nematode juveniles. Expression of a 14-3-3 gene in *H. glycines* has also been localized within genital primordia of infective J2 (E.L. Davis, unpublished). Interestingly, although the 14-3-3b protein was originally isolated from stylet secretions, no signal peptide for secretion was predicted from its coding sequence. Members of the 14-3-3 family are associated with parasites and have diverse roles in primary metabolism, regulation of cellular stress response and defense, organelle trafficking, and the cell cycle in which 14-3-3 often acts as an essential interacting protein or chaperone (Siles-Lucas and Gottstein 2003). All of these potential roles of 14-3-3 are mirrored in observed changes within nematode feeding cells, making the potential of a secreted 14-3-3 (e.g. 14-3-3b) from nematodes an interesting system for functional analyses.

4.4 Mitigating Host Response

4.4.1 Venom Allergen Proteins

The products of the parasitism genes listed above are similar to functionally characterized proteins from other organisms and promote the formulation of defined hypotheses about protein function during parasitism. There are some parasitism protein candidates, however, that are similar to known proteins but whose functions remain obscure. One intriguing group of parasitism proteins contains representatives from root-knot nematodes (Ding et al. 2000) and cyst nematodes (Gao et al. 2001b; Vanholme et al. 2005) that are collectively called venom allergen-like proteins (vaps). Genes encoding vaps are expressed in the subventral esophageal gland cells of these nematodes in the early stages of parasitism, but their secretion in plants and role(s) in parasitism are unclear. Gene sequences for these venom proteins were first described from hymenopteran insects (Fang et al. 1988), and vaps were also identified as secreted proteins (ASP) in the animal-parasitic nematode *Ancylostoma caninum* (Zhan et al. 2003). Genes encoding vaps have since been

found in other nematodes, including parasites as well as the free-living *C. elegans* (Mitreva et al. 2005b). The role of vaps in promoting parasitism is unclear since vaps of excretory-secretory origin have been demonstrated to stimulate mammalian immune response in animal-parasites (Jasmer et al. 2003). The seemingly ubiquitous nature of vaps among nematodes, even nonparasites, suggests a function basic to nematode biology that may have (as of yet, unknown) effects on parasitism.

4.4.2 Surface Defense

The hypodermis is a syncytial cell layer directly beneath the (nonliving) nematode cuticle that forms the new cuticle during molts and secretes a number of molecules for deposition on the cuticle surface of the nematode body (Bird and Bird 1991). Several genes expressed within the hypodermis encode proteins deposited on the cuticle surface that are in direct contact with host cells during nematode invasion of plant tissues. Among the dynamic mixture of proteins at the cuticle surface are proteins with potential roles in mitigating host defense response. Peroxidase genes are expressed in the potato cyst nematode hypodermis (Jones et al. 2004; Robertson et al. 2000), and the peroxidase proteins accumulate on the nematode body surface presumably to detoxify reactive oxygen species generated by the defense response of the host (Waetzig et al. 1999). An antiserum that bound to surface molecules of potato cyst nematode isolated cDNA expressing a homologue to the fatty acid- and retinol-binding (FAR) protein (Prior et al. 2001) family isolated from *C. elegans*. The FAR gene was expressed in the hypodermis of PCN, and its recombinant product demonstrated the ability to bind to retinol and fatty acids of C_{11} to C_{24} length. Recombinant FAR from PCN bound to two plant fatty acids, linolenic and linoleic acid, that are of significance as precursors of plant defense compounds and the jasmonic acid defense signaling pathway. Genes encoding SXP/RAL-2 proteins were expressed in the hypodermis and amphids of the potato cyst nematode (Jones et al. 2000) and exclusively in the subventral gland cells of J2 root-knot nematodes (Tytgat et al. 2005). The SXP-RAL-2 family is limited, to date, to both parasitic and nonparasitic nematodes and, like vaps, may play a fundamental functional role in nematode biology. Immunomodulation of vertebrate hosts has been achieved with SXP-RAL-2 of animal-parasitic nematodes (Wang et al. 1997), providing an undefined function in stimulating host response during parasitism.

4.4.3 Stealth Signals

Several molecules from nematodes with observable effects on plant cells have been reported, but the nature and origins of these molecules from nematodes are unclear. The isolation of secreted cytokinins from root-knot nematode juveniles in the absence of plant hosts (De Meutter et al. 2003) confirmed earlier evidence suggesting that nematodes produce cytokinin endogenously (Dimalla and Van Staden 1977). Since the pathway and tissue localization for cytokinin synthesis could not be

confirmed in the nematode, it was suggested that cytokinins of nematode origin may be an excreted waste product of nucleic acid degradation. This hypothesis relates to the localized hyperplasia that is a hallmark of gall formation, cell cycle regulation in feeding sites, and other cytokinin effects stimulated in roots by root-knot nematodes (Lohar et al. 2004). Incubation of roots of the legume *Lotus japonicus* in the presence of viable juveniles of root-knot nematode prior to infection stimulated identical cytoskeletal activity in root hairs as observed by treatment with rhizobial Nod factors (Weerasinghe et al. 2005). The "NemF" (Nem Factor) from root-knot nematodes also provided a response identical to Nod factor in Nod-receptor mutants, and a similar response to NemF was observed in root hairs of tomato. The identity of NemF and its origins in root-knot nematode have not been reported.

4.5 Bioactive Peptides

Perhaps the most interesting group of parasitism genes are those that encode signaling peptides. Secretions collected and fractionated from hatched juveniles of the potato cyst nematode contained a peptide or peptides of less than 3 kDa that induced mitogenic activity in tobacco leaf protoplasts and human peripheral blood mononuclear cells (Goverse et al. 1999). The nature and origins of this bioactive peptide or peptides from PCN are unknown. Two parasitism genes that encode secreted bioactive peptides produced in the esophageal gland cells of plant nematodes have been the subject of considerable characterization and functional analyses. HG-SYV46, the parasitism gene expressed most strongly in the dorsal gland cell of *H. glycines* during parasitism (Gao et al. 2003), was first isolated from a screen of an expressed *H. glycines* gland cell-specific cDNA library for signal peptides that function in secretion (Wang et al. 2001). Database searches of the complete predicted protein provided no significant homology, but the C-terminus of the SYV46 protein contained the consensus domain of known CLAVATA3/ESR-like (CLE) plant signaling peptides (Olsen and Skriver 2003). Plant CLV3 peptide regulates the balance of stem cell proliferation and differentiation in the shoot meristem through interactions with a CLAVATA1/CLAVATA2 receptor complex to negatively regulate expression of WUSCHEL (Fletcher 2002). Over-expression of HG-CLE in wild-type *Arabidopsis* negatively regulated *wus* expression in the shoot meristem (Mitchum et al. 2008; Wang et al. 2005) and promoted the *wus* phenotype, arrested shoot apical meristem (Fig. 4). Conversely, expression of HG-CLE in a *clv3*-deficient mutant was able to restore the wild-type number and size of floral organs in *Arabidopsis*. The data suggested that secreted HG-CLE may play a role in feeding cell differentiation in roots via direct interaction with a CLV1-like receptor or as a competitive inhibitor of CLE in roots that would augment wild-type differentiation of affected root cells (Davis and Mitchum 2005; Mitchum et al. 2008). Interestingly, the cloned *rhg1* SCN-resistance gene (Lightfoot and Meksem 2002) has homology to the *Xa21* resistance gene and the *CLV1* receptor gene reported in the public database and, like chorismate mutase, polymorphisms of *Hg-cle* correlate to SCN ability to infect soybean genotypes that contain different SCN resistance genes (M.G. Mitchum, pers. comm.).

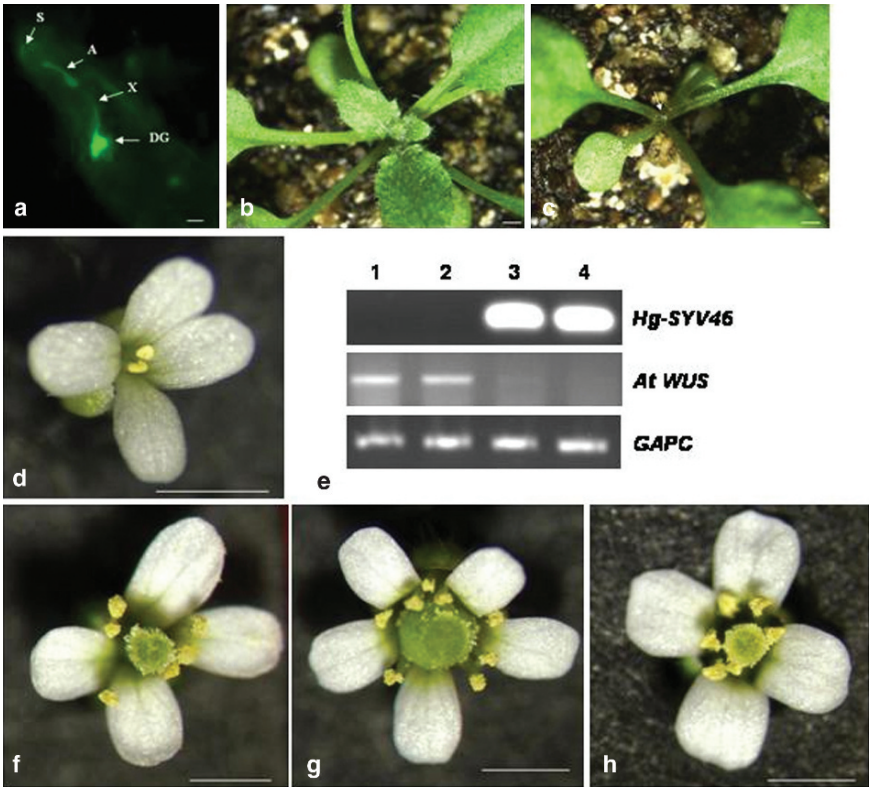

Fig. 4 Localization of HG-SYV46 in *Heterodera glycines* and effects of *Hg-SYV46* expression in transgenic *Arabidopsis thaliana*. (**a**) Polyclonal antibodies localized HG-SYV46 within the dorsal esophageal gland cell, its extension, and the gland cell ampulla at the base of the stylet (feeding spear) of a parasitic stage of *H. Glycines* dissected from a host plant root. (**b**) The developing shoot of a wild-type seedling (ecotype Columbia-0) at 20-days post-germination. (**c**) A *35S::Hg-SYV46* transgenic *Arabidopsis* plant at 20-days post-germination showing an arrested shoot apical meristem (*arrow*). (**d**) Flower of a *35S::Hg-SYV46* transgenic *Arabidopsis* plant showing a decreased number of stamens and missing carpels as compared to wild-type, a phenotype similar to a *wus* flower (Laux et al. 1996). (**e**) RT-PCR analysis of *Hg-SYV46* and *WUS* expression in the shoot apices of *Hg-SYV46* nonexpressed (*promoterless::Hg-SYV46,* lanes 1 & 2) and expressed (*35S::Hg-SYV46*, lanes 3 & 4) individual transgenic *Arabidopsis* lines from 10-day-old seedlings. Expression of the *Arabidopsis gapc* gene was used as an internal control. (**f**) A wild-type *Arabidopsis* flower containing four petals, six stamens, and two fused carpels. (**g**) Flower of an *Arabidopsis clv3-1* mutant (Clark et al. 1995) carrying more floral organs in all whorls than wild-type. (**h**) A fully restored flower of a *clv3-1* mutant with wild-type floral organ size and number obtained from a *35S::Hg-SYV46/clv3-1* transgenic plant. Abbreviations: DG = dorsal gland, X = extension, A = ampulla. *Scale bars* 0.1 mm in **a**, 1.0 mm in **b**–**d** and **f**–**h**. Reprinted from Wang et al. (2005) with permission from Blackwell Publishing Ltd

An expressed parasitism gene encoding a peptide (16D10) with CLE signature was also identified from the subventral esophageal gland cells of root-knot nematode (Huang et al. 2003). The mature 16D10 peptide contained only 13 amino acids and was immunodetected in secretions from the pre-infective juvenile stage of

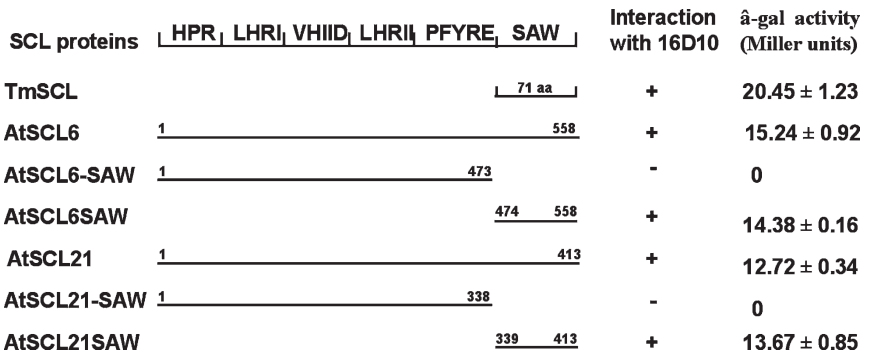

Fig. 5 Interaction of the root-knot nematode 16D10 peptide with plant SCARECROW-like (SCL) transcription factors. The schematic demonstrates the direct interaction of 16D10 with the SAW domain of tomato (tmscl) and *Arabidopsis* (atscl6 and atscl21) proteins in yeast. The specific domains of the SCL proteins represented schematically were tested to interact individually with 16D10. Positive interactions resulting in the activation of HIS3, ADE2, and lacz genes were detected by growth in the absence of histidine and adenine, and the beta-galactosidase activity presented here. Reprinted from Huang et al. (2006a) with permission from APS Press

root-knot nematode. Over-expression of 16D10 was not able to affect the floral phenotype of an *Arabidopsis clv3*-1 mutant as observed for *Hg-cle*, but expression of 16D10 significantly accelerated root growth in both whole *Arabidopsis* plants and tobacco hairy root assays (Huang et al. 2006a). The effects of 16D10 were root-specific and did not result in observable changes in root morphology except for accelerated root proliferation. A yeast two-hybrid screen and co-immunoprecipitation experiments using *Arabidopsis* over-expressing 16D10 demonstrated a specific interaction of the 16D10 peptide with the SAW domain (Fig. 5) of expressed plant SCARECROW-like (SCL) transcription factors. Members of the SCL family are primarily active in plant roots and regulate downstream pathways of root cell growth and differentiation (Pysh et al. 1999). The results of the root-knot nematode 16D10 experiments (Huang et al. 2006a) provide the first evidence that a secreted nematode parasitism gene product may regulate host gene activity via binding to an intracellular plant transcription factor to modulate root cell growth during feeding cell initiation.

5 Perspectives on Nematode Parasitism Genes

Molecules at the interface of the nematode and host are at the front lines of molecular communication and interaction during plant parasitism. Our emerging understanding of the functions of parasitism genes and genetic pathways that promote acquisition of nutriment from the living host suggests that they are diverse and tightly integrated with mechanisms adapted to sustain a parasitic lifestyle. For migratory nematodes that do not engage in a sustained interaction with host plant cells, the adaptations for

nutrient acquisition may be little more than enzymes that promote stylet penetration of host cells and digestion of host-derived nutrients. Migratory parasites that enter plant tissues face additional obstacles to feeding, including migration through different tissues and evasion of host defense (Hussey and Grundler 1998). Emerging information on expressed genes in parasitic stages of migratory endoparasitic nematodes (McCarter et al. 2005), such as *Pratylenchus* and *Bursaphelenchus*, and migratory stages of sedentary endoparasitic nematodes suggests that there are some common adaptations to navigate and survive within the host. Plant nematodes that form a sustained feeding relationship with host cells show another level of adaptive sophistication for parasitism that, at present, appears to be more advanced than the adaptations of migratory parasites (Baldwin et al. 2004; Davis et al. 2004). At the cellular level, this appears to be true even of ectoparasitic nematodes that form sedentary feeding relationships. The feeding of *Criconemella xenoplex* from the same subepidermal cell for days results in unique modifications of neighboring plasmodesmata for solute transport (Hussey et al. 1992a) and likely requires suppression of host defense at some level since plant callose deposition is stimulated around the nematode stylet (Hussey et al. 1992b). Host cellular response to feeding by the sedentary ectoparasite *Xiphinema* has striking similarities with giant-cells formed by *Meloidogyne* (Hussey and Grundler 1998). A number of expressed genes encoding secreted proteins have recently been identified from the pharyngeal gland region of *Xiphinema index* (Furlanetto et al. 2005). As the genomes of sedentary parasites are further explored, "generic" mechanisms underlying the foundations of sustained parasitic relationships may be revealed.

The root-knot and cyst nematodes have emerged as primary models of plant parasitism because of their economic significance (Barker 1998) and the extreme changes in host tissues that they induce (Hussey and Grundler 1998). In both molecular and structural aspects, plant response at these nematode feeding sites has similarities to other plant–microbe interactions (Davis and Mitchum 2005), but the cells induced for feeding within these sites are a unique adaptation to plant infection by nematodes. The growth and development of the sedentary stages of these species must be accommodated and tolerated by the invaded host tissues. Evidence described above suggests that molecules at the nematode body surface that were synthesized and secreted from the hypodermis or released from natural openings of the nematode body may have been adapted to mitigate response of host cells by contact or to mask recognition of the nematode as an invader. Few products of nematode metabolism or secondary pathways that play roles in parasitism have been isolated and characterized, but emerging genomic data and the potential of metabolomics may begin to uncover these potential adaptations. Our knowledge of nematode parasitism genes to date is likely far from exhaustive, and the data derived from plant nematode genome sequencing projects will most certainly illuminate our understanding of adaptations for parasitism. At present, parasitism gene study in nematodes has focused on products encoding expressed secretory proteins that may be direct or processed products of developmentally expressed genes, and the majority of these genes have been identified from the esophageal gland cells of tylenchid plant-parasites that have evolved elaborate and dynamic secretory activity

(Baum et al. 2006; Davis et al. 2004, 2008; Mitchum et al. 2007; Vanholme et al. 2004). The products of these gland cells are directed for secretion from the nematode stylet into the plant tissues in all stages of parasitism, including secretion into cells modified for nutrient ingestion by the nematode. The migratory stages of endoparasitic plant nematodes are dominated by the expression of parasitism genes encoding cell wall-modifying proteins that promote either intercellular or intracellular migration in conjunction with the mechanical force of stylet thrusts. The nematode genes encoding cellulases, pectinases, hemicellulases, and expansin have two primary criteria in common: (1) they are expressed in the subventral esophageal gland cells most active in the early stages of nematode infection and; (2) they all have striking sequence similarity to corresponding enzymes of microbial origin, suggesting horizontal acquisition of genes encoding cell wall-modifying proteins from soil microbes as a major driving force in the evolution of plant parasitism by nematodes (Baldwin et al. 2004; Davis et al. 2000; Jasmer et al. 2003; Ledger et al. 2006).

The transition from the migratory to the sedentary stages of parasitism is a continuum and may be the result of triggering a "developmental switch" akin to emergence from arrested development (dauer) upon environmental cues (Elling et al. 2007a; Mitreva et al. 2004). In some respects, this transition is mirrored in the expression of some parasitism genes within the esophageal gland cells (Davis et al. 2004; Hussey 1989). While the cell wall-modifying genes decrease in activity within the subventral gland cells during this transition, some subventral gland genes continue to be expressed throughout parasitism, and a few are expressed predominantly in the sedentary parasitic stages. Chorismate mutase is expressed in both dorsal and subventral gland cells throughout parasitism (Doyle and Lambert 2003), and the multiple roles of this pivotal metabolic enzyme may change in function as feeding sites are developed. A role for subventral gland parasitism gene products in feeding cell initiation is suggested by the interaction of 16D10 peptides with a plant transcription factor and its effects on root cell growth (Huang et al. 2006a). The activity and nature of multiple parasitism genes expressed in the nematode dorsal esophageal gland cell as feeding sites develop suggests significant and diverse "regulatory" roles for these secretions in parasitism (Gao et al. 2003; Huang et al. 2003). The nature of nematode dorsal gland parasitism gene products suggests multiple mechanisms for the nematode to modulate host feeding cell cycle, metabolism, transport, defense, gene expression, and production of a feeding tube. In contrast to the cell wall-modifying genes, very few of the candidate parasitism genes expressed in the dorsal gland cell have sequence similarity to genes reported from soil microbes. Some of these parasitism genes reflect adaptations of functional domains encoded by endogenous nematode genes for secretion and regulation of parasitism or potential convergent evolution to mimic the products of host plant genes. Polymorphisms observed among some nematode parasitism genes suggest that differential domains of these effectors may have experienced diversifying selection for alleles encoding virulence factors (Birch et al. 2006) that interact directly or indirectly with natural resistance genes. Concerning more global adaptations for parasitism, the observation that diverse suites of novel parasitism genes are expressed by root-knot and cyst nematodes may be reflective of the differential ontogeny of giant-cells and syncytia, respectively

(Davis and Mitchum 2005). The emerging data suggests that nematode parasitism gene products may interact with extracellular and intracellular host cell targets, both of which are accessible to the nematode stylet orifice. How the synthesis and secretion of the different parasitism proteins are regulated spatially and temporally within the same esophageal gland cell is unknown, but it would make a fascinating model for the study of secretory cell biology.

Current in vivo functional analyses of candidate parasitism genes are limited to two primary options, expression of the candidate gene in plants or disruption of the candidate gene in the nematode via gene silencing. Direct expression of candidate nematode genes in whole plants, roots, or cells can, and has, provided biological information relative to the plant–nematode interaction when an observable phenotype is produced (Doyle and Lambert 2003; Huang et al. 2006a; Wang et al. 2005). The activities of multiple members of parasitism gene families and potential interactions of nematode parasitism proteins released concomitantly into the host provide an added level of complexity to assess functional roles in parasitism. The potential to profile host gene expression upon expression of nematode parasitism genes offers the opportunity for comparative analyses to the host gene expression observed in nematode feeding sites. When they can be employed, the use of model plant species and their mutants are of immense benefit to more specifically define parasitism gene function. Although the dissection of individual components facilitates analysis of their function, it stands in contrast to the interaction of multiple components that contributes to the natural infection process. The appeal of gene silencing techniques is the potential to disrupt a single factor (or family) from the interaction and assess potential effects. As with gene expression in hosts, in the absence of high-throughput analyses, the potential success of gene silencing assays are predicated on the generation of an observable phenotype. These are not insurmountable obstacles, however, as the specific effects of some RNAi soaking assays on nematode infectivity of hosts have been observed (Bakhetia et al. 2005a; Chen et al. 2005; Rosso et al. 2005; Urwin et al. 2002). Improved efficiency, delivery, and targeting of gene silencing techniques for plant-parasitic nematodes, including host-derived RNAi (Gheysen and Vanholme 2007; Huang et al. 2006b; Steeves et al. 2006; Yadav et al. 2006), hold great promise for functional analyses of candidate nematode parasitism genes and potential applications for nematode management in crops.

Although the secretion of esophageal gland proteins into plant tissues by nematodes has been demonstrated in video and immunoassays (Doyle and Lambert 2003; Goellner et al. 2001; Jaubert et al. 2005; Wyss and Zunke 1986; Wyss et al. 1992; Wang et al. 1999), the intracellular localization of nematode secretions within feeding cells remains a technical challenge. The demonstration of functional nuclear localization signals (Elling et al. 2007b) and a nematode secretory peptide that can interact with a specific domain of a plant transcription factor (Huang et al. 2006a) have provided tempting models for direct analyses within feeding cells. Application of advanced microscopy that can detect modification or subcellular localization of nematode secretions and their interactions with host molecules within cells lies at the frontier of functional analyses of nematode parasitism. The products of nematode parasitism genes described in this treatise provide a launching pad for such further exploration.

References

Abad P et al. (2008) Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. Nature Biotech 26:909–915

Bakhetia M, Charlton W, Atkinson HJ, McPherson MJ (2005a) RNA interference of dual oxidase in the plant nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 18:099–1106

Bakhetia M, Charlton WL, Urwin PE, McPherson MJ, Atkinson HJ (2005b) RNA interference and plant parasitic nematodes. Trends Plant Sci 10:362–367

Baldwin JG, Nadler SA, Adams BJ (2004) Evolution of plant parasitism among nematodes. Annu Rev Phytopathol 42:83–105

Barker KR (1998) Introduction and synopsis of advancements in nematology. In: Barker KR, Pederson GA, Windham GL (eds) Plant nematode interactions. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, pp 1–20

Baum TJ, Davis EL, Hussey RS (2006) Root-knot and cyst nematode parasitism genes: the molecular basis of plant parasitism. In: Setlow J. K. (ed.), Genetic Engineering, Principles and Methods, vol 28, Springer Science + Business Media, Heidelberg, pp. 17–43

Bekal S, Niblack T, Lambert K (2003) A chorismate mutase from the soybean cyst nematode *Heterodera glycines* shows polymorphisms that correlate with virulence. Mol Plant Microbe Interact 16:439–446

Benfey PN, Chua NH (1990) The cauliflower mosaic virus-35S promoter – combinatorial regulation of transcription in plants. Science 25:959–966

Bera-Maillet C, Arthaud L, Abad P, Rosso MN (2000) Biochemical characterization of MI-ENG1, a family 5 endoglucanase secreted by the root-knot nematode *Meloidogyne incognita*. Eur J Biochem 267:3255–3263

Birch PRJ, Rehmany AP, Pritchard L, Kamoun S, Beynon JL (2006) Trafficking arms: oomycete effectors enter host plant cells. Trends Microbiol 14:8–11

Bird AF (1968) Changes associated with parasitism in nematodes. IV. Cytochemical studies on the ampulla of the dorsal gland of Meloidogyne javanica and on exudations from the buccal stylet. J Parasitol 54:879–890

Bird AF, Bird J (1991) The structure of nematodes. Academic Press, San Diego

Blanchard A, Esquibet M, Fouville D, Grenier E (2005) Ranbpm homologue genes characterized in the cyst nematodes *Globodera pallida* and *Globodera mexicana*. Physiol Mol Plant Pathol 67:15–22

Bohner S, Lenk I, Rieping M, Herold M, Gatz C (1999) Transcriptional activator TGV mediates dexamethasone-inducible and tetracycline inactivatable gene expression. Plant J 19:87–95

Chapman S, Kavanagh T, Baulcombe DC (1992) Potato virus X as a vector for gene expression in plants. Plant J 2:549–557

Chen Q, Rehman S, Smant G, Jones JT (2005) Functional analysis of pathogenicity proteins of the potato cyst nematode *Globodera rostochiensis* using RNAi. Mol Plant Microbe Interact 18:621–625

Cho HJ, Farrand SK, Noel GR, Widholm JM (2000) High-efficiency induction of soybean hairy roots and propagation of the soybean cyst nematode. Planta 210:195–204

Clark GB, Sessions A, Eastburn DJ, Roux SJ (2001) Differential expression of members of the annexin multigene family in *Arabidopsis*. Plant Physiol 126:1072–1084

Clark SE, Running MP, Meyerowitz EM (1995) *CLAVAT3* is a specific regulator of shoot and floral meristem development affecting the same process as *CLAVAT1*. Development 121: 2057–2067

Cosgrove DJ (2000) Loosening of plant cell walls by expansins. Nature 407:321–326

Davis EL, Mitchum MG (2005) Nematodes: sophisticated parasites of legumes. Plant Physiol 137:1182–1188

Davis EL, Hussey RS, Baum TJ, Bakker J, Schots A, Rosso MN, Abad P (2000) Nematode parasitism genes. Annu Rev Phytopathol 38:365–396

Davis EL, Hussey RS, Baum TJ (2004) Getting to the roots of parasitism by nematodes. Trends Parasitol 20:134–141

Davis EL, Hussey RS, Mitchum MG, Baum TJ (2008) Parasitism proteins in nematode–plant interactions. Curr Opinion Plant Biol 11:360–366

De Boer JM, Smant G, Goverse A, Davis EL, Overmars HA, Pomp HR, Van Gent Pelzer M, Zilverentant JF, Stokkermans J, Hussey RS, Gommers FJ, Bakker J, Schots A (1996) Secretory granule proteins from the subventral esophageal glands of the potato cyst nematode identified by monoclonal antibodies to a protein fraction from second-stage juveniles. Mol Plant Microbe Interact 9:39–46

DeBoer JM, Yan Y, Bakker J, Davis EL, Baum TJ (1998) *In situ* hybridization to messenger RNA of *Heterodera glycines*. J Nematol 30:309–312

DeBoer JM, Yan Y, Wang X, Smant G, Hussey RS, Davis EL, Baum TJ (1999) Developmental expression of secretory beta-1,4-endoglucanases in the subventral esophageal glands of *Heterodera glycines*. Mol Plant Microbe Interact 12:663–669

De Meutter J, Vanholme B, Bauw G, Tytgat T, Gheysen G, Gheysen G (2001) Preparation and sequencing of secreted proteins from the pharyngeal glands of the plant parasitic nematode *Heterodera schachtii*. Mol Plant Pathol 2:297–301

De Meutter J, Tytgat T, Witters E, Gheysen G, Van Onckelen H, Gheysen G (2003) Identification of cytokinins produced by the plant parasitic nematodes *Heterodera schachtii* and *Meloidogyne incognita*. Mol Plant Pathol 4:271–277

Deubert KH, Rohde RA (1971) Nematode enzymes. In: Zuckerman BM, Mai WF, Rohde RA (eds) Plant parasitic nematodes, vol 2. Academic Press, New York, pp 73–90

Dimalla GG, Van Staden J (1977) Cytokinins in the root-knot nematode, *Meloidogyne incognita*. Plant Sci Lett 10:25–29

Ding X, Shields J, Allen R, Hussey RS (1998) A secretory cellulose-binding protein cDNA cloned from the root-knot nematode (*Meloidogyne incognita*). Mol Plant Microbe Interact 11:952–959

Ding X, Shields J, Allen R, Hussey RS (2000) Molecular cloning and characterization of a venom allergin AG5-like cDNA from *Meloidogyne incognita*. Int J Parasitol 30:77–81

Dong K, Opperman CH (1997) Genetic analysis of parasitism in the soybean cyst nematode *Heterodera glycines*. Genetics 146:1311–18

Doyle EA, Lambert KN (2003) *Meloidogyne javanica* chorismate mutase 1 alters plant cell development. Mol Plant Microbe Interact 16:123–131

Dubreuil G, Magliano M, Deleury E, Abad P, Rosso MN (2007) Transcriptome analysis of root-knot nematode functions induced in the early stages of parasitism. New Phytol 176:426–436

Elling AA, Mitreva M, Recknor J, Gai X, Martin J, Maier TR, McDermott JP, Hewezi T, Bird DM, Davis EL, Hussey RS, Nettleton D, McCarter JP, Baum TJ (2007a) Divergent evolution of arrested development in the dauer stage of *Caenorhabditis elegans* and the infective stage of *Heterodera glycines*. Genome Biol 8:R211

Elling AA, Davis EL, Hussey RS, Baum TJ (2007b) Active uptake of cyst nematode parasitism proteins into the plant cell nucleus. Int J Parasitol 37:1269–1279

Estelle M (2001) Proteases and cellular regulation in plants. Curr Opin Plant Biol 4:254–260

Fang K, Vitale M, Fehlner P, King T (1988) cDNA cloning and primary structure of a white-face hornet venom allergen, antigen 5. Proc Natl Acad Sci (USA) 85:895–899

Fioretti L, Warry A, Porter A, Haydock P, Curtis R (2001) Isolation and localization of an annexin gene (*gp-nex*) from the potato cyst nematode, *Globodera pallida*. Nematology 3:45–54.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391:806–11

Fletcher JC (2002) Shoot and floral meristem maintenance in *Arabidopsis*. Annu Rev Plant Biol 53:45–66

Furlanetto C, Cardle L, Brown DJF, Jones JT (2005) Analysis of expressed sequence tags from the ectoparasitic nematode *Xiphinema index*. Nematology 7:95–104

Gao B, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS (2001a) Identification of putative parasitism genes expressed in the esophageal gland cells of the soybean cyst nematode, *Heterodera glycines*. Mol Plant Microbe Interact 14:1247–1254

Gao B, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS (2001b) Molecular characterisation and expression of two venom allergen-like secretory protein genes in *Heterodera glycines*. Int J Parasitol 31:1617–1625

Gao B, Allen R, Maier T, McDermott JP, Davis EL, Baum TJ, Hussey RS (2002) Characterisation and developmental expression of a chitinase gene in *Heterodera glycines*. Int J Parasitol 32:1293–1300

Gao BL, Allen R, Maier T, Davis EL, Baum TJ, Hussey RS (2003) The parasitome of the phytonematode *Heterodera glycines*. Mol Plant Microbe Interact 16:720–726

Gao B, Allen R, Davis EL, Baum TJ, Hussey RS (2004a) Developmental expression and biochemical properties of a ß-1,4-endoglucanase family in the soybean cyst nematode, *Heterodera glycines*. Mol Plant Pathol 5:93–104

Gao B, Allen R, Davis EL, Baum TJ, Hussey RS (2004b) Molecular characterisation and developmental expression of a cellulose-binding protein gene in the soybean cyst nematode *Heterodera glycines*. Int J Parasitol 34:1377–1383

Gheysen G, Fenoll C (2002) Gene expression in nematode feeding sites. Annu Rev Phytopathol 40:191–219

Gheysen G, Vanholme B (2007) RNAi from plants to nematodes. Trends Biotechnol 25:89–92

Gleason CA, Liu QL, Williamson VM (2008) Silencing a candidate nematode effector gene corresponding to the tomato resistance gene *Mi*-1 leads to acquisition of virulence. Mol Plant Microbe Interact 21:576–585

Goellner M, Smant G, DeBoer JM, Baum TJ, Davis EL (2000) Isolation of beta-1,4 endoglucanase genes of *Globodera tabacum* and their expression during parasitism. J Nematol 32:154–165

Goellner M, Wang XH, Davis EL (2001) Endo-beta-1,4-glucanase expression in compatible plant–nematode interactions. Plant Cell 13:2241–2255

Goverse A, Davis EL, Hussey RS (1994) Monoclonal antibodies that bind to the esophageal glands and stylet secretions of *Heterodera glycines*. J Nematol 26:251–259

Goverse A, Rouppe van der Voort J, Roupe van der Voort C, Kavelaars A, Smant G, Schots A, Bakker J, Helder J (1999) Naturally induced secretions of the potato cyst nematode co-stimulate the proliferation of both tobacco leaf protoplasts and human peripheral blood mononuclear cells. Mol Plant Microbe Interact 12:872–881

Goverse A, Engler JD, Verhees J, van der Krol S, Helder J, Gheysen G (2000) Cell cycle activation by plant parasitic nematodes. Plant Mol Biol 43:747–761

Hofmann A, Delmer DP, Wlodawer A (2003) The crystal structure of annexin Gh1 from *Gossypium hirsutum* reveals an unusual S3 cluster. Eur J Biochem 270:2557–2564

Hotopp JC, Clark ME, Oliveira DC, Foster JM, Fischer P, Torres MC, Giebel JD, Kumar N, Ishmael N, Wang S, Ingram J, Nene RV, Shepard J, Tomkins J, Richards S, Spiro DJ, Ghedin E, Slatko BE, Tettelin H, Werren JH (2007) Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Science 317:1753–1756

Huang G, Gao B, Maier T, Allen R, Davis EL, Baum TJ, Hussey RS (2003) A profile of putative parasitism genes expressed in the esophageal gland cells of the root-knot nematode, *Meloidogyne incognita*. Mol Plant Microbe Interact 16:376–381

Huang G, Dong R, Maier T, Allen R, Davis EL, Baum TJ, Hussey RS (2004) Use of solid-phase subtractive hybridization for the identification of parasitism gene candidates from the root-knot nematode *Meloidogyne incognita*. Mol Plant Pathol 5:217–222

Huang G, Dong R, Allen R, Davis EL, Baum TJ, Hussey RS (2005) Two chorismate mutase genes from the root-knot nematode *Meloidogyne incognita*. Mol Plant Pathol 6:23–30

Huang G, Dong R, Allen R, Davis EL, Baum TJ, Hussey RS (2006a) A root-knot nematode secretory peptide functions as a ligand for a plant transcription factor. Mol Plant-Microbe Interact 19:463–470

Huang G, Allen R, Davis EL, Baum TJ, Hussey RS (2006b) Engineering broad root-knot resistance in transgenic plants by RNAi knockout of a conserved and essential root-knot nematode parasitism gene. Proc Natl Acad Sci USA 103:14302–14306

Hussey RS (1989) Disease-inducing secretions of plant-parasitic nematodes. Annu Rev Phytopathol 27:123–141

Hussey RS, Grundler FM (1998) Nematode parasitism of plants. In: Perry RN, Wright DJ (eds) Physiology and biochemistry of free-living and plant parasitic nematodes. CAB International Press, Wallingford, UK, pp 213–243

Hussey RS, Mims CW (1991) Ultrastructure of feeding tubes formed in giant-cells induced in plants by the root-knot nematode *Meloidogyne incognita*. Protoplasma 162:99–107

Hussey RS, Mims CW, Westcott SW (1992a) Ultrastructure of root-cortical cells parasitized by the ring nematode *Criconemella xenoplax*. Protoplasma 167:55–65

Hussey RS, Mims CW, Westcott SW (1992b) Immunocytochemical localization of callose in root-cortical cells parasitized by the ring nematode *Criconemella xenoplax*. Protoplasma 171:1–6

Janjusevic R, Abramovitch RB, Martin GB, Stebbins CE (2006) A bacterial inhibitor of host programmed cell death defenses is an E3 ubiquitin ligase. Science 311:222–226

Janssen J, Bakker J, Gommers FJG (1991) Mendelian proof for a gene-for-gene relationship between virulence of *Globodera rostochiensis* and the H1 resistance gene in *Solanum tuberosum* ssp. andigena CPC 1673. Rev Nématol 14:207–211

Jasmer DP, Goverse A, Smant G (2003) Parasitic nematode interactions with mammals and plants. Annu Rev Phytopathol 41:245–270

Jaubert S, Ledger TN, Laffaire JB, Piotte C, Abad P, Rosso MN (2002) Direct identification of stylet secreted proteins from root-knot nematodes by a proteomics approach. Mol Biochem Parasitol 121:205–211

Jaubert S, Laffaire JB, Ledger TN, Escoubas P, Amri EZ, Abad P, Rosso MN (2004) Comparative analysis of two 14-3-3 homologues and their expression pattern in the root-knot nematode *Meloidogyne incognita*. Int J Parasitol 34:873–880

Jaubert S, Milac AL, Petrescu AJ, de Almeida-Engler J, Abad P, Rosso MN (2005) In planta secretion of a calreticulin by migratory and sedentary stages of root-knot nematode. Mol Plant Microbe Interact 18:1277–1284

Jones JT, Smant G, Blok VC (2000) SXP/RAL-2 proteins of the potato cyst nematode *Globodera rostochiensis*: secreted proteins of the hypodermis and amphids. Nematology 2:887–893

Jones JT, Furlanetto C, Bakker E, Banks B, Blok V, Chen Q, Phillips M, Prior A (2003) Characterisation of a chorismate mutase from the potato cyst nematode *Globodera pallida*. Mol Plant Pathol 4:43–50

Jones JT, Reavy B, Smant G, Prior A (2004) Glutathione peroxidases of the potato cyst nematode *Globodera rostochiensis*. Gene 324:47–54

Keen NT, Roberts PA (1998) Plant parasitic nematodes: digesting a page from the microbe book. Proc Natl Acad Sci (USA) 95:4789–4790

Kikuchi T, Shibuya H, Jones JT (2005) Molecular and biochemical characterization of an endo-beta-1,3-glucanase from the pinewood nematode *Bursaphelenchus xylophilus* acquired by horizontal gene transfer from bacteria. Biochem J 389:117–125

Kikuchi T, Shibuya H, Aikawa T, Jones JT (2006) Cloning and characterization of pectate lyases expressed in the esophageal gland of the pinewood nematode *Bursaphelenchus xylophilus*. Mol Plant Microbe Interact 19:280–287

Kudla U, Qin L, Milac A, Kielak A, Maissen C, Overmars H, Popeijus H, Roze E, Petrescu A, Smant G, Bakker J, Helder J (2005) Origin, distribution and 3D-modeling of Gr-EXPB1, and expansin from the potato cyst nematode *Globodera rostochiensis*. FEBS Lett 579:2451–2457

Lambert KN, Allen KD, Sussex IM (1999) Cloning and characterization of an esophageal gland specific chorismate mutase from the phytoparasitic nematode *Meloidogyne javanica*. Mol Plant Microbe Interact 12:328–335

Lambert KN, Bekal S, Domier LL, Niblack TL, Noel GR, Smyth CA (2005) Selection of *Heterodera glycines chorismate mutase-1* alleles on nematode-resistant soybean. Mol Plant Microbe Interact 18:593–601

Laux T, Mayer KFX, Berger J, Jurgens G (1996) The *WUSCHEL* gene is required for shoot and floral meristem integrity in *Arabidopsis*. Development 122:87–96

Ledger TN, Jaubert S, Bosselut N, Abad P, Rosso MN (2006) Characterization of a new b-1,4 endoglucanase gene from the root-knot nematode *Meloidogyne incognita* and evolutionary scheme for phytonematode family-5 glycosyl hydrolases. Gene 382:121–128

Lee S, Lee EJ, Yang EJ, Lee JE, Park AR, Song WH, Park OK (2004) Proteomic identification of annexins, calcium-dependant membrane binding proteins that mediate osmotic stress and abscissic acid signal transduction in Arabidopsis. Plant Cell 16:1378–1391

Lightfoot D, Meksem K (2002) Isolated polynucleotides and polypeptides relating to loci underlying resistance to soybean cyst nematode and soybean sudden death syndrome and methods employing the same. US Pat Application Publ #2002144310

Lilley CJ, Bakhetia M, Charlton WL, Urwin PE (2007) Recent progress in the development of RNA interference for plant-parasitic nematodes. Mol Plant Pathol 8:701–711

Lohar DP, Schaff JE, Laskey JG, Kieber JJ, Bilyeu KD, Bird DM (2004) Cytokinins play opposite roles in lateral root formation, and nematode and Rhizobial symbioses. Plant J 38:203–214

Maizels RM, Balic A, Gomez-Escobar N, nair M, Taylor MD, Allen JE (2004) Helminth parasites – masters of regulation. Immunol Rev 201:89–116

McCarter JP, Bird DM, Mitreva M (2005) Nematode gene sequences: update for December 2005. J Nematol 37:417–421

McClure MA, von Mende N (1987) Induced salivation in plant-parasitic nematodes. Phytopathol 77:1463–1469

Michalak M, Robert Parker JM, Opas M (2002) Ca2+ signaling and calcium binding chaperones of the endoplasmic reticulum. Cell Calcium 32:269–278

Mitchum MG, Hussey RS, Davis EL, Baum TJ (2007) Application of biotechnology to understand pathogenesis of nematode plant pathogens. In: Punja ZK, DeBoer S, Sanfacon H (eds) Biotechnology & plant disease management. CABI International, Wallingford, UK, pp 58–86

Mitchum MG, Wang X, Davis EL (2008) Diverse and conserved roles of CLE peptides. Curr Opinion Plant Biol 11:75–81

Mitreva M, McCarter JP, martin J, Dante M, Wylie T, Chiapelli B, Pape D, Clifton SW, Nutman TB, Waterson RH (2004) Comparative genomics of gene expression in the parasitic and free-living nematodes *Strongyloides stercoralis* and *Caenorhabditis elegans*. Genome Res 14:209–220

Mitreva M, Blaxter ML, Bird DM, McCarter JP (2005a) Comparative genomics of nematodes. Trends Genet 21:573–581

Mitreva M, McCarter JP, Arasu P, Hawdon J, Martin J, Dante M, Wylie T, Xu J, Stajich JE, Kapulkin V, Clifton SW, Waterson RH, Wilson R (2005b) Investigating hookworm genomes by comparative analysis of two Ancylostoma species. BMC Genomics 6:58

Mitreva-Dautova M, Roze E, Overmars H, De Graff L, Schots A, Helder J, Goverse A, Bakker J, Smant G (2006) A symbiont-independent endo-1,4-beta-xylanase from the plant-parasitic nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 19:521–529

Neveu C, Jaubert S, Abad P, Castagnone-Sereno P (2003) A set of genes differentially expressed between avirulent and virulent *Meloidogyne incognita* near-isogenic lines encode secreted proteins. Mol Plant Microbe Interact 16:1077–1084

Nielsen H, Engelbrecht J, Brunak S, von Heijne G (1997) Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng 10:1–6

Novina CD, Sharp PA (2004) The RNAi revolution. Nature 430:161–164

Olsen AN, Skriver K (2003) Ligand mimicry? A plant-parasitic nematode polypeptide with similarity to CLAVATA3. Trends Plant Sci 8:55–57

Opperman CH, Bird DMcK, Schaff JE (2008) Genomic analysis of the root-knot nematode genome. Plant Cell Monogr., doi:10.1007/7089_2008_37

Padidam M, Gore M, Lu DL, Smirnova O (2003) Chemical-inducible, ecdysone receptor-based gene expression system for plants. Transgenic Res 12:101–109

Patel N (2008) Functional analyses of cyst nematode parasitism genes. PhD Dissertation, North Carolina State University

Perry RN, Zunke U, Wyss U (1989) Observations on the response of the dorsal and subventral oesophageal glands of *Globodera rostochiensis* to hatching stimulation. Rev Nematol 12:91–96

Popeijus H, Overmars H, Jones J, Blok V, Goverse A, Helder J, Schots A, Bakker J, Smant G (2000) Degradation of plant cell walls by a nematode. Nature 406:36–37

Prior A, Jones JT, Blok VC, Beauchamp J, McDermott L, Cooper A, Kennedy MW (2001) A surface-associated retinol- and fatty acid-binding protein (Gp-FAR-1) from the potato cyst nematode *Globodera pallida*: lipid binding activities, structural analysis and expression pattern. Biochem J 356:387–394

Pysh LD, Wysocka-Diller JW, Cmilleri C, Bouchez D, Benfey PN (1999) The *GRAS* gene family in *Arabidopsis*: sequence characterization and basic expression analysis of the *SCARECROW-LIKE* genes. Plant J 18:111–119

Qin L, Smant G, STokkermans J, Bakker J, Schots A, Helder J (1998) Cloning of a trans-spliced glyceraldehydes-3-phosphate-dehydrogenase gene from the potato cyst nematode *Globodera rostochiensis* and expression of its putative promoter region in *Caenorhabditis elegans*. Mol Biochem Parasitol 96:59–67

Qin L, Overmars H, Popeijus H, Roupe Van Der Voort JNAM, Groenink W, van Koert P, Schots A, Bakker J, Smant G (2000) An efficient cDNA-AFLP-based strategy for the identification of putative pathogenicity factors from the potato cyst nematode *Globodera rostochiensis*. Mol Plant Microbe Interact 13:830–836

Qin L, Prins P, Jones JT, Popeijus H, Smant G, Bakker J, Helder J (2001) GenEST, a powerful bi-directional link between cDNA sequence data and gene expression profiles generated by cDNA-AFLP. Nucleic Acids Res 29:1616–1622

Qin L, Kudla U, Roze EHA, Goverse A, Popeijus H, Nieuwland J, Overmars H, Jones JT, Schots A, Smant G, Bakker J, Helder J (2004) Plant degradation: A nematode expansin acting on plants. Nature 427:30

Robertson L, Robertson W, Sobczak M, Helder J, Tetaud E, Ariyanayagam M, Ferguson M, Fairlamp A, Jones JT (2000) Cloning, expression and functional characterization of a peroxiredoxin from the potato cyst nematode *Globodera rostochiensis*. Mol Biochem Parasitol 111:41–49

Romero RM, Roberts MF, Phillipson JD (1995) Chorismate mutase in microorganisms and plants. Phytochem 40:1015–1025

Rosso MN, Dubrana MP, Cimbolini N, Jaubert S, Abad P (2005) Application of RNA interference to root-knot nematode genes encoding esophageal gland proteins. Mol Plant Microbe Interact 18:615–620

Roze E, Hanse B, Mitreva M, Vanholme B, Bakker J, Smant G (2008) Mining the secretome of the root-knot nematode *Meloidogyne chitwoodi* for candidate parasitism genes. Mol Plant Pathol 9:1–10

Scholl EH, Thorne JL, McCarter JP, Bird DM (2003) Horizontally transferred genes in plant-parasitic nematodes: a high-throughput genomic approach. Genome Biol 4:R39

Semblat JP, Rosso MN, Hussey RS, Abad P, Castagnone-Sereno P (2001) Molecular cloning of a cDNA encoding an amphid-secreted putative avirulence protein from the root-knot nematode *Meloidogyne incognita*. Mol Plant Microbe Interact 14:72–79

Shpigel E, Roiz L, Goren R, Shoseyov O (1998) Bacterial cellulose-binding domain modulates in vitro elongation of different plant cells. Plant Physiol 117:1185–94

Siles-Lucas M, Gottstein B (2003) The 14-3-3 protein: a key molecule in parasites as in other organisms. Trends Parasitol 19:575–581

Smant G, Goverse A, Stokkermans JPWG, De Boer JM, Pomp H, Zilverentant JF, Overmars HA, Helder J, Schots A, Bakker J (1997) Potato root difusate-induced secretion of soluble, basic proteins originating from the subventral oesophageal glands of potato cyst nematodes. Phtyopathol 87:839–845

Smant G, Stokkermans JPWG, Yan YT, de Boer JM, Baum TJ, Wang XH, Hussey RS, Gommers FJ, Henrissat B, Davis EL, Helder J, Schots A, Bakker J (1998) Endogenous cellulases in animals: Isolation of beta-1,4-endoglucanase genes from two species of plant-parasitic cyst nematodes. Proc Natl Acad Sci USA 95:4906–4911

Steeves RM, Todd TC, Essig JS, Trick HN (2006) Transgenic soybeans expressing siRNAs specific to a major sperm protein gene suppress *Heterodera glycines* reproduction. Funct Plant Biol 33:991–999

Suchitra S, Jochi P (2005) Characterization of *Haemonchus contortus* calreticulin suggests its role in feeding and immune evasion by the parasite. Biochim Biophys Acta 1722:293–303

Timmons L, Fire A (1998) Specific interference by ingested dsRNA. Nature 395:854
Tytgat T, Vanholme B, De Meutter J, Claeys M, Couvreur M, Vanhoutte I, Gheysen G, Van Criekinge W, Borgonie G, Coomans A, Gheysen G (2004) A new class of ubiquitin extension proteins secreted by the dorsal pharyngeal gland in plant parasitic cyst nematodes. Mol Plant Microbe Interact 17:846–852
Tytgat T, Vercauteren I, Vanholme B, De Meutter J, Vanhoutte I, Gheysen G, Borgonie G, Coomans A, Gheysen G (2005) An SXP/RAL-2 protein produced by the subventral pharyngeal glands in the plant parasitic root-knot nematode *Meloidogyne incognita*. Parasitol Res 95:50–54
Urwin PE, Moller SG, Lilley CJ, McPherson MJ, Atkinson HJ (1997) Continual green-fluorescent protein monitoring of cauliflower mosaic virus 35S promoter activity in nematode-induced feeding cells in *Arabidopsis thaliana*. Mol Plant Microbe Interact 10:394–400
Urwin PE, Lilley CJ, Atkinson HJ (2002) Ingestion of double-stranded RNA by preparasitic juvenile cyst nematodes leads to RNA interference. Mol Plant Microbe Interact 15:747–752
Valentine T, Shaw J, Blok VC, Phillips MS, Oparka KJ, Lacomme C (2004) Efficient virus-induced gene silencing in roots using a modified tobacco rattle virus vector. Plant Physiol 136:3999–4009
Vanholme B, De Meutter J, Tytgat T, Van Montagu M, Coomans A, Gheysen G (2004) Secretions of plant-parasitic nematodes: a molecular update. Gene 332:13–27
Vanholme B, Mitreva MD, Van Criekinge W, Logghe M, Bird D, McCarter JP, Gheysen G (2005) Detection of putative secreted proteins in the plant-parasitic nematode *Heterodera schachtii*. Parasitol Res 98:414–424
Vanholme B, Mitreva M, Van Criekinge W, Logghe M, Bird D, McCarter JP, Gheysen G (2006) Detection of putative secreted proteins in the plant-parasitic nematode *Heterodera schachtii*. Parasitol Res 98:414–424
Veech JA, Starr JL, Nordgren RM (1987) Production and partial characterization of stylet exudates from adult females of *Meloidogyne incognita*. J Nematol 19:463–468
Waetzig GH, Sobczak M, Grundler FMW (1999) Localization of hydrogen peroxide during the defense response of *Arabidopsis thaliana* against the plant-parasitic nematode *Heterodra glycines*. Nematology 1:681–686
Wang SH, Zheng HJ, Dissanayake S, Chen WF, Tao ZH, Lin SZ, Piessens WF (1997) Evaluation of recombinant chitinase and SXP1 antigens as antimicrofilarial vaccines. Am J Trop Med Hyg 56:474–481
Wang X, Meyers DM, Baum TJ, Smant G, Hussey RS, Davis EL (1999) In planta localization of a ß-1,4-endoglucanse secreted by *Heterodera glycines*. Mol Plant Microbe Interact 12:64–67
Wang X, Allen R, Ding XF, Goellner M, Maier T, de Boer JM, Baum TJ, Hussey RS, Davis EL (2001) Signal peptide-selection of cDNA cloned directly from the esophageal gland cells of the soybean cyst nematode *Heterodera glycines*. Mol Plant Microbe Interact 14:536–544
Wang X, Mitchum MG, Gao BL, Li C, Diab H, Baum TJ, Hussey RS, Davis EL (2005) A parasitism gene from a plant-parasitic nematode with function similar to *CLAVATA3/ESR (CLE)* of *Arabidopsis thaliana*. Mol Plant Pathol 6:187–191
Weerasinghe R, Bird DM, Allen NS (2005) Root-knot nematodes and bacterial Nod factors elicit common signal transduction events in *Lotus japonicus*. Proc Natl Acad Sci (USA) 102:3147–3152
Wesley SV, Helliwell CA, Smith NA, Wang M, Rouse DT, Liu Q, Gooding PS, Singh SP, Abbott D, Stoutjesdijk PA, Robinson SP, Gleave AP, Green AG, Waterhouse PM (2001) Construct design for efficient, effective and high-throughput gene silencing in plants. Plant J 27:581–590
Wilde A, Lizaragga SB, Zhang L, Wiese C, Gliksman NR, Walczak CE, Zheng Y (2001) Ran stimulates spindle assembly by altering microtubule dynamics and the balance of motor activities. Nature Cell Biol 3:221–227
Wyss U, Zunke U (1986) Observations on the behaviour of second stage juveniles of *Heterodera schachtii* inside host roots. Rev Nematol 9:153–165
Wyss U, Grundler FMW, Munch A (1992) The parasitic behaviour of second stage juveniles of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. Nematologica 38:98–111

Yadav BC, Veluthambi K, Subramaniam K (2006) Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. Mol Biochem Parasitol 148:219–222

Yan YT, Smant G, Stokkermans J, Qin L, Helder J, Baum T, Schots A, Davis E (1998) Genomic organization of four beta-1,4-endoglucanase genes in plant-parasitic cyst nematodes and its evolutionary implications. Gene 220:61–70

Yan Y, Smant G, Davis EL (2001) Functional screening yields a new beta-1,4 endoglucanase gene from *Heterodera glycines* that may be the product of a recent gene duplication. Mol Plant Microbe Interact 14:63–71

Zhan B, Liu Y, Badamchian M, Williamson A, Feng J, Loukas A, Hawdon JM, Hotez PJ (2003) Molecular characterization of the *Ancylostoma*-secreted protein family from the adult stage of *Ancylostoma caninum*. Int J Parasitol 33:897–907

Zuo J, Niu QW, Chua NH (2000) An estrogen-receptor-based transactivator XVE mediates highly inducible gene expression in transgenic plants. Plant J 24:265–273