

# NEMATODE PARASITISM GENES

---

Eric L. Davis

*Department of Plant Pathology, Campus Box 7616, North Carolina State University, Raleigh, North Carolina 27695; e-mail: eric\_davis@ncsu.edu*

Richard S. Hussey

*Department of Plant Pathology, 2309 Miller Plant Science Building, University of Georgia, Athens, Georgia 30602-7274; e-mail: hussey@uga.cc.uga.edu*

Thomas J. Baum

*Department of Plant Pathology, 351 Bessey Hall, Iowa State University, Ames, Iowa 50011; e-mail: tbaum@iastate.edu*

Jaap Bakker and Arjen Schots

*Department of Nematology, Wageningen University and Research Centre, Binnenhaven 10, 6709 PD Wageningen, The Netherlands; e-mail: Jaap.Bakker@MEDEW.NEMA.WAU.NL; Arjen.Schots@LMA.NEMA.WAU.NL*

Marie-Noëlle Rosso and Pierre Abad

*Institut National de la Recherche Agronomique, Laboratoire de Biologie des Invertébrés, 123 Boulevard Francis Meilland, 06600 Cedex Antibes, France; e-mail: rosso@antibes.inra.fr; abad@antibes.inra.fr*

**Key Words** functional genomics, gene evolution, horizontal gene transfer, secretory glands, plant resistance

■ **Abstract** The ability of nematodes to live on plant hosts involves multiple parasitism genes. The most pronounced morphological adaptations of nematodes for plant parasitism include a hollow, protrusible stylet (feeding spear) connected to three enlarged esophageal gland cells that express products that are secreted into plant tissues through the stylet. Reverse genetic and expressed sequence tag (EST) approaches are being used to discover the parasitism genes expressed in nematode esophageal gland cells. Some genes cloned from root-knot (*Meloidogyne* spp.) and cyst (*Heterodera* and *Globodera* spp.) nematodes have homologues reported in genomic analyses of *Caenorhabditis elegans* and animal-parasitic nematodes. To date, however, the candidate parasitism genes endogenous to the esophageal glands of plant nematodes (such as the  $\beta$ -1,4-endoglucanases) have their greatest similarity to microbial genes, prompting speculation that genes for plant parasitism by nematodes may have been acquired by horizontal gene transfer.

## CONTENTS

INTRODUCTION . . . . .	366
NEMATODE PARASITISM OF PLANTS . . . . .	367
DIRECT MOLECULAR ANALYSIS . . . . .	370
GENETIC MODELS OF PLANT PARASITISM BY NEMATODES . . . . .	376
STRUCTURE, REGULATION, AND FUNCTIONAL ANALYSIS OF PARASITISM GENES . . . . .	378
ORIGINS OF PARASITISM GENES . . . . .	382
TARGETING THE PRODUCTS OF NEMATODE PARASITISM GENES . . . . .	385
CONCLUDING REMARKS . . . . .	387

## INTRODUCTION

Most nematodes are not parasites. The vast majority of nematode species are microbivores, fungivores, predators, and omnivores that live in a variety of terrestrial, aquatic, and marine environments (6). The minority of nematode species that comprises the parasites of plants and animals, however, has staggering health, ecological, and economic impacts (9, 80). Understanding the genetic adaptations underlying the evolution of parasitism by nematodes is not only fascinating biology, but it will undoubtedly reveal potential targets to combat nematode parasitism that are of paramount importance to successful medicine and sustainable agriculture. “Parasitism” may be defined in several ways, and unfortunately, a lack of consensus about the meanings of host-parasite (pathogen) terminology still exists in plant pathology (23). For simplicity, we use Webster’s definition of a *parasite* as “an organism living in or on another living organism, obtaining from it part or all of its organic nutriment, and commonly exhibiting some degree of adaptive structural modification” (62). This broad definition encompasses a wide range of potential nematode *parasitism genes* that have evolved specifically, or perhaps were “procured” and modified from other successful parasitic organisms, to promote parasitism in a host. Nematodes should be considered first as parasites, and if disease results in the host, the parasites become *pathogens* (129). The products of nematode parasitism genes may be manifested as morphological structures that provide access to parasitism of a particular host (e.g. a stylet) or they may play critical physiological roles in the interaction of the nematode with its host.

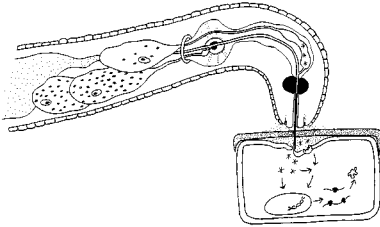
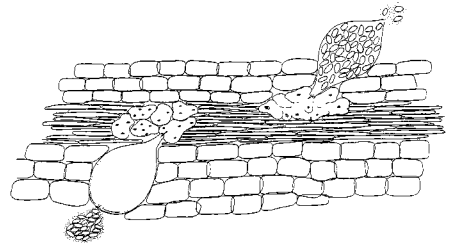
A large volume of (mostly descriptive) research has been conducted on the nature of plant-nematode interactions, and readers are referred to several recent reviews on this topic (14, 72, 120, 137). Plant nematodes are obligate parasites—some species have evolved rather simple feeding strategies while other nematode species are highly adapted for more sophisticated parasitic relationships with host plants. A majority of research has focused upon plant response to nematode parasitism, primarily the complex modifications that some plant-parasitic nematodes induce in host plant cells and plant resistance to nematode challenge. Recent

research is now providing insights into the molecular and genetic basis of the “nematode side” of plant-nematode interactions. Nematode parasitism genes may be active in any or all parts of the parasitic cycle of plant nematodes (Figure 1), including “preparasitic” life stages (before invasion of the plant) and “parasitic” life stages (after invasion of the plant). Forward genetic investigations of the interactions of cyst nematodes with resistant plant genotypes are being combined with physical maps of nematode genomes and cloning strategies to identify nematode virulence genes (15, 112). The identification of genes encoding bioactive molecules from nematodes that initiate and maintain successful parasitic interactions with host plants is another area of active investigation, primarily by reverse-genetic approaches (72). The technologies of genomics are rapidly providing scientists with the means to compare gene structure, organization, and function across different genomes, and the completion of the entire genome sequence of the microbivorous nematode, *Caenorhabditis elegans*, will be invaluable to the study of nematode parasitism genes (27). This opportunity is already being realized by genomic analyses of animal parasites, including the Filarial Nematode Genome Project (16, 80). It is likely that some parasitism genes have evolved from “basic” nematode genes, and that orthologues of these genes exist across nematode genomes. Fundamental mechanisms of parasitism may have been retained between plant- and animal-parasitic nematodes akin to those that have been demonstrated between bacteria that are pathogenic on plants and animals (33). Intriguing new evidence, however, suggests another potential source from which nematodes may have acquired specific genes for plant parasitism—horizontal gene transfer (123, 142).

## NEMATODE PARASITISM OF PLANTS

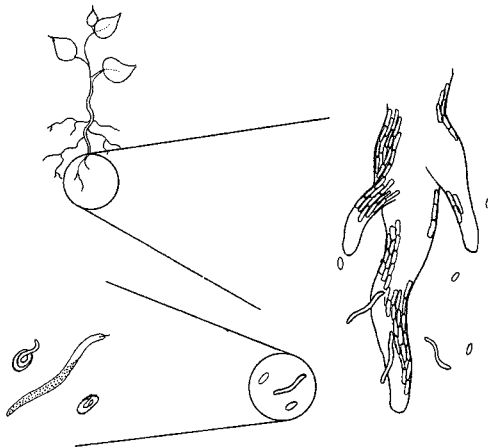
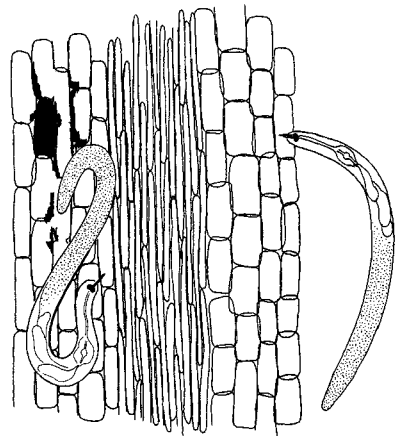
Plant-parasitic nematodes have evolved diverse parasitic strategies and feeding relationships with their host plants to obtain nutrients that are necessary for development and reproduction. The vast majority of plant-parasitic nematode species are soil-dwelling and feed from plant roots (Figure 1). These biotrophic parasites, depending upon species, feed from the cytoplasm of unmodified living plant cells or have evolved to modify plant cells into elaborate discrete feeding cells. Plant-parasitic nematodes use a hollow, protrusible feeding structure, called a stylet, to penetrate the wall of a plant cell, inject gland secretions into the cell, and withdraw nutrients from the cytoplasm. Migratory feeding nematodes remove cytoplasm from the parasitized cell, frequently causing cell death, and then move to another cell to repeat the feeding process. Other nematodes become sedentary and feed from a single cell or a group of cells for prolonged periods of time. For this sustained feeding, the sedentary parasites dramatically modify root cells of susceptible hosts into elaborate feeding cells, including modulating complex changes in cell morphology, function, and gene expression. These feeding cells become the sole source of nutrients for sedentary endoparasites such as *Meloidogyne* (root-knot nematode) or *Heterodera* and *Globodera* (cyst nematode) species. Similarly, in

- Active feeding by nematode via the stylet
- Feeding cells serve as a nutrient sink for nematode
- Nematode stimulus maintains feeding site
- Feeding tubes aid ingestion of nutrients
- Successful nematode growth and reproduction



- Nematode signals trigger feeding site formation
- Esophageal gland secretions released through stylet
- Interaction of plant and nematode signals
- Gene expression is modified in parasitized cells
- Avirulent nematodes elicit defense in resistant genotypes

- Ectoparasites feed externally by inserting stylet
- Endoparasites enter roots to feed
- Mechanical and/or enzyme-aided migration within roots
- Nematodes select specific cells for feeding
- Resistant response to avirulent nematodes



- Egg hatch is influenced by root exudates
- Motile nematodes active in soil environment
- Nematodes respond to root signals
- Soil microbial activity affects nematodes
- Nematodes recognize specific root tissues

**Figure 1** Progressive stages of plant parasitism by nematodes (from bottom).

sedentary ectoparasites such as the ring nematode, *Criconemella xenoplax*, a single feeding cell is utilized as a nutrient source for several days before the nematode moves on to establish another feeding site (72).

In addition to the protrusible stylet, nematodes in the orders Tylenchida and Aphelenchida have evolved a well-developed esophagus for feeding on plants (Figure 1). The esophagus has a muscular metacorpus containing a triradiate pump chamber and three large and complex secretory gland cells (50, 73). The transcriptionally active gland cells, one dorsal (DG) and two subventral (SvG), are the principal source of the secretions involved in plant parasitism. Each gland is a single large, specialized secretory cell with a cytoplasmic extension that terminates in a storage ampulla which is connected to the esophageal lumen by an elaborate valve (1, 50, 73). Secretory proteins are synthesized in the nuclear region of the gland cell and stored in spherical Golgi-derived membrane-bounded granules which are transported along microtubules in the gland cell extension to the ampullae. During secretion, the gland cell is triggered to rapidly release the secretory proteins from the granules by exocytosis into the membranous end-sac of the valve where the proteins pass through a duct to enter the lumen of the esophagus to be injected through the stylet into host tissue.

Critical unresolved questions in the study of nematode esophageal gland secretions are the nature and number of different secretory proteins packaged in the secretory granules and the temporal changes in the kinds of proteins secreted during the parasitic cycle. The core of secretory granules typically is a large volume of highly concentrated protein, and the number of different secretory proteins in the matrix can vary with gland cell type and parasitic stage (24). Specific compartmentalization of one secretory protein within the matrix of granules formed in the SvG cells of second-stage juveniles (J2) of the root-knot nematode, *Meloidogyne incognita*, has been documented (75). Morphological changes in esophageal gland cells are correlated with the developmental phases in the life cycle of root-knot and cyst nematodes. The SvG cells are the most active glands in infective J2, but following the onset of parasitism, the DG cell is stimulated to increase production of secretory granules and becomes the predominate gland in the parasitic stages (13, 73). These changes in the esophageal gland cells during the parasitic cycle indicate various roles for the gland secretory proteins during different stages of parasitism. Changes in secretory antigens observed within both the SvG and DG of root-knot and cyst nematodes throughout the parasitic cycle have also been documented that support changing roles of the gland cell secretions during nematode feeding and development (34, 64). The relative importance of secretions from the SvG versus the DG in host-nematode interactions has been debated (70, 139), but the recent discovery that  $\beta$ -1,4-endoglucanases (cellulases) are synthesized in SvG of cyst nematodes and secreted through the nematode's stylet in planta unequivocally establishes a role for SvG secretions in plant parasitism (123, 135).

Root-knot and cyst nematodes have the most evolutionarily advanced mode of parasitism of the plant-parasitic nematodes. These nematodes have evolved to alter gene expression in specific root cells to modify them into very specialized and

complex feeding cells (14). Infective J2 penetrate behind the root tip and migrate intercellularly by separating cells at the middle lamella (root-knot nematodes) or intracellularly by rupturing cell walls (cyst nematodes) through the cortex to the vascular tissue (138, 139). Although migration within root tissue involves stylet thrusting to weaken the cell walls, cyst nematode J2 secrete cellulases through the stylet to facilitate degradation of the cellulose within the walls (123, 135). When J2 reach the appropriate vascular tissue, products of the glands are secreted through the stylet to induce the transformation of recipient cells in susceptible plants into metabolically active feeding cells, called syncytium (cyst nematodes) or giant-cells (root-knot nematodes). These unidentified gland secretions modify, directly or indirectly, gene expression to induce profound morphological, physiological, and molecular changes in the recipient host cells to enable them to function as a continuous source of nutrients for the parasitic stages. Cell fusion following cell wall degradation gives rise to the syncytium, whereas abnormal cell growth following repeated mitosis uncoupled from cytokinesis produces the giant-cells. These large, multinucleate feeding cells possess thickened cell walls that are remodeled to form elaborate ingrowths and a dense granular cytoplasm with an increased number of organelles and small vacuoles. A number of plant genes with known or putative functions are up- or down-regulated in these feeding cells, suggesting that root-knot and cyst nematodes induce transcriptional changes in the parasitized plant cells (14, 52, 53, 59, 69, 96).

Although the mechanism(s) by which these nematodes alter plant gene expression is unknown, strong evidence suggests that products of the esophageal gland cells that are secreted through the stylet cause parasitized root cells to differentiate into unique feeding cells. While it must be considered that secretions from nematode amphids and other body orifices, and molecules that comprise the nematode surface coat, may interact with host plant cells, the contents of the esophageal glands cells that are secreted through the nematode stylet represent the most advanced adaptation for plant parasitism by nematodes. The key to understanding nematode parasitism of plants and the molecular triggers that alter gene expression to transform host cells into feeding sites is to expand our knowledge of the nature and function of components of nematode stylet secretions. Cloning nematode genes encoding esophageal gland cell secretory proteins is critical for determining the role of different stylet secretions in plant parasitism. In the following sections of this review we focus primarily on the approaches and current progress in identifying parasitism genes of plant nematodes in the order Tylenchida, with particular emphasis on the root-knot and cyst nematodes.

## DIRECT MOLECULAR ANALYSIS

The isolation of nematode parasitism genes by direct analysis of gene expression and translation during the parasitic cycle represents a relatively rapid and direct means to identify putative parasitism genes as compared to forward genetic

approaches. Direct molecular analyses often rely on assumptions made about critical events in the nematode parasitic cycle. Fortunately, a wealth of information is available that describes parasitism by nematodes in great detail. The products of some nematode parasitism genes will be involved in the exchange of signals and activity of parasitic mechanisms that have evolved specifically for parasitism of a host. Genes involved in controlling basic components of the nematode life cycle which have been adapted and integrated with the parasitic cycle are indirectly essential for parasite success, but they do not have a direct role in promoting plant parasitism by nematodes. The most compelling tissues to look for plant nematode parasitism genes are the esophageal gland cells which have been dramatically adapted for enhanced secretory activity that is directly involved in plant parasitism (70). Indeed, the only putative parasitism genes cloned from plant-parasitic nematodes as of this writing have been from the esophageal gland cells (42, 88, 110, 123). The true functions of these and any nematode parasitism genes remains putative, at present, until techniques are developed with plant-parasitic nematodes to complete the process of reverse genetics or to intervene in the activity of target gene products to assess function (see below). The use of "model" nematode systems (i.e. *C. elegans* and filarial nematodes) can aid in this process, although the isolation and analysis of some nematode genes specifically adapted for parasitism of plants will not be suited for these model systems.

Analyses of stylet secretions produced in the esophageal gland cells of plant-parasitic nematodes accelerated with the advent of contemporary molecular techniques (72). Previous studies of the esophageal glands and the limited amount of stylet secretions that could be obtained from root-knot nematodes indicated that a mixture of proteins (some glycosylated), but not nucleic acids, were present in the secretions (70). The presence of proteins in nematode stylet secretions was also confirmed using *in vitro* systems designed to chemically stimulate the production of stylet secretions from root-knot and cyst nematodes (34, 64, 93). At least 10 protein bands, and the activity of proteases and superoxide dismutase, have been observed in analyses of stylet secretions from J2 of the potato cyst nematode, *Globodera rostochiensis*, that were stimulated by incubation in 5-methoxy DMT oxalate (109). Nematode stylet secretions produced *in vitro*, and various preparations of the esophageal gland regions of root-knot and cyst nematodes, also have been used as immunogens to generate panels of monoclonal antibodies that bound specifically to different esophageal gland antigens (2, 35, 36, 64, 71). The monoclonal antibodies have been used either to isolate different esophageal gland antigens for direct analyses or to screen cDNA expression libraries constructed from cyst and root-knot nematodes to isolate the corresponding secretion genes.

A monoclonal antibody [7A9, see (35)] to a SvG protein of the root-knot nematode *M. incognita* was used to isolate a cDNA clone (*sec-1*) that had moderate similarity to the rod portion of myosin heavy chains (105). Since the secretion of SEC-1 from the nematode could not be verified, it was hypothesized that SEC-1 was involved in the movement of secretory granules in the esophageal gland cells rather than being a secretory protein itself. In the soybean cyst nematode,

*Heterodera glycines*, a SvG-specific monoclonal antibody [MAb 9C2, see (64)] was used to isolate a cDNA clone (*svg-1*) that had homology to a group of heavily O-glycosylated secreted proteins called mucins (X Wang & EL Davis, unpublished data). Interestingly, the surface mucins of the animal-parasitic nematode *Toxocara canis* have been implicated in interactions with host defense mechanisms (58). A partial cDNA (*drs-1*) was isolated from a preparasitic J2 cDNA expression library of *H. glycines* using a dorsal gland-specific monoclonal antibody [MAb 5B9, see (64)]. Although the predicted open reading frame of the *drs-1* partial cDNA was greater than 300 amino acids, no homology of *drs-1* to other reported genes was detected in database searches (140).

The  $\beta$ -1,4-endoglucanase (cellulase) genes cloned from cyst nematodes represent the most successful use of an esophageal gland-specific monoclonal antibody to isolate nematode parasitism genes (123). A monoclonal antibody (MGR 48) that bound specifically to SvG antigens in several species of cyst nematodes (36) was used to affinity-isolate the antigens from large-scale preparations of proteins from *G. rostochiensis* and *H. glycines* (123). Degenerate oligonucleotides were developed to the N-terminal amino acid sequences of each isolated MGR 48 antigen and two cDNA clones were derived from each nematode species using a 3' RACE technique. Database searches of each cDNA sequence had homology to the Family 5 bacterial  $\beta$ -1,4-endoglucanases. Both *G. rostochiensis* and *H. glycines* had a cDNA (*eng-1*) that contained a secretion signal peptide, catalytic domain, peptide linker, and a cellulose-binding domain (CBD). The *eng-2* gene of *G. rostochiensis* was missing the CBD, and the *eng-2* gene of *H. glycines* was missing the CBD and peptide linker. mRNA in situ hybridizations of the *eng* probes bound specifically within the SvG of cyst nematodes (37, 123). Cellulolytic activity was demonstrated in overexpressed cloned *eng* products, and polyclonal sera raised to recombinant ENG proteins bound specifically within the nematode SvG. Genomic clones of the cyst nematode *eng* genes contained an intron/exon structure typical of eukaryotic genes (142). These data combined confirmed that the cyst nematode endoglucanases were endogenous. More recently, similar cyst nematode endoglucanase genes have been isolated from *Globodera tabacum* (60) and *Heterodera schachtii* (39). Using conserved regions of nematode, fungal, and bacterial endoglucanase genes, a PCR-based approach was used to identify cellulase genes in *M. incognita* (110). These same PCR primers have most recently been used to isolate putative cellulase genes from plant-parasitic nematodes of diverse parasitic habits including *Pratylenchus agilis*, *Paratrichodorus minor*, *Bursaphelenchus xylophilus*, *Rotylenchulus reniformis*, and *Ditylenchus dipsaci* (Y Yan, MN Rosso & EL Davis, unpublished data). The cloning of the endoglucanase genes verifies much earlier observations of cellulase activity in plant-parasitic nematodes (41). These earlier reports also suggest that other enzymes that degrade plant cell constituents may be among the secreted products of nematode parasitism genes.

Genomics, especially the generation of cDNA libraries and expressed sequence tags (ESTs) from parasitic nematodes, represents a powerful and comprehensive

approach to isolate nematode parasitism genes. The most basic approach is to construct cDNA libraries from specific life stages of whole nematodes and sequence as many random ESTs as possible (15: J Jones, H Popeijus, J Bakker & A Schots, unpublished data). The ESTs may be used directly as “anchors” on physical maps of the nematode genome, and in addition, database searches may quickly reveal expressed genes that have an apparent role in parasitism. This latter approach has been accomplished by analysis of ESTs from a preparasitic J2 cDNA library of *G. rostochiensis* (100a, 101). A full-length cDNA encoding a predicted peptide of 261 amino acids was obtained that had strong homology to reported fungal pectate lyases (E.C. 4.2.2.2.). The predicted protein had a secretion signal peptide, and analyses are under way to identify if the pectate lyase may be secreted from the nematodes (i.e. via the esophageal glands and stylet). The recent cloning of a putative pectate lyase cDNA from *M. javanica* and localization of the transcript within the nematode’s esophageal glands (K Lambert, personal communication) suggests the potential secretion of a pectate lyase from the nematode stylet during plant parasitism.

Random sequencing of ESTs from a selected nematode life stage may reveal potential parasitism genes (i.e. pectate lyase), and it may be coupled with cDNA-AFLP analyses (5) to select candidate nematode parasitism genes from the EST database that are expressed in a stage-specific manner (101a). It has been reported that the SvG of *G. rostochiensis* J2 within eggs are activated by hydration (99), but the addition of potato root diffusate is required to increase DG activity and stimulate hatch of *G. rostochiensis* J2 (4, 99). cDNA-AFLP analysis comparing these different stages of egg hatch by *G. rostochiensis* has revealed genes that were expressed specifically in J2 upon hatch in potato root diffusate, and the full-length sequences were obtained using an EST database of a cDNA library of whole *G. rostochiensis* J2 (101a). Conversely, data obtained by the EST approach are also being analyzed for potential stage-specific expression using cDNA-AFLP. mRNA in situ hybridization (37) is now being employed with some of the candidate parasitism genes isolated by the cDNA-AFLP/EST approach to determine if the genes are expressed within the nematode esophageal gland cells.

Differential gene expression between preparasitic and parasitic nematode life stages has been analyzed using a RNA fingerprinting technique (42, 44). A cDNA (*mi-msp-1*) encoding a putative secretory venom allergin AG5-like protein with strong similarity to *Ancylostoma*-secreted protein 2 (67) was obtained from *M. incognita* using this protocol (44). A cDNA coding for a cellulose-binding protein also was isolated from *M. incognita* (*mi-cbp-1*) from an elevated transcript level in parasitic J2 (42). The CBP product is specifically expressed in the SvG of *M. incognita*. The N-terminal region of the predicted peptide had no similarity to known proteins, but the C terminus has strong homology to a cellulose-binding domain. Secretion of this protein through the nematode stylet was confirmed in vitro. Although in planta secretion seems likely, in vivo analyses must be conducted to test this hypothesis. The role of CBP remains elusive at this time. Interestingly,

a recombinant cellulose-binding domain (CBD) derived from the cellulolytic bacterium *Clostridium cellulovorans* was found to modulate the elongation of different plant cells in vitro (119). This finding suggests a possible role of the CBP in host modifications associated with root-knot nematode parasitism.

Gene products with obvious (putative) functions in plant parasitism (i.e. cellulases, pectinases) can be relatively easily identified from random ESTs derived from preparations of whole nematodes if abundant transcripts are present in the parasitic stage(s) chosen for analysis. Expressed nematode genes that are rare, or genes with obscure functions in plant parasitism, will be more difficult to isolate since virtually no idea of their identity exists at present. The isolation of candidate nematode parasitism genes can be enhanced by comparative analysis of gene expression in different nematode life stages, such as the cDNA-AFLP and RNA fingerprinting approaches described above. EST analyses targeting specific nematode tissues (i.e. esophageal gland cells) that express products likely to be involved in plant-nematode interactions can narrow this focus even further. In one example, esophageal gland regions from *Meloidogyne javanica* were excised and cDNA was prepared from this tissue by RT-PCR (88). This cDNA pool was differentially screened against cDNA from the (glandless) nematode tail region to isolate genes expressed specifically in the nematode esophageal gland region. A full-length cDNA clone was obtained that had homology to a bacterial chorismate mutase (CM). Tissue-specific expression of *mj-cm-1* has been localized to the esophageal gland cells of parasitic *M. javanica* by mRNA in situ hybridization and with antisera generated to recombinant MJ-CM-1 protein. Chorismate mutase is an enzyme associated with the shikimate pathway leading to the synthesis of phenylalanine and tyrosine (61). Interestingly, the shikimate pathway has not been shown to be present in nematodes or other animals. Introduction of MJ-CM-1 into the cytosol of an initial feeding cell could potentially alter the spectrum of chorismate-dependent compounds, which, among other functions, are involved in cell wall formation, hormone biosynthesis, and synthesis of defense compounds.

The differential screening of cDNA generated from the esophageal gland and tail regions of *M. javanica* was a tissue-specific approach to isolating candidate nematode parasitism genes (88), but the gland region cDNA was contaminated with expressed genes extracted from nontarget tissues immediately surrounding the esophageal gland cells. To avoid contamination from extraneous tissues, a microaspiration technique used to obtain the contents of individual nematode esophageal gland cells (118) is now being coupled with a protocol designed to generate cDNA from individual cells by RT-PCR (83). mRNA isolates from transcriptionally active gland cells of a range of parasitic stages of *M. incognita* and *H. glycines* have been pooled and used to generate esophageal gland cell-specific cDNA libraries that provide a comprehensive profile of nematode esophageal gland gene expression during plant parasitism (43, 134). ESTs may be sequenced directly from random clones within each gland-specific library, or alternatively, microarrays (DNA chips) gridded with this comprehensive gland gene profile could be used to differentiate nematode gland gene expression at any selected parasitic stage

(104). In addition, genes encoding secreted peptides can be selected from these libraries using a secretion-specific vector expressed in yeast (85). Initial results of ESTs analyzed from esophageal gland-specific cDNA libraries from *M. incognita* and *H. glycines* include sequences with similarity to genes identified in *C. elegans*, some sequences with similarity to bacterial genes, and some of the isolated ESTs have no homology to any reported genes (TJ Baum, EL Davis & RS Hussey, unpublished data). It is envisioned that improved bioinformatics systems such as PFAM peptide analysis (48), and the development of efficient and definitive functional assays for putative plant-parasitic nematode parasitism genes, will advance rapidly within the next few years to confirm the identity of "unknown" coding sequences.

Expressed genes (ESTs) with homology to genes identified in *C. elegans* have also been isolated from investigations of the genome of *Brugia malayi*, the animal-parasitic nematode that is the model of the Filarial Nematode Genome Project (16, 25). The availability of the genome sequence of *C. elegans* and the difficulties in analyses of obligate parasite genomes have prompted a "gene discovery" (EST) approach as the initial phase of filarial genome analysis rather than large-scale genome sequencing (16, 80). cDNA libraries constructed from various parasitic stages of *Brugia* have been used to analyze at least 16,000 ESTs. Not only do many *Brugia* ESTs have homologues in *C. elegans*, but analysis of one 65-kb DNA stretch surrounding a putative macrophage migration inhibition factor (*mif*) gene in *B. malayi* had conserved synteny and gene order with its counterpart in *C. elegans* (16). Nevertheless, a number of isolated ESTs in *Brugia* have no apparent homologue in *C. elegans*, and it may be considered that these types of genes are candidate adaptations for parasitism. Homologues of genes involved in parasitism among different species of animal-parasitic nematodes have been discovered (80, 107), and it behooves investigators of plant-parasitic nematodes to search for commonalities in these parasitic processes. *Trichinella spiralis*, for example, is an animal-parasitic nematode that modifies host muscle cells into elaborate feeding sites for intracellular parasitism (78) with striking similarity to the feeding sites (syncytia) formed from plant cells for parasitism by cyst nematodes. Antigens of *T. spiralis* origin co-localize with nuclei of infected host cells (143), but it remains in question whether the origin of the antigens is from the stichocytes (a multicellular organ similar to the esophageal gland cells of plant-parasitic nematodes) of *T. spiralis* (40, 79). Do the nuclear antigens of *T. spiralis* represent a direct regulation of host cell gene expression, and are analogous mechanisms present in plant-parasitic nematodes? Numerous analyses have been conducted on the excretory-secretory (ES) products of parasitic nematodes, and the genes encoding specific ES antigens are being isolated using ES-specific antibodies (16, 56, 67, 92). An elegant adaptation of this procedure was used to isolate genes encoding secreted antigens from a tissue-specific cDNA library constructed from gut tissue of *Haemonchus contortus* (107). Antigens secreted from nematodes that may interact with host cell receptors may also be deposited on the surface of both animal- and plant-parasitic nematodes (17, 66) or be present in secretions from nematode

chemosensory organs (98). It has been demonstrated that some nematode parasites of animals change their surface coat to alter host response to parasite invasion, and that the use of surface (*srf*) mutants of *C. elegans* may be useful in identifying these mechanisms (17). It seems reasonable to assume that some fundamental parallels in the alteration of the nematode surface and/or secretory products exist among nematodes that parasitize animals and those nematodes that parasitize plants.

## GENETIC MODELS OF PLANT PARASITISM BY NEMATODES

Analysis of mutants has been an extremely powerful approach to unravel complex biological mechanisms in organisms such as *C. elegans*, *Arabidopsis thaliana*, and *Drosophila melanogaster*. Unfortunately, the artificial generation of plant-parasitic nematode mutants altered in their parasitic behavior is still technically challenging and in most cases, presumably lethal. Thus, plant nematologists have to confine their studies to the genetic variation offered by nature. A well-known group of naturally occurring variants among plant-parasitic nematodes are those revealed by their (in)ability to reproduce on host plants that carry major resistance genes. The data suggest that most of the reported variants in nematode virulence can be explained by gene-for-gene relationships with their hosts, similar to what is observed with many microbial plant pathogens (7). For one nematode/plant combination, a gene-for-gene relationship has been confirmed by genetic analyses of both interacting partners. Virulence tests of 15 F<sub>2</sub> lines, obtained by selfing of the F<sub>1</sub> of a cross between a virulent and avirulent line, showed that virulence in *G. rostochiensis* towards the *H1* gene in potato is controlled by a single recessive gene (77). Although Mendelian proof for both interacting partners remains scarce, evidence is accumulating that such gene-for-gene mechanisms are common among plant/nematode interactions.

At present more than 25 major resistance genes (R-genes) against nematodes have been mapped (82). With the exception of the first nematode R-gene identified, *Hs1<sup>pro-1</sup>*, the other cloned nematode R-genes share various structural features with other plant disease resistance genes that operate in gene-for-gene relationships (7, 28, 49, 137). Several nematode R-genes are members of a family characterized by a nucleotide-binding site (NBS) and leucine-rich repeats (LRRs) (87, 95). Recent cloning of the potato cyst nematode resistance gene *Gpa2* also revealed NBS and LRR domains (131). Interestingly, the *Gpa2* gene has a remarkably high homology with the virus resistance gene *Rx*. Various studies have shown that *Rx*-mediated resistance against potato virus X is a gene-for-gene mechanism in which the R-gene encodes a putative receptor that recognizes the viral coat protein as an avirulence gene product (11).

A major challenge in plant nematology is to identify the avirulence gene products of parasitic nematodes. To reach this goal, various research groups have conducted selections of virulent and avirulent nematode lines. Such lines have

been established for *H. schachtii* (89), *M. incognita* (31), *H. glycines* (45), and *G. rostochiensis* (77). Root-knot nematodes have been subjected to rigorous selection experiments to generate parasitic variants in these asexual nematode species. Selection experiments with *M. incognita* against the *Mi* resistance gene of tomato showed a slow, but progressive increase in the proportion of virulent nematodes after each generation, suggesting a polygenic inheritance (31). However, this slow progressive increase of *M. incognita* virulence on *Mi* is in contrast with recent selection experiments of *M. javanica* against the *Mi* gene (137) and *M. chitwoodi* on *Solanum fendleri*, carrying the resistance gene *Rmc2* (76). These latter experiments produced complete virulence after only one or two selection cycles of reproduction of nematodes on resistant plants. Such apparent ease to select for virulence suggests a simple inheritance of the virulence character, possibly monogenic recessive as observed in many other resistance-breaking pathogens (121). On the other hand, it has also been observed that for certain R-genes the selection of virulent lines fails. Selection for virulence in *M. incognita* against two autodiploid resistant lines in pepper, HD149 carrying the *Me3* gene and HD330 carrying the *Me1* gene, showed that only *Me3*-virulent populations can be obtained, whereas the *Me1* gene cannot be circumvented, despite strong selection pressure (29).

One strategy to isolate and characterize (a)virulence gene products is to construct a linkage map and to screen for tightly linked markers, which can be used as a starting point for positional cloning of (a)virulence genes. This approach has been initiated for *H. glycines* (15) and *G. rostochiensis* (112). For *G. rostochiensis*, a novel type of linkage analyses was developed involving a "pseudo-F<sub>2</sub>" mapping strategy. This approach enables linkage mapping in non-inbred species for which individual genotypes are not accessible for extensive marker analysis. This pseudo-F<sub>2</sub> mapping strategy resulted in nine linkage groups, which correspond to the nine chromosomes of *G. rostochiensis* (112, 113). The maximum genetic length of *G. rostochiensis* was estimated to approximate 650 cM and the estimated physical size was estimated at  $8 \times 10^7$  bp, similar to the genome of *C. elegans*. The low kilobase/centimorgan (kb/cM) ratio of the *Globodera* genome should facilitate the positional cloning of nematode (a)virulence genes.

The advantage of positional cloning is that it requires no assumptions with regard to the molecular nature of the (a)virulence gene product. However, this approach is not feasible for parthenogenic species such as *M. incognita*. The only way to isolate (a)virulence genes from *M. incognita* is by rigorous differential molecular analyses of virulent and avirulent lines. Fortunately, various near-isogenic lines of *M. incognita* have been developed that differ in their ability to overcome the *Mi* resistance gene (30). Two-dimensional gel electrophoresis of soluble proteins and DNA fingerprinting techniques have revealed a number of interesting polymorphisms between these virulent and avirulent lines (30, 117). As these avirulence genes are cloned and identified, their products may be expressed in planta in a *Mi* background to evaluate function in the form of incompatibility (i.e. a hypersensitive response).

Not all plant resistance to nematodes can be explained by a classical gene-for-gene relationship with dominant resistance genes in the plant and dominant avirulence genes in the nematodes. For example, the interaction between *H. glycines* and soybean may deviate from this model (144). Analysis of inbred lines of *H. glycines* suggests that single independent recessive genes govern nematode ability to reproduce on two resistant soybean genotypes, and an independent dominant *H. glycines* gene confers this ability on a third resistant soybean genotype (45). Another example is resistance in carrot against *M. hapla*, where resistance is mediated by two recessive genes (133).

At present, the products encoded by nematode (a)virulence genes remain a mystery. It may be useful to conceptualize the products of nematode (a)virulence genes as being analogous to those defined in bacterial pathogens of plants. It has been demonstrated that the products of some bacterial *avr* genes actually are slight modifications of gene products necessary for successful infection (parasitism) of plants (33, 75a). Likewise, modification of the products of parasitism genes of nematodes may give rise to molecules that are recognized directly or indirectly by corresponding plant resistance genes to promote avirulence. Conversely, gene products that confer avirulence in nematodes may have no mechanistic role in the process of parasitism by nematodes, but they can interact directly or indirectly with specific plant resistance genes during the parasitic interaction. It should also be considered that the products of some nematode parasitism genes may act to directly or indirectly suppress plant defense responses, and that alterations or deletions of these nematode gene products may promote incompatibility.

## STRUCTURE, REGULATION, AND FUNCTIONAL ANALYSIS OF PARASITISM GENES

The developmental regulation and the detailed characterizations that have been conducted with genes cloned from the esophageal gland cells of plant-parasitic nematodes make them good models for analysis of gene structure, regulation, and function. The *sec-1* cDNA of *M. incognita*, a putative myosin heavy chain peptide, was the first transcript identified from the esophageal gland cells of a plant-parasitic nematode (105). The structure of the corresponding *sec-1* gene contained nine short introns that show an average AT content of 77%. Intron size and composition, as well as splice sites and the polyadenylation signal of *sec-1*, were similar to features of genes of *C. elegans* (21). *mj-cm-1*, the putative chorismate mutase gene isolated from the esophageal glands of *M. javanica* (88), contains two introns and at least one splice site that closely matches the consensus splice sequence in *C. elegans*. The mRNA of both *sec-1* and *mj-cm-1* were found to be *trans*-spliced with a leader sequence (SL1) commonly found in transcripts of *C. elegans* and other nematodes (19, 21). The spliced leader of *sec-1*, however, had one nucleotide substitution compared to SL1 and was designated as SL1M (86, 105), and this same SL1M spliced leader was also found on *mj-cm-1* (88). Both the canonical SL1 and SL1M

have been detected in other genes expressed within *M. javanica* (86). The potential utility of the spliced leader sequence in an expressed-gene cloning strategy has been demonstrated in the construction of cDNA libraries from parasitic nematodes (20, 88). How useful this strategy will be in cloning expressed parasitism genes in plant nematodes is unclear since relatively few full-length transcripts from plant-parasitic nematodes have been analyzed to date.

The nematode cellulase genes have been characterized in depth in terms of structure and expression. The deduced protein sequences of the ENG-1 and ENG-2 catalytic domains are highly conserved (from 80% to 97% identity) within *G. rostochiensis* and *H. glycines* (123). Inter-genus comparisons revealed 72% to 78% nucleic acid identity between the catalytic domains of *Heterodera* and *Globodera* cellulases (123), but only 48% nucleic acid identity between the *Meloidogyne* cellulase catalytic domains and *hg-eng-1* (110). The 5'-flanking regions of the four cyst nematode cellulase genes had signature sequences typical of eukaryotic promoter regions, including TATA boxes, bHLH-type transcription factor binding sites, and putative silencer, repressor, and enhancer elements (142). No conspicuous similarity was found between the 5'-flanking regions of *hg-eng-1* and *hg-eng-2*. In contrast, the 5'-flanking region of *gr-eng-1* and *gr-eng-2* were highly similar, with 88% nucleic acid identity in the 322-bp region preceding the putative transcription start point. All four cyst nematode cellulase genes have the same, rarely used polyadenylation and cleavage signal sequence 5'-GATAAA-3'.

The cellulase genes of *G. rostochiensis* and *H. glycines*, as well as six genomic fragments of cellulase genes isolated from *M. incognita*, are interspersed by introns, which are similar in size to those of *C. elegans* (MN Rosso & P Abad, unpublished data; 142). No interspecific sequence homology is found among introns of nematode cellulase genes, but in contrast, conserved sequence homologies do exist between corresponding introns of different cellulase genes within a nematode species. Although the number of introns is significantly different between cyst and root-knot cellulase genes, the intron positions in the encoded cellulase peptide sequence are identical. In addition, the cellulase genes appear to be present in multiple copies in the genomes of cyst and root-knot nematodes. The presence of highly conserved introns in several genes suggests that the multiple copies evolved at least partly by an amplification process from a common ancestor gene. In all cyst and root-knot cellulase genes reported, *cis*-splicing mainly uses the consensus splice site sequence GU-AG (21). Only a few introns use GC as a 5'-splicing donor sequence instead of GU (142). cDNA analysis has shown that the *trans*-spliced SL1 or SL1M sequences found in some transcripts of plant-parasitic nematode genes are absent from nematode endoglucanase cDNAs (110, 123).

Extensive expression analyses have been conducted with the endoglucanase genes of *H. glycines*. In situ hybridizations using riboprobes specific for the *eng-1* and *eng-2* cellulase transcripts suggest that both cellulases in *H. glycines* are expressed in concert in postembryonic developmental stages (38). Cellulase transcripts first appear in the SvG of J2 within the eggshell shortly before hatching and remain abundant until the start of the third-stage (J3) male and female life stages

of *H. glycines*. Cellulase gene expression is not detected in late-J3 male or female life stages or in any subsequent developing female stage. Interestingly, however, cellulase gene expression is re-initiated in the SvG of fourth-stage (J4) and adult males of *H. glycines*. Contrary to observations in females of *H. glycines*, cellulase mRNAs were detectable by RT-PCR in adult females of *M. incognita* (110). A potential role of *M. incognita* cellulases in the formation of a canal through root gall tissue for deposition of eggs onto the root surface appears plausible but has yet to be demonstrated. At the protein level, the expression profile of the two types of *H. glycines* cellulases was confirmed with specific antibodies (38). These expression profiles are similar to previously performed Western blot (122) and immunohistochemical analyses (36) of *G. rostochiensis*. Specific antisera were also used to detect HG-ENG2 secreted in planta along the migration path of *H. glycines* J2 in soybean roots (135). The data on cellulase expression and secretion in planta are probably sufficient to postulate that cellulases aid in nematode penetration, migration, and emigration of host roots, and consequently, are involved in mediating plant parasitism. However, in plant nematology, "proof-of-concept" of gene/protein function is difficult to achieve due to a lack of a functional mutant analysis and complementation scheme. Plant nematologists have no options available at present to complete reverse-genetic approaches to determine the effects of the disruption of wild-type genes. Furthermore, genetic approaches are of limited use in most cases because of a lack of the necessary genetic variability in available nematode populations. Only in the event that natural phenotypic differences in parasitism are observable (i.e. avirulence) can genetic concepts be explored and exploited (see above).

Modifications of functional assays used for *C. elegans* genes are being explored to develop methods to determine the function(s) of isolated plant nematode parasitism genes. An efficient transformation system for plant-parasitic nematodes would allow the expression of cloned genes in different genetic backgrounds (94). For example, a gene suspected to confer avirulence to a certain host resistance gene could be expressed in virulent nematode strains, and the virulence/avirulence of transgenic nematode lines could be assessed. Suspected regulatory regions of plant-parasitic nematode genes could be fused to reporter genes like green-fluorescent protein (GFP), and promoter activities could be monitored non-destructively throughout the nematode life cycle. Translational fusions to GFP could potentially be used to temporally and spatially localize nematode gene products secreted into plant tissue (63, 94). Although the GFP-reporter approach would not clearly document gene functions, it would, however, shed light on the expression patterns and perhaps in planta localization of products of genes-of-interest. The activity of a *G. rostochiensis* promoter in *C. elegans* was the first documented evidence of promoter function from a plant-parasitic nematode (102). Putative promoter regions have also been identified for the cellulase genes of cyst nematodes, and the expression patterns of these genes suggest that the expression of these promoters could be SvG-specific (142). In *C. elegans*, transformation is achieved by microinjection of gene constructs into the gonads of the hermaphrodite (94).

Transgenes are transmitted to the progeny as extrachromosomal arrays and, as such, are transient. In a small percentage of cases, transgene(s) are integrated into a chromosome, which gives rise to a stable transgenic line. The transformation protocols in *C. elegans* are not directly applicable to the obligate sedentary parasitic cyst and root-knot nematodes, unfortunately, so several alternative strategies are being investigated to genetically transform these plant-parasitic nematodes. One strategy involves the microinjection of male testis of *H. glycines* and allowing males to mate to noninjected females (CH Opperman, unpublished data). In another attempt, J4 and adult females of *H. schachtii* are being injected with a candidate transgene (TJ Baum, unpublished data). A third approach employs a ballistic delivery of DNA-coated tungsten particles into embryonating eggs of *H. glycines* and *M. javanica*. (Y Yan & EL Davis, unpublished data; P Abad, unpublished data). Transformation of plant-parasitic nematodes with anti-sense constructs of target nematode genes driven by appropriate promoters could be used, in theory, to inhibit gene activity and establish proof-of-concept of function.

A second methodology to directly inhibit gene activity that is both powerful and efficient is double-stranded (ds) RNA-mediated interference (RNAi) of gene expression as demonstrated in *C. elegans* (55). In this methodology, dsRNA complementary to a gene-of-interest is injected into the target nematode. As a consequence, activity of the gene-of-interest is transiently abolished in the treated animal. Two distinct advantages provided by RNAi analyses include the following: (a) The dsRNA does not have to be injected into the nematode germ line to exert inhibitory effects in tissues distal to the injection site (i.e. RNAi does not require successful transformation), and (b) the inhibitory effects of injected dsRNA can be realized in one or more subsequent nematode generations derived from the injected parent. RNAi designed to knock-out parasitism gene function in nematodes can be assayed directly for its effects on plant parasitism. The limited number of affected individuals recovered from RNAi treatment of nematodes, and the inherent variability in plant root infection assays, make this approach technically challenging. It also will be important to monitor the effects of RNAi by mRNA in situ hybridization and/or antibody probes specific to the target gene and product to confirm inhibition. This confirmation has been conducted successfully in organisms other than *C. elegans* (114, 146). To date, dsRNA complementary to *hg-eng-1* has been injected into developing females of *H. schachtii* (TJ Baum, unpublished data) and dsRNA complementary to *mi-cbp-1* has been injected into developing females of *M. incognita* (RS Hussey, unpublished data). Progeny were tested for changes in either cellulase expression or nematode parasitism, respectively. At the time of writing, no evidence of a functional RNAi scheme has been obtained for plant-parasitic nematodes.

An alternative method to assess gene function is to identify specific inhibitors (effectors) that alter the activity of nematode parasitism gene products. Molecules that bind to and inactivate cyst nematode cellulases are being assayed for their effects on plant parasitism, and additionally, such strategies may be progenitors of novel nematode control mechanisms in crop plants (see below). Conversely,

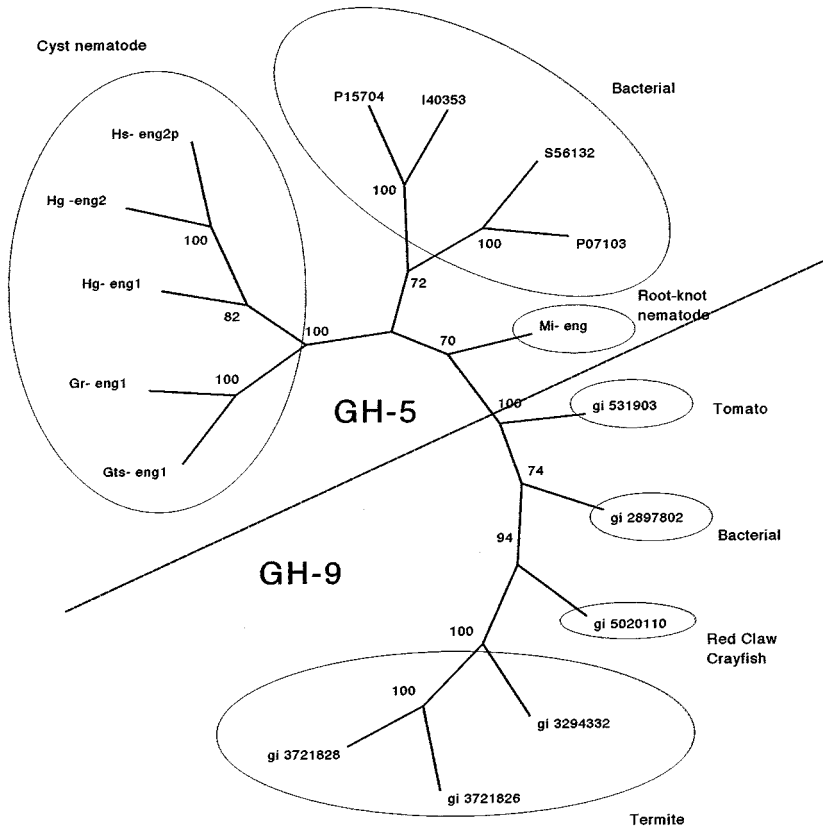
transformation of plant cells with putative nematode parasitism genes driven by appropriate promoters may be used to monitor observable effects of the expressed nematode gene on plant cell phenotype. This type of analysis has recently been conducted successfully to demonstrate that a bacterial (*Xanthomonas citri*) pathogenicity gene (*pthA*) can elicit plant cell division, enlargement, and death when expressed in recipient plant cells (47). It is also conceivable that the expressed products of nematode parasitism genes could be microinjected into plant cells to assess effects on plant cell phenotype (72). It is unknown, however, if the plant cell targets of the products of nematode parasitism genes are extracellular, within the plant cell cytosol, or localized within any number of subcellular compartments. Plant cell targets for the products of nematode parasitism genes may be isolated by using the putative nematode parasitism gene in a modified yeast two-hybrid screen with cDNA from healthy host plant cells (54). Most recently, an elegant assay has demonstrated that a low-molecular-weight peptide (<3 kDa) component of the stylet secretions of *G. rostochiensis* could co-stimulate the proliferation of tobacco cell protoplasts in the presence of phytohormones (65). This suggests that the activity of plant constituents (i.e. signal molecules) is critical to the parasitic interaction, and that the products of nematode parasitism genes may be processed or may be components of biosynthetic pathways.

## ORIGINS OF PARASITISM GENES

Genes evolved from nematode ancestors of contemporary species are one likely origin of nematode parasitism genes. In this regard, the value of the information generated in the *C. elegans* Genome Sequencing Project (27) to identify genes basic to the biology of plant-parasitic nematodes cannot be overemphasized. It is already clear that some *C. elegans* genes match those identified in plant-parasitic nematodes (EL Davis, RS Hussey & TJ Baum, unpublished data; G Smant, A Schots & J Bakker, unpublished data). In other instances, however, such as the cellulase genes of cyst and root-knot nematodes, no significant similarity to any *C. elegans* gene can be found. It is unclear, at present, to what extent genes identified in *C. elegans* may have been adapted for plant parasitism, and to what extent genes for parasitism may potentially have been acquired from other sources. It is likely that genes fundamental to the life cycle of all nematodes are similar between *C. elegans* and parasitic nematodes, and that orthologues of selected *C. elegans* genes may be isolated from plant nematodes by direct molecular analyses. For example, genes for putative guanylyl cyclase chemoreceptors (*gcy* genes) cloned from *H. glycines* (141) have strong similarity to the conserved catalytic and single transmembrane domains of *C. elegans* *gcy* genes (145). The putative extracellular (receptor) regions of the guanylyl cyclases of *H. glycines* are significantly diverged from those of *C. elegans*, however, leading to speculation that the *H. glycines* GCY may be adapted to receive a plant signal. The largest group of genes discovered in *C. elegans* are those encoding seven-transmembrane domain proteins, many of

which are putative chemoreceptors (8). This seems a fruitful area to investigate potential chemoreceptors in parasitic nematodes. Orthologues of other candidate genes from *C. elegans* that may be present in plant-parasitic nematodes include, for example, genes involved in hatching (*hch*), dauer formation (*daf*), or sex determination (*xol*, *her*, *fem*), as has been suggested (15, 108). The search for orthologues of these genes in tylenchid plant-parasitic nematodes has been frustrated, thus far, by a lack of sufficient similarity in nucleotide sequence to obtain definitive homologues of these genes by hybridization or PCR-based techniques. Considerable sequence divergence in putative orthologues of these genes may be partly responsible for these phenomena since, in one estimate, the Rhabditida and Tylenchida are separated by an evolutionary distance of more than 300 million years (100). A recent molecular evolutionary analysis of the Nematoda suggests that the tylenchids are more closely related to the cephalobids than to the rhabditids (18). This same molecular phylogeny also suggests that plant parasitism may have arisen independently at least three times during the course of nematode evolution. The presence of similar cellulase genes in *Paratrichodorus* and several tylenchid nematode species suggests, however, that some genes from an ancient ancestor of all of these nematodes may have been retained in all lineages leading to plant parasitism.

In theory, two evolutionary pathways may have led to the predicted phylogenetic position of the nematode cellulases (Figure 2). First, the genes encoding the nematode cellulases and bacterial cellulases may have evolved from an ancient cellulase gene in a common ancestor of bacteria and nematodes. The catalytic domains in glycosyl hydrolases that are categorized into the same gene family are thought to have evolved from a common ancestor (68). Members of the same gene family share their secondary and tertiary protein structure, the stereochemical outcome of the hydrolysis reaction, and biochemical specificity. This observation implies the existence of a strong evolutionary constraint on these enzymes. An unprecedented sequence convergence to the extent found between bacterial and nematode cellulases, however, is a very unlikely explanation (46). The nematode endoglucanases clearly show the highest similarity with bacterial endoglucanases, which could also point to a horizontal gene transfer from bacteria to an ancestor of the cyst nematode species. Interestingly, some of the tentative cases of horizontal gene transfer between bacteria, fungi, and plants include glycosyl hydrolases (32, 103). It is not likely that several independent transfers have taken place after the divergence of *G. rostochiensis*, *H. glycines*, and *M. incognita* because of the conserved intron positions in the genes (110, 142). No additional clues for a horizontal gene transfer are present in the current data. The molar G+C content of the coding regions of the nematode endoglucanases are within the range of that in bacterial endoglucanases (*Erwinia* spp. and *Pseudomonas* spp.), but is not deviant from the molar G+C content normally found in nematodes (123, 142). In addition, the nematode endoglucanase genes have introns, splicing features, and flanking regions that show a eukaryotic signature. This means that any speculation on a horizontal transfer of the cellulase into the nematode genomes is based on primary amino acid sequence only. The recent discoveries of cellulase genes in



**Figure 2** Phylogenetic analysis of beta-1,4-endoglucanases of glycosyl hydrolase families 5 (GH-5) and 9 (GH-9). The initial alignment of the amino acid sequences of the catalytic domains of the cellulase genes was made using the Clustal W1.7 algorithm. The phylogenetic tree was constructed based on maximum parsimony (PROTPARS) criteria. No outgroup for both GH-5 and GH-9 cellulases is known, hence, the tree is unrooted. Relative support for the different nodes was assessed using 500 bootstrap replicates with 9 random additional replicates for each bootstrap replicate. The cellulases are indicated using either their gene names (nematode cellulases) or their primary accession numbers.

other species of plant-parasitic nematodes (see above), in termites (128), and in crayfish (26) will aid in comparative analyses to test the hypothesis of an ancient horizontal gene transfer between bacteria and nematodes.

It is impossible to provide conclusive evidence for a horizontal gene transfer from one organism to the germ line of another organism. Therefore various lines of evidence are usually necessary to build a convincing case. Examples of putative cases of horizontal gene transfer have been described from prokaryote to prokaryote, from eukaryote to prokaryote, and from prokaryote to eukaryote (124, 126).

In practice, the transfer of genetic material between bacteriophage and bacteria, or between *Agrobacterium* and host plant cells, represents our (now routine) exploitation of natural gene transfer. However, there is no confirmed example of acquisition of a gene by horizontal transfer from bacteria or fungi to an animal. No obvious physical mechanism is apparent for potential gene transfer from bacteria to nematodes, although it is plausible that genes were (somehow) procured and retained in a bacterial-feeding ancestor of modern nematodes. The apparent transfer of double-stranded RNA (dsRNA) across the gut of *C. elegans* after ingestion of *E. coli* producing the dsRNA, and the resultant dsRNA suppression of the complimentary gene in distal target tissues within the nematode (127), may suggest that genetic material could have been transferred in some similar fashion during evolution. Alternatively, the potential presence of bacterial symbionts in nematode ancestors, such as the *Wolbachia* symbiont present in some filarial nematodes (16), may represent a source for transfer of bacterial genes to nematodes. Bacterial symbionts within the esophageal gland cells or other tissues of contemporary plant-parasitic nematodes, however, have not been detected.

Recent discoveries that other genes expressed in the esophageal gland cells of plant-parasitic nematodes have their strongest similarities to bacterial genes lend support to the hypothesis that parasitism genes in plant nematodes may have been acquired, at least in part, by gene transfer from microorganisms that inhabit the same parasitic niche. The *mj-cm-1* and *mi-cbp-1* genes both have their strongest similarities to bacterial genes (42, 88). Complementation of a bacterial mutant with *mj-cm-1* has been used to provide functional analysis of this gene (88) and may be indicative of the bacterial origin of this gene. Some ESTs recently analyzed from the esophageal gland cell-specific cDNA libraries of *M. incognita* and *H. glycines* have their strongest similarities to bacterial genes, and hybridizations of a subset of these ESTs on Southern blots of nematode DNA provide preliminary evidence that the expressed gland genes are of nematode origin (EL Davis, RS Hussey & TJ Baum, unpublished data).

## TARGETING THE PRODUCTS OF NEMATODE PARASITISM GENES

Understanding the origins, structures, and functions of the products of nematode parasitism genes will undoubtedly present new targets to interfere with nematode parasitism of plants. Nematode attack of plants may be thwarted when the nematodes are still in the soil, or alternatively, new mechanisms of defense may be introduced (bioengineered) into plants for nematodes to encounter as they parasitize prospective hosts. It is conceivable that adaptive processes like nematode egg hatch and chemotaxis (and the gene products that control them) may be vulnerable to target-specific chemicals, bioengineered rhizosphere microorganisms, or transgenic plants that exude inhibitory compounds (81, 98). Most effort to date, however, has focused upon the disruption of nematode feeding in transgenic plants,

and readers are referred to two recent reviews on this topic (3, 132). Efforts to isolate natural plant resistance genes, and their corresponding nematode virulence genes, seek to transfer natural resistance genes into new plant genotypes and/or to modify the resistance to make it more broad-spectrum and durable. Efforts to develop novel transgenic mechanisms of resistance to nematodes in plants must combine appropriate temporal and spatial expression with carefully selected effector transgenes. Effector transgenes that target plant processes to disrupt feeding site formation by nematodes must be very tightly expressed to avoid damage to the plant or potential gene silencing in subsequent plant generations. Transgenes that target nematodes should be relatively innocuous to plants, but they must be chosen wisely to avoid potential harmful effects to nontarget organisms (including humans) and the environment, and to prevent the rapid evolution of nematode genotypes that are resistant to the effector. The use of expressed toxins, for example, might be subject to both of these problems. The most successful nematode-oriented strategy to date has been to target nematode gut proteinases with specific inhibitors to purportedly disrupt proper digestion by feeding nematodes (3, 91). A modified oryzacystatin (*Oc-1Δ86*), expressed in transgenic plants, reduced both the development and fecundity of *G. rostochiensis* and *M. incognita* (3).

The proteinase inhibitor approach targets a basic component of nematode metabolism as opposed to a fundamental adaptation for plant parasitism. As progress in the identification of nematode parasitism gene products proceeds, it is envisioned that inhibition of multiple fundamental mechanisms of parasitism by nematodes will present an effective and durable means to develop and deploy transgenic plant resistance to nematodes. Attempts are already in progress to inhibit the activity of nematode esophageal gland cell gene products that are secreted into plant tissues during parasitism. These investigations serve both as assays to infer nematode parasitism gene function and as potential means to develop novel nematode management tactics. The expression of specific antibody genes in plants (plantibodies) has been demonstrated to modulate the activity of target molecules and to provide resistance to viral pathogens and phytoplasmas (90, 125, 136). Plantibodies have been developed that bind specifically to a dorsal gland antigen in *M. incognita* (10). Single-chain variable fragments (scFv) of chosen antibodies are smaller molecules that can overcome potential difficulties in antibody assembly in plants yet retain antibody binding specificity and affinity (125). The ability to target scFv molecules to the intra- or extracellular spaces of plant cells is an important advancement to direct plantibody activity to where target nematode stylet secretions are released (116). ScFv plantibodies that bind to specific esophageal antigens of *M. incognita* and *G. rostochiensis* have been developed (111, 115) and transgenic plants are currently being evaluated. In addition to plantibodies, the expression of inhibitory peptides that bind to and inactivate nematode secretory gene products that are essential for plant parasitism represents an alternative means to develop novel nematode control. Peptides are relatively small molecules that can have high binding affinities and strong activities. Several classes of naturally occurring plant defense peptides have been reported that provide defense against both insects and pathogens (57). Synthetic and natural

combinatorial libraries are emerging as reservoirs to be screened to obtain batteries of new bioactive molecules that bind to and inactivate target molecules (12, 106). This novel approach is now under way to identify peptides that inhibit the activity of *H. glycines* cellulases (X Wang & EL Davis, unpublished data).

In theory, effector molecules may be designed to specifically interfere with the interaction of any secreted nematode parasitism gene product and its host target molecule. It is inherently advantageous if the target of an effector is external to the nematode body, so that potential barriers to ingestion or transport of effectors to internal body tissues can be avoided. Studies suggest that the feeding tubes formed within feeding cells of *Meloidogyne* and *Heterodera* species act as molecular sieves (51, 74) and may limit the size of ingested compounds to less than 40 kDa (22, 63, 130)—an important consideration for effectors targeted to the nematode gut. Likewise, effector molecules that target internal body tissues must be able to cross the nematode body wall or gut lining to reach the appropriate internal tissues to exert their effects. In contrast, access of effector molecules to products secreted from the nematode, components of the nematode surface, or targets exposed to the environment in natural body openings should be relatively unimpeded and present a greater opportunity for successful intervention in nematode parasitism of plants.

## CONCLUDING REMARKS

The cloning and characterization of genes that promote nematode parasitism of plants is in its early stages, but already, some promising research directions and unexpected results have been realized. Genetic analyses will be merged with physical maps of plant-parasitic nematode genomes to isolate nematode (a)virulence genes, but it is unclear if these genes will represent a subset of modified parasitism genes or if they will have functions unrelated to plant parasitism. Direct molecular analyses of genes expressed in the nematode esophageal gland cells whose products are secreted into plant tissue during parasitism is proving to be a fruitful area of investigation. The isolation of nematode cellulase genes that are specifically expressed in the subventral gland cells and demonstration of the secretion of the cellulase into plant tissue is verification that the products of the glands are involved in plant parasitism. A surprising result is that the nematode cellulase genes, and several other genes cloned from nematode esophageal gland cells, have striking similarities to microbial genes (84), suggesting that some nematode parasitism genes may have been acquired by ancient horizontal gene transfer. If this tempting hypothesis is correct, it is likely that expanses of chromatin, rather than single genes, would have been transferred to nematodes. As genetic and physical mapping of the genomes of plant-parasitic nematodes progresses, it will be interesting to see if functionally coupled nematode parasitism genes are clustered within the genome (97). Initial evidence of the genome organization of the cellulases genes of *H. glycines* indicates that at least two cellulase genes are present in tandem (Y Yan & EL Davis, unpublished data). If expanses of chromatin have been acquired from microbial sources, it may be considered that all or part of some

“pathogenicity islands” (33) may have been exchanged and usurped for nematode parasitism of plants. The application of genomics to the study of nematode parasitism genes will help to address these questions and allow the isolation of additional nematode parasitism genes to progress. The development of efficient assays for functional analysis of isolated parasitism genes will be of paramount importance to understanding the evolution and complexity of plant parasitism by nematodes.

## ACKNOWLEDGMENTS

The authors are indebted to Dr. Geert Smant of Wageningen University for his analysis and construction of the cellulase phylogenetic tree presented in Figure 2, and for his helpful input in preparing the text of this manuscript. The authors thank Vickie Brewster for creating Figure 1 to author specifications. We kindly acknowledge those scientists willing to share unpublished data with the authors, and the many dedicated individuals in our respective labs that have generated some of the knowledge that we share with you here. Support of international travel for collaborative research among the authors was provided by NATO grant # CRG972256.

**Visit the Annual Reviews home page at [www.AnnualReviews.org](http://www.AnnualReviews.org)**

## LITERATURE CITED

1. Anderson RV, Byers JR. 1975. Ultrastructure of the esophageal procorpus in the plant parasitic nematode, *Tylenchorhynchus dubius*, and functional aspects in relation to feeding. *Can. J. Zool.* 53:1581–95
2. Atkinson HJ, Harris PD, Halk EU, Novitski C, Leighton-Sands J, et al. 1988. Monoclonal antibodies to the soya bean cyst nematode, *Heterodera glycines*. *Ann. Appl. Biol.* 112:459–69
3. Atkinson HJ, Lilley CJ, Urwin PE, McPherson MJ. 1998. Engineering resistance to plant-parasitic nematodes. See Ref. 98a, pp. 382–413
4. Atkinson HJ, Taylor JD, Fowler M. 1987. Changes in the second stage juveniles of *Globodera rostochiensis* prior to hatching in response to potato root diffusate. *Ann. Appl. Biol.* 110:105–14
5. Bachem CWB, van der Hoeven RS, de Bruijn SM, Vreugdenhil D, Zabeau M, et al. 1996. Visualization of differential gene expression using a novel method of DNA fingerprinting based on AFLP: analysis of gene expression during potato tuber development. *Plant J.* 9:745–53
6. Baldwin JG, Nadler SA, Wall DH. 2000. Nematodes—pervading the earth and linking all life. In *Nature and Human Society, the Quest for a Sustainable World*, ed. P Raven, T Williams. Proc. Forum, 28–30 Oct. 1997, Washington, DC: Natl. Acad. Press, pp. 176–91.
7. Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar SP. 1997. Signaling in plant-microbe interactions. *Science* 276: 726–33
8. Bargmann CI. 1998. Neurobiology of the *Caenorhabditis elegans* genome. *Science* 282:2028–33
9. Barker K. 1998. Introduction and synopsis of advancements in nematology. In *Plant Nematode Interactions*, ed. KR Barker, GA Pederson, GL Windham, pp. 1–20. Madison, WI: Am. Soc. Agron., Crop Sci. Soc. Am., Soil Sci. Soc. Am.

10. Baum, TJ, Hiatt A, Parrott WA, Pratt LH, Hussey RS. 1996. Expression in tobacco of a functional monoclonal antibody specific to stylet secretions of the root-knot nematode. *Mol. Plant-Microbe Interact.* 9:382–87
11. Bendahmane A, Kanyuka K, Baulcombe D. 1999. The *Rx* gene from potato controls separate virus resistance and cell death responses. *Plant Cell* 11:781–92
12. Beste G, Schmidt FS, Stibora T, Skerra A. 1999. Small antibody-like proteins with prescribed ligand specificities derived from the lipocalin fold. *Proc. Natl. Acad. Sci. USA* 96:1898–903
13. Bird AF. 1983. Changes in the dimensions of the esophageal glands in root-knot nematodes during the onset of parasitism. *Int. J. Parasitol.* 13:343–48
14. Bird DM. 1996. Manipulation of host gene expression by root-knot nematodes. *J. Parasitol.* 82:881–88
15. Bird DM, Opperman CH, Jones SJM, Baillie DL. 1999. The *Caenorhabditis elegans* genome: a guide in the post genomics age. *Annu. Rev. Phytopathol.* 37:247–65
16. Blaxter M, Aslett M, Guiliano D, Daub J, The Filarial Genome Project. 1999. Parasitic helminth genomes. *Parasitology* 118:S39–51
17. Blaxter M, Bird D. 1997. Parasitic nematodes. See Ref. 107a, pp. 851–78
18. Blaxter ML, De Ley P, Garey JR, Liu LX, Scheldeman P, et al. 1998. A molecular evolutionary framework for the phylum Nematoda. *Nature* 392:71–75
19. Blaxter ML, Liu LX. 1996. Nematode spliced leaders: function, evolution, and utility. *Int. J. Parasitol.* 26:1025–33
20. Blaxter ML, Raghavan N, Ghosh I, Guiliano D, Lu W, et al. 1996. Genes expressed in *Brugia malayi* infective third stage larvae. *Mol. Biochem. Parasitol.* 77:77–93
21. Blumenthal T, Steward K. 1997. RNA processing and gene structure. See Ref. 107a, pp. 117–45
22. Bockenhoff A, Grundler FMW. 1994. Studies on the nutrient uptake by the beet cyst nematode *H. schachtii* by in situ microinjection of fluorescent probes into the feeding structures in *Arabidopsis thaliana*. *Parasitology* 109:249–54
23. Bos L, Parlevliet JE. 1995. Concepts and terminology on plant/pest relationships: toward consensus in plant pathology and crop protection. *Annu. Rev. Phytopathol.* 33:69–102
24. Burgess TL, Kelly RB. 1987. Constitutive and regulated secretion of proteins. *Annu. Rev. Cell Biol.* 3:243–93
25. Bürglin TR, Lobos E, Blaxter ML. 1998. *Caenorhabditis elegans* as a model for parasitic nematodes. *Int. J. Parasitol.* 28:395–411
26. Byrne KA, Lehnert SA, Johnson SE, Moore SS. 1999. Isolation of a cDNA encoding a putative cellulase in the red claw crayfish *Cherax quadricarinatus*. *Gene* 239:317–24
27. *C. elegans* Sequencing Consortium. 1998. Genome sequence of the nematode *Caenorhabditis elegans*: a platform for investigating biology. *Science* 282:2012–18
28. Cai D, Kleine M, Kilfe S, Harloff HJ, Sandal NN, et al. 1997. Positional cloning of a gene for nematode resistance in sugar beet. *Science* 275:832–34
29. Castagnone-Sereno P, Bongiovanni M, Palloix A, Dalmasso A. 1996. Selection for *Meloidogyne incognita* virulence against resistance genes from tomato and pepper and specificity of the virulence/resistance determinants. *Eur. J. Plant Pathol.* 102:585–90
30. Castagnone-Sereno P, Rosso MN, Bongiovanni M, Dalmasso A. 1995. Electrophoretic analysis of near-isogenic avirulent and virulent lineages of the parthenogenetic root-knot nematode *Meloidogyne incognita*. *Physiol. Mol. Plant Pathol.* 47:293–302
31. Castagnone-Sereno P, Wajnberg E, Bongiovanni M, Leroy F, Dalmasso A. 1994.

- Genetic variation in *Meloidogyne incognita* virulence against the tomato *Mi* resistance gene: evidence from isofemale line selection studies. *Theor. Appl. Genet.* 88:749–53
32. Chen H, Li XL, Ljungdahl LG. 1997. Sequencing of a 1,3-1,4-beta-D-glucanase (lichenase) from the anaerobic fungus *Orpinomyces* strain PC-2: properties of the enzyme expressed in *Escherichia coli* and evidence that the gene has a bacterial origin. *J. Bacteriol.* 179:6028–34
  33. Collmer A. 1998. Determinants of pathogenicity and avirulence in plant pathogenic bacteria. *Curr. Opin. Plant Biol.* 1(4):329–35
  34. Davis EL, Allen R, Hussey RS. 1994. Developmental expression of esophageal gland antigens and their detection in stylet secretions of *Meloidogyne incognita*. *Fundam. Appl. Nematol.* 17:255–62
  35. Davis EL, Aron LM, Pratt LH, Hussey RS. 1992. Novel immunization procedures used to develop monoclonal antibodies that bind to specific structures in *Meloidogyne* spp. *Phytopathology* 82:1244–50
  36. de Boer JM, Smant G, Goverse A, Davis EL, Overmars HA, et al. 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
  37. de Boer JM, Yan Y, Bakker J, Davis EL, Baum TJ. 1998. *In situ* hybridization to messenger RNA of *Heterodera glycines*. *J. Nematol.* 30:309–12
  38. de Boer JM, Yan Y, Wang X, Smant G, Hussey RS, et al. 1999. Developmental expression of secretory beta-1,4-endoglucanases in the subventral esophageal glands of *Heterodera glycines*. *Mol. Plant-Microbe Interact.* 12:663–69
  39. de Meutter J, Tytgat T, van der Schueren E, Smant G, Schots A, et al. 1998. Analysis of *Heterodera schachtii* cellulase. *Proc. Int. Nematol. Symp., 24th, Dundee, Scotland*, p. 24 (Abstr.)
  40. Despommier DD, Gold AM, Buck SW, Capo V, Silberstein D. 1990. *Trichinella spiralis*: secreted antigen of the infective L<sub>1</sub> larva localizes to the cytoplasm and nucleoplasm of infected host cells. *Exp. Parasitol.* 71:27–38
  41. Deubert KH, Rohde RA. 1971. Nematode enzymes. In *Plant Parasitic Nematodes*, ed. BM Zuckerman, WF Mai, RA Rohde, 2:73–90. New York: Academic
  42. 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.* 11:952–59
  43. Ding X, Shields J, Allen R, Hussey RS. 1998. Secretion gene profile of the dorsal gland cell in the root-knot nematode (*Meloidogyne incognita*). *Phytopathology* 88(9S):S22 (Abstr.)
  44. Ding X, Shields J, Allen R, Hussey RS. 2000. Molecular cloning and characterisation of a venom allergin AG5-like cDNA from *Meloidogyne incognita*. *Int. J. Parasitol.* 30:77–81
  45. Dong K, Opperman CH. 1997. Genetic analysis of parasitism in the soybean cyst nematode *Heterodera glycines*. *Genetics* 146:1311–18
  46. Doolittle RF. 1994. Convergent evolution: the need to be explicit. *Trends Biochem. Sci.* 19:15–18
  47. Duan YP, Castaneda A, Zhao G, Erdos G, Gabriel DW. 1999. Expression of a single, host-specific, bacterial pathogenicity gene in plant cells elicits division, enlargement, and cell death. *Mol. Plant-Microbe Interact.* 12:556–60
  48. Eddy SR. 1998. Multiple-alignment and sequence searches. *Trends Genet. (Suppl.) Trends Guide Bioinformat.* 14:15–18
  49. Ellis J, Jones D. 1998. Structure and function of proteins controlling strain-specific pathogen resistance in plants. *Curr. Opin. Plant Biol.* 1:288–93

50. Endo BY. 1984. Ultrastructure of esophageus of larvae of the soybean cyst nematode, *Heterodera glycines*. *Proc. Helminthol. Soc. Wash.* 51:1–24
51. Endo BY. 1991. Ultrastructure of initial responses of susceptible and resistant soybean roots to infection by *Heterodera glycines*. *Rev. Nematol.* 14:73–94
52. Favery B, Lecomte P, Gil N, Bechtold N, Bouchez D, et al. 1998. *RPE*, a plant gene involved in early developmental steps of nematode feeding cells. *EMBO J.* 17:6799–811
53. Fenoll C, Aristizabal FA, Sanz-Alferez S, del Campo FF. 1997. Regulation of gene expression in feeding sites. See Ref. 53a, pp. 133–49
- 53a. Fenoll C, Grundler FMW, Ohl SA, eds. 1997. *Cellular and Molecular Aspects of Plant-Nematode Interactions*. Dordrecht, The Netherlands: Kluwer
54. Fields S, Song OK. 1989. A novel genetic system to detect protein-protein interactions. *Nature* 340:245–46
55. Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, et al. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391:806–11
56. Frank, GR, Wisniewski N, Brandt KS, Carter CRD, Jennings NS, et al. 1998. Molecular cloning of the 22–24 kDa excretory-secretory 22U protein of *Dirofilaria immitis* and other filarial nematode parasites. *Mol. Biochem. Parasitol.* 98:297–302
57. Garcia-Olmedo F, Molina A, Alamillo JM, Rodriguez-Palenzuela P. 1998. Plant defense peptides. *Biopolymers* 47:479–91
58. Gems D, Maizels RM. 1996. An abundantly-expressed mucin-like protein from *Toxocara canis* infective larvae: the precursor of the larval surface coat protein. *Proc. Natl. Acad. Sci. USA* 93:1665–70
59. Gheysen G, de Almeida Engler J, van Montagu M. 1997. Cell cycle regulation in nematode feeding sites. See Ref. 53a, pp. 120–32
60. Goellner, M, Smant G, deBoer JM, Baum TJ, Davis EL. 2000. Isolation and expression of beta-1,4-endoglucanase genes of *Globodera tabacum* during parasitism. *J. Nematol.* 32:In press
61. Goodman RN, Király Z, Wood KR. 1986. *The Biochemistry and Physiology of Plant Disease*. Columbia, MO: Univ. Missouri Press. 433 pp.
62. Gove PB, ed. 1976. *Webster's Third New International Dictionary of the English Language, Unabridged*. Springfield, MA: Merriam. 2662 pp.
63. Goverse A, Biesheuvel J, Wijers G-J, Gommers FJ, Bakker J, et al. 1998. *In planta* monitoring of the activity of two constitutive promoters, CaMV 35S and TR2', in developing feeding cells induced by *Globodera rostochiensis* using green fluorescent protein in combination with confocal laser scanning microscopy. *Physiol. Mol. Plant Pathol.* 52:275–84
64. Goverse A, Davis EL, Hussey RS. 1994. Monoclonal antibodies that bind to the esophageal glands and stylet secretions of *Heterodera glycines*. *J. Nematol.* 26:251–59
65. Goverse A, Rouppe van der Voort J, Rouppe van der Voort C, Kavelaars A, Smant G, et al. 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.* 12:872–81
66. Gravato Nobre MJ, Evans K. 1998. Plant and nematode surfaces: their structure and importance in host-parasite interactions. *Nematologica* 44:103–24
- 66a. Hacker J, Blum-Oehler G, Mühldorfer I, Tschäpe H, 1997. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. *Mol. Microbiol.* 23:1089–97
67. Hawdon JM, Narasimhan S, Hotez PJ.

1999. *Ancylostoma* secreted protein 2: cloning and characterization of a second member of a family of nematode secreted proteins from *Ancylostoma caninum*. *Mol. Biochem. Parasitol.* 99:149–65
68. Henrissat B, Bairoch A. 1993. New families in the classification of glycosyl hydrolases based upon amino acid sequence similarities. *Biochem. J.* 293:781–88
69. Hermsmeier D, Hart JK, Byzova M, Rodermeil SR, Baum TJ. 2000. Changes in mRNA abundance within *Heterodera schachtii*-infected roots of *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* 13:390–15
70. Hussey RS. 1989. Disease-inducing secretions of plant-parasitic nematodes. *Annu. Rev. Phytopathol.* 27:123–41
71. Hussey RS, Davis EL. 2000. Nematode esophageal glands and plant parasitism. In *Nematology, Advances and Perspectives*. Vol. 1: *Nematode Morphology, Physiology, and Ecology*. Tsinghua, China: Tsinghua Univ. Press. In press
72. Hussey RS, Grundler FMW. 1998. Nematode parasitism of plants. See Ref. 98a, pp. 213–43
73. Hussey RS, Mims CW. 1990. Ultrastructure of esophageal glands and their secretory granules in the root-knot nematode *Meloidogyne incognita*. *Protoplastas* 156:9–18
74. Hussey RS, Mims CW. 1991. Ultrastructure of feeding tubes formed in giant-cells induced in plants by the root-knot nematode *Meloidogyne incognita*. *Protoplastas* 162:99–107
75. Hussey RS, Paguio OR, Seabury F. 1990. Localization and purification of a secretory protein from the esophageal glands of *Meloidogyne incognita* with a monoclonal antibody. *Phytopathology* 80:709–14
- 75a. Jackson RW, Athanassopoulos E, Tsiamis G, Mansfield JW, Sesma A, et al. 1999. Identification of a pathogenicity island, which contains genes for virulence and avirulence, on a large native plasmid in the bean pathogen *Pseudomonas syringae* pathovar phaseolicola. *Proc. Natl. Acad. Sci.* 96:10875–80
76. Janssen GJW, Scholten OE, van Norel A, Hoogendoorn CJ. 1998. Selection of virulence in *Meloidogyne chitwoodi* to resistance in the wild potato *Solanum fendleri*. *Eur. J. Plant Pathol.* 104:645–51
77. 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. Nematol.* 14:207–11
78. Jasmer DP. 1993. *Trichinella spiralis* infected skeletal muscle cells arrest in G<sub>2</sub>/M and cease muscle cell gene expression. *J. Cell Biol.* 121:785–93
79. Jasmer DP, Yao S, Vassilatis D, Despommier DD, Neary SM. 1994. Failure to detect *Trichinella spiralis* p43 in isolated host nuclei and in irradiated larvae of infected muscle cells which express the infected cell phenotype. *Mol. Biochem. Parasitol.* 67:225–34
80. Johnston DA, Blaxter ML, Degraeve WM, Foster J, Ivans AC, et al. 1999. Genomics and the biology of parasites. *BioEssays* 21:131–47
81. Jones PW, Tylka GL, Perry RN. 1998. Hatching. See Ref. 98a, pp. 181–212
82. Jung C, Daguang C, Kleine M. 1998. Engineering nematode resistance in crop species. *Trends Plant Sci.* 3:266–71
83. Karrer, EE, Lincoln JE, Hogenhout S, Bennett AB, Bostock RM, et al. 1995. *In situ* isolation of mRNA from individual plant cells: Creation of cell-specific cDNA libraries. *Proc. Natl. Acad. Sci. USA* 92:3814–18
84. Keen NT, Roberts PA. 1998. Plant parasitic nematodes: digesting a page from the microbe book. *Proc. Natl. Acad. Sci. USA* 95:4789–90
85. Klein RD, Gu X, Goddard A, Rosenthal A. 1996. Selection for genes encoding

- secreted proteins and receptors. *Proc. Natl. Acad. Sci. USA* 93:7108–13
86. Koltai H, Spiegel Y, Blaxter ML. 1997. Regulated use of an alternative spliced leader exon in the plant-parasitic nematode *Meloidogyne javanica*. *Mol. Biochem. Parasitol.* 86:107–10
87. Lagudah ES, Moullet O, Appels R. 1997. Map-based cloning of a gene sequence encoding a nucleotide-binding domain and leucine-rich region at the *Cre3* nematode resistance locus of wheat. *Genome* 40:659–65
88. 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.* 12:328–36
89. Lange W, Müller J, De Bock TSM. 1993. Virulence in the beet cyst nematode (*Heterodera schachtii*) versus some alien genes for resistance in beet. *Fund. Appl. Nematol.* 16:447–54
90. Le Gall F, Bové JM, Garnier M. 1998. Engineering of a single-chain variable fragment (scFv) antibody specific for the solbur phytoplasma (Mollicute) and its expression in *Escherichia coli* and tobacco plants. *Appl. Environ. Microbiol.* 64:4566–72
91. Lilley CJ, Urwin PE, Atkinson HJ. 1999. Characterization of plant nematode genes: identifying targets for a transgenic defence. *Parasitology* 118:S63–72
92. Lopez de Mendoza ME, Curtis RHC, Gowen S. 1999. Identification and characterization of excreted-secreted products and surface coat antigens of animal and plant-parasitic nematodes. *Parasitology* 118:397–405
93. McClure MA, Von Mende N. 1987. Induced salivation in plant-parasitic nematodes. *Phytopathology* 77:1463–69
94. Mello C, Fire A. 1995. DNA transformation. In *Methods in Cell Biology*. Vol. 48. *Caenorhabditis elegans: Modern Biological Analysis of an Organism*. ed. HF Epstein, DC Shakes, pp. 451–82. San Diego: Academic
95. Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, et al. 1998. The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* 10:1307–19
96. Opperman CH, Taylor CG, Conkling MA. 1994. Root-knot nematode-directed expression of a plant root-specific gene. *Science* 263:221–23
97. Overbeek R, Fonstein M, D'Souza M, Pusch GD, Maltsev N. 1999. The use of gene clusters to infer functional coupling. *Proc. Natl. Acad. Sci. USA* 96:2896–901
98. Perry RN. 1996. Chemoreception in plant parasitic nematodes. *Annu. Rev. Phytopathol.* 34:181–99
- 98a. Perry RN, Wright DJ, eds. 1998. *The Physiology and Biochemistry of Free-Living and Plant-Parasitic Nematodes*. Wallingford, UK: CABI Publ.
99. 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
100. Poinar GO. 1983. *A Natural History of Nematodes*. Englewood Cliffs, NJ: Prentice-Hall. 323 pp.
- 100a. Popeijus H, Overmars H, Jones J, Blok V, Goverse A, et al. 2000. Non-symbiotic degradation of plant cell walls by animals. *Nature*. In press
101. Popeijus HE, Smant G, Jones J, Helder J, Schots A, et al. 1999. Identification of a pectate lyase (4.2.2.2) from the plant parasitic nematode *Globodera rostochiensis*. *Int. Congr. Mol. Plant-Microbe Interact.*, 9<sup>th</sup>, Abstr. 5.1. Amsterdam, The Netherlands
- 101a. Qin L, Overmars HA, Popeijus H, Schots A, Bakker J, et al. 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.* In press
102. Qin L, Smant G, Stokkermans J, Bakker J, Schots A, et al. 1998. Cloning of a trans-spliced glyceraldehyde 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
103. Quillet L, Barry S, Labedan B, Petit F, Guespin-Michel J. 1995. The gene encoding the beta-1,4-endoglucanase (Cela) from *Myxococcus xanthus*: evidence for independent acquisition by horizontal transfer of binding and catalytic domains from actinomycetes. *Gene* 158:23–29
104. Ramsey G. 1998. DNA chips: state-of-the-art. *Nat. Biotechnol.* 16:40–44
105. Ray C, Abbott AG, Hussey RS. 1994. Trans-splicing of a *Meloidogyne incognita* mRNA encoding a putative esophageal gland protein. *Mol. Biochem. Parasitol.* 68:93–101
106. Reed, JD, Edwards DL, Gonzalez CF. 1997. Synthetic peptide combinatorial libraries: a method for the identification of bioactive peptides against phytopathogenic fungi. *Mol. Plant-Microbe Interact.* 10:537–49
107. Rehman A, Jasmer DP. 1998. A tissue specific approach for analysis of membrane and secreted protein antigens from *Haemonchus contortus* gut and its application to diverse nematode species. *Mol. Biochem. Parasitol.* 97:55–68
- 107a. Riddle DL, Blumenthal T, Meyer BJ, Priess JR, eds. 1997. *C. elegans II*. New York: Cold Spring Harbor Lab. Press
108. Riddle DL, Georgi LL. 1990. Advances in research on *Caenorhabditis elegans*: application to plant parasitic nematodes. *Annu. Rev. Phytopathol.* 28:247–69
109. Robertson L, Robertson WM, Jones JT. 1999. Direct analysis of the secretions of the potato cyst nematode *Globodera rostochiensis*. *Parasitology* 119:167–76
110. Rosso MN, Favery B, Piotte C, Arthaud L, de Boer JM, et al. 1999. Isolation of a cDNA encoding a beta-1,4-endoglucanase in the root-knot nematode *Meloidogyne incognita* and expression analysis during plant parasitism. *Mol. Plant-Microbe Interact.* 12:585–91
111. Rosso, MN, Schouten A, Roosien J, Borst-Vrensens T, Hussey RS, et al. 1996. Expression and functional characterization of a single chain FV antibody directed at secretions involved in plant nematode infection process. *Biochem. Biophys. Res. Commun.* 220:255–63
112. Rouppe van der Voort JNAM, Eck H van, Zandvoort P van, Overmars H, Helder J, et al. 1999. Linkage analysis by genotyping sibling populations: a genetic map of the potato cyst nematode using a “pseudo-F2” mapping strategy. *Mol. Gen. Genet.* 261:1021–31
113. Rouppe van der Voort JNAM, Van Enkevort LJG, Pijnacker LP, Helder P, Gommers FJ, et al. 1996. Chromosome number of the potato cyst nematode *Globodera rostochiensis*. *Fund. Appl. Nematol.* 19:369–74
114. Sanchez Alvarado A, Newmark PA. 1999. Double-stranded RNA specifically disrupts gene expression during planarian generation. *Proc. Natl. Acad. Sci. USA* 96:5049–54
115. Schouten A, Roosien J, de Boer JM, Wilmink A, Rosso MN, et al. 1997. Improving scFv antibody expression levels in the plant cytosol. *FEBS Lett.* 415:235–41
116. Schouten A, Roosien J, van Engelen FA, de Jong G, Borst-Vrensens A, et al. 1996. The C-terminal KDEL sequence increases expression levels of a single-chain antibody designed to be targeted to both the cytosol and the secretory

- pathway in transgenic tobacco. *Plant Mol. Biol.* 30:781–93
117. Semblat JP, Abad P, Castagnone P. 1999. Molecular analysis of (a)virulence in root-knot nematodes *Meloidogyne* spp. *Abstr. Int. Congr. Mol. Plant-Microbe Interact.*, 9<sup>th</sup>, Amsterdam, July 25–30
118. Shields JP, Ding X, Hussey RS. 1998. Microaspiration of esophageal gland contents from plant-parasitic nematodes. *J. Nematol.* 30:515 (Abstr.)
119. Shpigel E, Roiz L, Goren R, Shoseyov O. 1998. Bacterial cellulose-binding domain modulates in vitro elongation of different plant cells. *Plant Physiol.* 117(4):1185–94
120. Sijmons PC, Atkinson HJ, Wyss U. 1994. Parasitic strategies of root nematodes and associated host cell responses. *Annu. Rev. Phytopathol.* 32:235–59
121. Simms EL. 1996. The evolutionary genetics of plant pathogen systems. *Bioscience* 46:136–45
122. Smant G, Govere A, Stokkermans JPWG, de Boer JM, Pomp HR, et al. 1997. Potato root diffusate-induced secretion of soluble, basic proteins originating from the subventral esophageal glands of potato cyst nematodes. *Phytopathology* 87:839–45
123. Smant G, Stokkermans JPWG, Yan Y, de Boer JM, Baum TJ, et al. 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* 95:4906–11
124. Smith MW, Feng DF, Doolittle RF. 1992. Evolution by acquisition: the case for horizontal gene transfer. *Trends Biochem. Sci.* 17:489–93
125. Stiekema WJ, Bosch D, Wilmink A, deBoer JM, Schouten A, et al. 1997. Towards plantibody-mediated resistance against nematodes. See Ref. 53a, pp. 262–74
126. Syvanen M. 1994. Horizontal gene transfer: evidence and possible consequences. *Annu. Rev. Genet.* 28:237–61
127. Timmons L, Fire A. 1998. Specific interference by ingested dsRNA. *Nature* 395:854
128. Tokuda G, Lo N, Watanabe H, Slaytor M, Matsumoto T, et al. 1999. Metazoan cellulase genes from termites: intron/exon structures and sites of expression. *Biochim. Biophys. Acta* 1447:146–59
129. Triantaphyllou AC. 1987. Genetics of nematode parasitism of plants. In *Vistas on Nematology*, ed. JA Veech, DW Dickson, pp. 354–63. Hyattsville, MD: Soc. Nematol.
130. 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
131. Vossen vander E, Rouppe van der Voort JNAM, Kanyuka K, Bendahmane A, Sandbrink H, et al. 2000. The potato cyst nematode resistance gene *Gpa2* and the virus resistance gene *Rx* form two specificities within a single NBS-LRR resistance gene cluster in potato. *Nat. Biotechnol.* In press
132. Vrain TC. 1999. Engineering synthetic resistance against nematodes, a review. *J. Nematol* 31:424–36
133. Wang M, Goldman IL. 1996. Resistance to root knot nematode (*Meloidogyne hapla* Chitwood) in carrot is controlled by two recessive genes. *J. Hered.* 87:119–23
134. Wang X, Ding X, Maier T, Goellner M, Baum TJ, et al. 1999. A novel method to isolate expressed esophageal gland secretory genes from *Heterodera glycines*. *Phytopathology* 89 (6S):S82–83 (Abstr.)
135. Wang X, Meyers D, Yan Y, Baum T, Smant G, et al. 1999. *In planta*

- localization of a  $\beta$ -1,4-endoglucanase secreted by *Heterodera glycines*. *Mol. Plant-Microbe Interact.* 12:64–67
136. Whitlam GC, Cockburn W. 1996. Antibody expression in transgenic plants. *Trends Plant Sci.* 1:268–72
137. Williamson VM. 1998. Root-knot nematode resistance genes in tomato and their potential for future use. *Annu. Rev. Phytopathol.* 36:277–93
138. Wyss U. 1992. Observations on the feeding behavior of *Heterodera schachtii* throughout development, including events during molting. *Fund. Appl. Nematol.* 15:75–89
139. Wyss U, Grundler FMW, Münch A. 1992. The parasitic behavior of second-stage juveniles of *Meloidogyne incognita* in roots of *Arabidopsis thaliana*. *Nematologica* 38:98–111
140. Yan Y, Davis EL. 1997. Characterization of secretory protein genes from the soybean cyst nematode. *Phytopathology* 87(6S):S106 (Abstr.)
141. Yan Y, Davis EL. 1999. A putative chemosensory receptor gene in the soybean cyst nematode. *Phytopathology* 89 (6S):S87 (Abstr.)
142. Yan Y, Smant G, Stokkermans J, Qin L, Helder J, et al. 1998. Genomic organization of four  $\beta$ -1,4-endoglucanase genes in plant-parasitic cyst nematodes and its evolutionary implications. *Gene* 220:61–70
143. Yao C, Jasmer DP. 1998. Nuclear antigens in *Trichinella spiralis* infected muscle cells: nuclear extraction, compartmentalization and complex formation. *Mol. Biochem. Parasitol.* 92:207–18
144. Young ND. 1996. QTL mapping and quantitative disease resistance in plants. *Annu. Rev. Phytopathol.* 34:479–501
145. Yu S, Avery L, Baude E, Garbers DL. 1997. Guanylyl cyclase expression in specific sensory neurons: a new family of chemosensory receptors. *Proc. Natl. Acad. Sci. USA* 94:3384–87
146. Zamore PD, Tuschl T, Sharp PA, Bartel DP. 2000. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell* 101:25–33