

Chapter 17

Genomics of the Soybean Cyst Nematode-Soybean Interaction

Melissa G. Mitchum and Thomas J. Baum

Introduction

The soybean cyst nematode (SCN), *Heterodera glycines*, is a problem worldwide wherever soybean is grown and consistently is the most economically important pathogen of soybean in the United States. It is estimated that this microscopic roundworm causes nearly \$1 billion annually in yield losses to US soybean producers. SCN-resistant cultivars serve as the primary means of management; however, genetic heterogeneity of SCN populations allows this pathogen to readily overcome resistance. In addition, a lack of understanding of nematode virulence has hampered the ability of researchers to devise novel management tactics. In recent years, however, an increase in genomic analyses of both soybean and *H. glycines* has begun to provide the necessary tools for developing a better understanding of this complex plant-nematode interaction. Expressed sequence tags (ESTs), generated from cDNA libraries representing different *H. glycines* and soybean developmental stages are a powerful tool for identifying genes important in soybean-SCN interactions. More than 22,000 *H. glycines* and 350,000 soybean ESTs have been deposited to publicly available databases. Moreover, the soybean and *H. glycines* EST datasets were used to develop microarray platforms representing 7,530 SCN and 35,611 soybean probe sets for comprehensive profiling of nematode and plant gene expression changes during parasitism. Soybean and SCN genetic and physical maps are under development and genome sequencing projects are underway for both organisms. These community efforts promise to provide both the tools and genome-wide catalogues of soybean and nematode genes for both functional and comparative analysis that should reveal additional, novel insight into mechanisms of *H. glycines* parasitism of soybean. In this chapter, recent genomics advances in our understanding of the soybean-SCN interaction are highlighted.

M.G. Mitchum

Christopher S. Bond Life Sciences Center, Division of Plant Sciences, University of Missouri-Columbia, MO 65211, USA
e-mail: goellnerm@missouri.edu

SCN Biology

The soybean cyst nematode is an obligate sedentary endoparasite. Sedentary endoparasitic nematodes penetrate host roots and migrate to the vascular tissue where they become sedentary and begin feeding (Hussey and Grundler, 1998). As with all plant-parasitic nematodes, SCN uses a hollow, protrusible stylet to pierce the plant cell wall, secrete proteins produced in the esophageal gland cells directly into host roots, and withdraw cellular contents. The details of the soybean cyst nematode life cycle (reviewed in Niblack et al., 2006) are depicted in Fig. 17.1 and are briefly described here. Infective second-stage juveniles (J2) hatch from eggs in the soil and locate host plant roots through attraction to diffusates. J2 mechanically penetrate the cell wall using their stylets while secreting cell wall-hydrolyzing enzymes to facilitate migration towards the root vasculature. Once the juvenile reaches the vasculature of the root, it selects an individual cell to begin the formation of a unique feeding structure called a syncytium (Fig. 17.2), which consists of hundreds of fused and metabolically highly active root cells. As the juvenile feeds, its body swells and at this point in its life cycle the parasitic juvenile is completely dependent

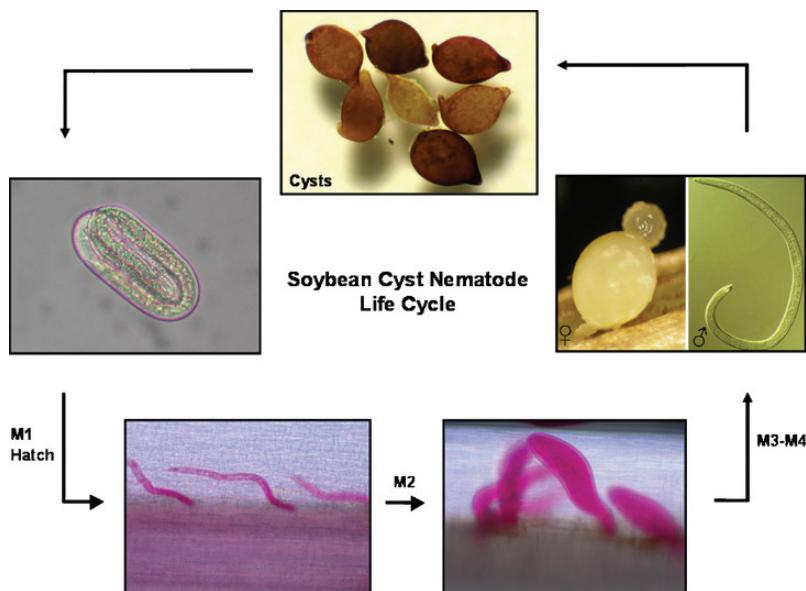


Fig. 17.1 Life cycle of *Heterodera glycines*, the soybean cyst nematode. Second-stage juveniles (J2) hatch from eggs in the soil and are attracted to soybean roots. The J2 penetrates into the roots and migrates towards the vascular cylinder where it selects a cell to initiate the formation of a syncytium. The nematode proceeds through three more molts to the adult male and female life stages. Following fertilization, the adult female secretes a small number of eggs in a gelatinous matrix outside her body while the majority of the eggs are retained within the female uterus. The dead female body forms the cyst to protect the eggs in the soil until favorable conditions arise. The average life cycle takes 25–30 days for completion under optimal conditions. M = molt (See also Color Insert)

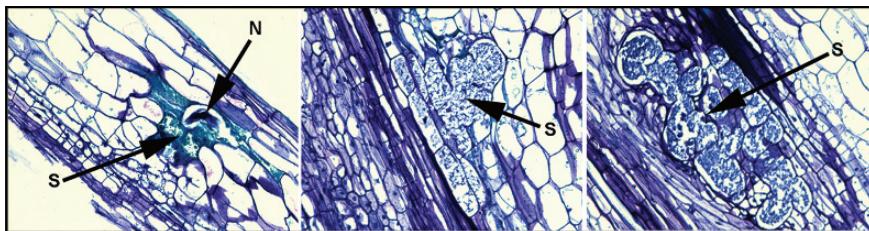


Fig. 17.2 Thin sections showing development of a syncytium at 2, 5, and 10 days post-inoculation of soybean roots with soybean cyst nematode second-stage juveniles. Reproduced with permission from Ithal et al., 2007a, Parallel genome-wide expression profiling of host and pathogen during soybean cyst nematode infection of soybean, *Molecular Plant-Microbe Interactions* 20: 293–305 (See also Color Insert)

on the successful formation of feeding cells to obtain the nutrients necessary to meet the increased energy demands required to proceed through three more molts to the adult male or female life stages. The soybean cyst nematode reproduces by obligate amphimixis, which requires that adult males regain their vermiform shape and motility and migrate out of the root to fertilize females protruding from the root surface. Fertilized females produce up to a few hundred eggs, most of which are retained within the uterus. When the female dies, her body serves as a cyst to protect the eggs in the soil from adverse environmental conditions in the absence of a host for many years until conditions are favorable.

SCN has two subventral (SvG) and a single dorsal (DG) esophageal gland cell (Fig. 17.3). Proteins originating in the SCN esophageal gland cells are secreted through the nematode stylet into host roots to metabolically and developmentally reprogram normal soybean root cells for the formation of syncytia (Fig. 17.2; Davis et al., 2000, 2004; Baum et al., 2007). Syncytia serve as major sinks for metabolites that are withdrawn by the feeding nematodes using their stylets. The syncytium forms through coordinated dissolution of plant cell walls, a process that is thought to be mediated by proteins of plant origin (Goellner et al., 2001). Protoplast fusion of adjacent cells results in the formation of a multinucleate syncytium made up of hundreds of cells. The nuclei within the syncytium are polyploid due to repeated rounds of endoreduplication and take on an amoeboid shape with a prominent nucleolus. The large central vacuole is dispersed into several smaller vacuoles, organelles proliferate, cell walls thicken, and there is an observed increase in cytoplasmic density (Hussey and Grundler, 1998). Cell wall ingrowths form along walls adjacent to the vasculature, typical of transfer cells, which increases the plasma membrane surface area for nutrient uptake (Jones and Northcote, 1972).

SCN Parasitism Gene Identification

The observed physiological and molecular changes of host root cells that occur during nematode feeding cell formation have long been considered to be a direct result of proteins secreted from the nematode stylet into plant tissues (Williamson and

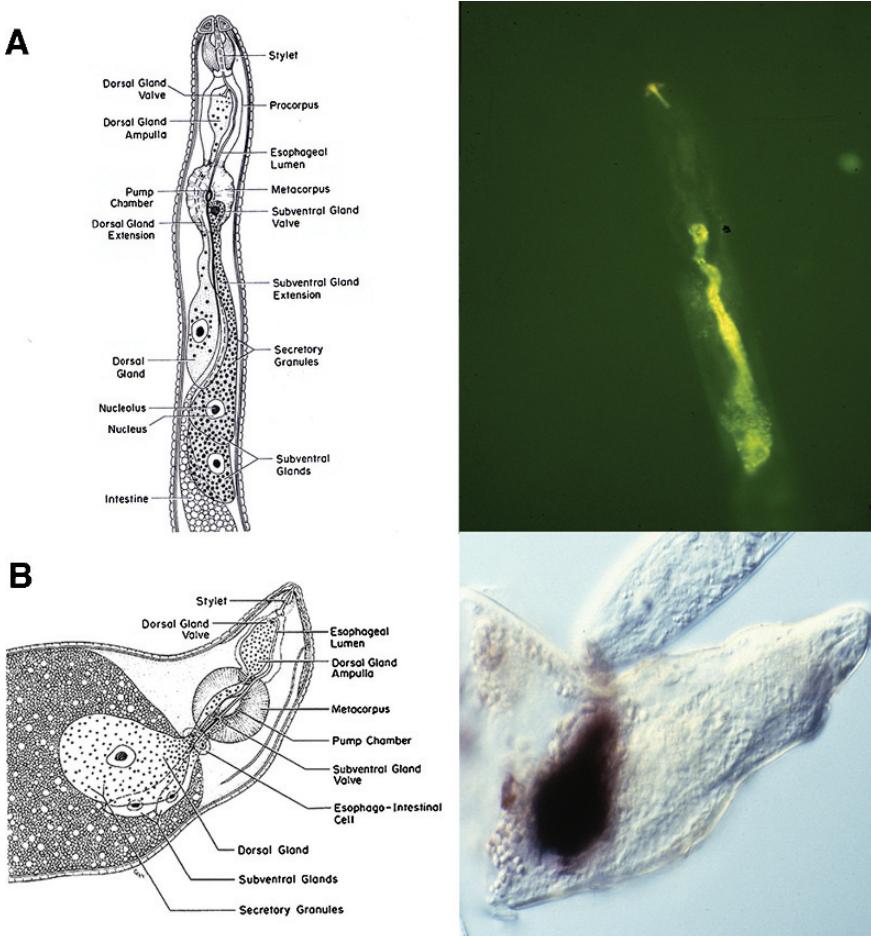


Fig. 17.3 Illustrations of the three soybean cyst nematode esophageal gland cells during second-stage juvenile (**A**, left panel) and adult female (**B**, left panel) life stages and corresponding parasitism gene expression confirmed by either immunolocalization of the protein to the two subventral glands (**A**, right panel) or *in situ* hybridization to detect mRNA accumulation in the dorsal gland (**B**, right panel). Immunolocalization and *in situ* pictures courtesy of Eric Davis and Richard Hussey, respectively. Drawings reprinted, with permission, from the *Annual Review of Phytopathology*, Volume 27 ©1989 by Annual Reviews, www.annualreviews.org (See also Color Insert)

Hussey, 1996). Stylet-secreted proteins are encoded by nematode parasitism genes that are expressed in the three esophageal gland cells. In general, parasitism genes are defined as genes that encode secretions from a nematode with a direct role in parasitism (Davis et al., 2004). Over the course of the last decade, a combination of genomic and proteomic approaches led to tremendous advancements in identifying nematode parasitism gene candidates (PGC) (Davis et al., 1994; Goverse et al., 1994; Hussey et al., 1990; Ding et al., 1998; Lambert et al., 1999; Ding et al., 2000;

Qin et al., 2000; de Meutter et al., 2001; Gao et al., 2001a,b; Wang et al., 2001; Jaubert et al., 2002; Grenier et al., 2002; Gao et al., 2003; Huang et al., 2003, 2004; Tytgat et al., 2004; Blanchard et al., 2005; Vanholme et al., 2005), resulting in the identification of key molecules involved in parasitism.

Direct targeting of the esophageal gland cells has proven to be the most successful strategy for the identification of SCN PGCs (Wang et al., 2001; Gao et al., 2001a, 2003). Using a microaspiration approach, the gland cell contents from SCN parasitic life stages were extracted and used to generate gland-enriched cDNA libraries. Sequencing and bioinformatic analyses of the gland-enriched cDNA libraries led to the identification of proteins with predicted secretion signal peptides. Following confirmation of gland-specific expression by *in situ* mRNA hybridization or immunolocalization (Fig. 17.3), more than 60 SCN parasitism gene candidates were identified (Wang et al., 2001; Gao et al., 2001a, 2003). Significantly, this provided the first snapshot of the SCN parasitome, i.e., the transcriptome of the esophageal gland cells coding for putative stylet-secreted proteins.

Bioinformatic Analysis of SCN Parasitism Gene Sequences

Sequence annotations of the more than 60 SCN parasitism gene candidate sequences identified to date, predict functions for a small subset of the protein products including those with roles in cell wall degradation, metabolism, and molecular mimicry of host proteins involved in cellular signaling and protein degradation pathways. Readers are referred to several comprehensive reviews of this subject (Davis et al., 2004; Davis and Mitchum, 2005; Baum et al., 2007). Interestingly, more than 70 % of the identified PGCs lack homology with any sequences in existing databases and were termed “pioneers” (Wang et al., 2001; Gao et al., 2001a, 2003). The following sections provide a brief review of some of the predicted roles of SCN parasitism proteins, which have provided new insight into our understanding of SCN parasitism with regard to cell wall modification during infection, metabolic and developmental reprogramming of host cells, and manipulation of host defense mechanisms. Readers are referred to Baum et al. (2007) for a detailed description of the molecular approaches currently being used to advance our understanding of SCN parasitism protein function.

SCN Parasitism Protein Function

Cell Wall Modification

The structural barrier of the plant cell wall presents a formidable challenge to an SCN infective J2. However, the nematode is well-equipped with a stylet for mechanical penetration and the ability to secrete an array of cell wall degrading enzymes (CWDEs) to facilitate penetration and migration through host root tissues. Genes

encoding beta-1,4-endoglucanases (ENGs; EGases) and pectate lyases were cloned from SCN (Smant et al., 1998; Yan et al., 1998, 2001; De Boer et al., 2002; Gao et al., 2002) and secretion of EGases was detected along the migratory path of juveniles invading host roots (Wang et al., 1999). Expression of the SCN *ENG* genes was shown to be developmentally regulated. That is, *ENG* expression is high in juveniles within eggs prior to hatching and in hatched juveniles prior to root penetration. However, expression declines substantially in parasitic juvenile stages as the nematode establishes a feeding site suggesting a minimal role, if any, in syncytium formation (de Boer et al., 1999; Gao et al., 2004a). The *ENG* genes are also expressed in adult vermiform males that regain mobility for migration out of the root to fertilize the females (de Boer et al., 1999). Gene sequences coding for secreted cellulose binding domain (CBD) proteins were also identified in SCN (Gao et al., 2003; Gao et al., 2004b). However, the function of the SCN CBD remains unknown. Recombinant plant and bacterial CBDs, however, were shown to modulate cell elongation and growth in vitro (Shpigel et al., 1998; Safra-Dassa et al., 2006).

Despite the mounting evidence that plant-parasitic nematodes secrete a complex mixture of CWDEs to facilitate penetration and migration through host root tissues, there is very little evidence to support a role for these proteins in the induction and formation of feeding cells. On the contrary, cell wall modifications within feeding cells appear to be the result of direct or indirect activation of plant cell wall modifying proteins (CWMPs) including EGases, expansins, pectin acetyl esterases, and xyloglucan endotransglycosylases (Goellner et al., 2001; Vercauteren et al., 2002; Wieczorek et al., 2006; Ithal et al., 2007a,b; Wang et al., 2007) that likely act in a controlled, coordinated fashion to regulate feeding cell formation.

Metabolic Reprogramming

Once a SCN juvenile has selected a cell near the vasculature to feed from, there is evidence to suggest that it secretes molecules to redirect host cell metabolism to establish a syncytium. Chorismate mutase, an enzyme of the shikimate pathway, was shown to be expressed within the esophageal gland cells of SCN (Bekal et al., 2003; Gao et al., 2003). The shikimate pathway in plants produces essential aromatic amino acids required by the nematode that can only be obtained from their diet. Chorismate mutase converts plastid-derived chorismate to prephenate to provide precursors for the synthesis of the aromatic amino acids, phenylalanine, tyrosine and tryptophan. The aromatic amino acids can then serve as precursors for the production of molecules with defined roles in plant-microbe interactions including auxin, flavonoids, salicylic acid, and phytoalexins. A cytosolic branch of the shikimate pathway for the production of aromatic amino acids from chorismate also exists. Therefore, nematode secretion of chorismate mutase into the host cytoplasm may be used to developmentally reprogram or suppress the accumulation of plant defense compounds produced in the plastidal branch of the shikimate pathway by increasing flux through the cytosolic branch. Consistent with this hypothesis,

overexpression of a root-knot nematode (RKN) chorismate mutase gene in soybean hairy roots resulted in phenotypes characteristic of auxin deficiency, including lateral root termination and impaired vasculature development (Doyle and Lambert, 2003). These phenotypes could be rescued by application of exogenous IAA suggesting that the transgenic roots overexpressing the RKN chorismate mutase were indeed auxin deficient. Additional studies will be needed to determine exactly how SCN may be altering the regulation of the shikimate pathway and potentially other metabolic pathways to establish a compatible interaction.

Ligand Mimicry

The dedifferentiation of soybean cells into syncytia likely requires an exchange of signals between SCN and the initial cell selected to establish a feeding site. Recent evidence suggests that SCN has evolved secreted mimics of plant signals to reprogram host cell development for syncytium formation providing the first insight into the “putative” signal or signals that are exchanged between soybean and SCN. The first nematode gene encoding a putative mimic of a plant protein was identified from SCN (Wang et al., 2001). The gene coded for a small, secreted protein that contained a C-terminal domain with similarity to plant CLAVATA3/ESR-related (CLE) peptides (Olsen and Skriver, 2003). The SCN CLE was shown to be expressed in the dorsal gland cell (Wang et al., 2001) when the nematode is actively initiating or feeding from syncytia and is either absent or expressed at extremely low levels in non-feeding life stages (Wang et al., 2006) supporting a role for CLEs in syncytium induction, function, or maintenance. Constitutive overexpression of the SCN CLE gene in Arabidopsis produced a *wuschel* (*wus*)-like phenotype (Brand et al., 2000; Hobe et al., 2003) characteristic of CLV3 overexpression (Wang et al., 2005). Consistent with this result, the SCN CLE could complement a *clv3* mutant (Wang et al., 2005). These studies suggest that nematode CLE peptides may have functional similarity to plant CLE peptides. More recent data indicate that there are two predominant forms of SCN CLEs that differ only in a variable domain immediately upstream of the CLE domain (Gao et al., 2003; Wang et al., 2006). Interestingly, these two SCN CLEs give different overexpression phenotypes in Arabidopsis (Wang et al., 2006) suggesting that they may have evolved different functions. Significantly, this work documents the first identification of a functional CLE domain outside of the plant kingdom and suggests that nematodes may be co-opting host developmental programs for syncytium formation through ligand mimicry (Wang et al., 2005).

Molecular Mimicry of Host Ubiquitination Pathway Components

Candidate SCN parasitism genes coding for secreted proteins with homology to ubiquitin extension, S-phase kinase associated protein 1 (SKP-1)-like, and RING-H2 proteins were also identified (Gao et al., 2003; reviewed in Davis et al., 2004).

Interestingly, these proteins are components of the ubiquitination pathway in eukaryotes; a pathway that targets proteins for degradation and is used as a mechanism for post-translational gene regulation. Recently, it was shown that a number of different pathogens can deliver effectors into host plant cells that can mimic the function of components of host E3 ubiquitin ligase complexes. Ubiquitination-mediated protein degradation was shown to play a role in regulating defense responses in plants and the secretion of host E3 ubiquitin ligase complex mimics may be one mechanism used by pathogens to manipulate plant defense responses to their own advantage (Zeng et al., 2006). The identification of SCN-secreted proteins with homology to proteins of the E3 ubiquitin ligase complex suggests that plant-parasitic cyst nematodes may also be equipped with the ability to mimic the host ubiquitination pathway to establish successful parasitic associations.

(A) *virulence Proteins*

Bona fide avirulence proteins have not been identified from plant-parasitic nematodes. However, effector molecules coded for by nematode parasitism genes are strong avirulence protein candidates. Recent hypotheses suggest that variants of plant-pathogen effector molecules with a central role in virulence function as avirulence factors depending on the genetic context of the host plant (Birch et al., 2006). Recent data indicate that the *H. glycines* chorismate mutase gene (*Hg-cm-1*) may play a role in virulence. Polymorphisms in *Hg-cm-1* were identified that correlate with virulence on a set of soybean cyst nematode-resistant soybean lines (Bekal et al., 2003) and the *Hg-cm-1A* allele was preferentially selected on the germplasm PI88788, the most common source of soybean cyst nematode resistance (Lambert et al., 2005). Similarly, polymorphisms were identified in the SCN *CLE* genes among soybean cyst nematode inbred populations that differ in their ability to parasitize resistant soybean implicating these proteins in virulence (Wang et al., 2006). In addition, small cysteine-rich proteins (<150aas), well known for their role as avirulence factors and/or elicitors of plant defense responses (Birch et al., 2006), are among the gland-expressed parasitism gene candidates (Gao et al., 2003).

SCN Genomics

In addition to targeting the identification and function of a select set of nematode genes, such as the parasitism genes described above, large-scale genomic approaches to elucidate various aspects of SCN biology and host plant interactions are well underway. The recent construction of SCN life stage-specific cDNA libraries provided the template that generated a collection of 20,100 expressed sequence tags (ESTs) to add to the existing SCN sequences previously deposited in GenBank (Parkinson et al., 2003, 2004; Elling et al., 2007). These publicly known EST sequences form 6,860 contigs, which likely reflect close to a third of all SCN

genes assuming the number of genes in SCN to be conserved with that of the free-living nematode *Caenorhabditis elegans* (~19,000 genes). Publicly available EST sequences were used by Affymetrix Inc. to develop the Soybean Genome Array GeneChip, which contains 7,530 SCN probe sets in addition to 37,593 soybean and 15,800 *Phytophthora sojae* probe sets. Use of this genomic resource for the study of SCN biology was first reported by Ithal et al. (2007a) who established expression profiles for known SCN parasitism genes during early events of the SCN life cycle. Subsequently, the Soybean Genome Array Gene Chip was used to determine the first profile of global gene expression changes throughout all major SCN life stages (eggs, infective J2, parasitic J2, J3, J4, and virgin females), excluding adult males (Elling et al., 2007). The authors demonstrated the utility of the dataset by comparing the mechanism of arrested development in SCN infective juveniles with that of the dauer stage of *C. elegans* (Elling et al., 2007). Undoubtedly, this dataset provides a valuable community resource to dissect a variety of other aspects of SCN biology and comparative genomics of nematodes (Mitrova et al., 2005). Several groups also initiated the development of genetically homogeneous SCN populations, molecular markers, and genetic maps to facilitate map-based cloning efforts (Dong and Opperman, 1997; Atibalentja et al., 2005). SCN BAC libraries were constructed and are being used to develop a SCN physical map anchored to the genetic map (Lambert et al., 2006) providing a solid framework for the public SCN genome sequencing projects that are in progress (Lambert et al., 2006). These efforts will no doubt distinguish SCN as an invaluable model plant-parasitic cyst nematode.

Genetic Analysis of the Soybean-SCN Interaction

Our understanding of SCN virulence (i.e., the ability of SCN to evolve to either evade or overcome host plant resistance) is limited and continues to present a challenge for SCN management which relies heavily on natural host plant resistance. Extensive genetic variability exists within field populations of SCN. This variability is currently described as HG types (for *Heterodera glycines* type) which define SCN populations' virulence on a set of seven resistant soybean indicator lines (Niblack et al., 2002, 2006). Genetic analysis of both soybean and SCN suggest that there are multiple genes in both the plant and nematode for resistance and virulence, respectively. However, on a genetic level there are several examples of plant-nematode interactions that were shown to follow the gene-for-gene hypothesis. This was demonstrated for the potato cyst nematode, *G. rostochiensis*, interaction with potato carrying the *H1* resistance gene (Janssen et al., 1991) and the root-knot nematode, *Meloidogyne incognita*, interaction with tomato carrying the *Mi* resistance gene (Milligan et al., 1998). Several plant resistance genes that confer resistance to plant-parasitic nematodes were cloned and characterized (Cai et al., 1997; Milligan et al., 1998; van der Vossen et al., 2000; Ernst et al., 2002; Paal et al., 2004). Despite these successes, soybean genes that confer resistance to SCN have not been cloned. Nevertheless, multiple quantitative trait loci (QTLs) conferring

resistance to individual SCN populations in numerous soybean germplasm sources were mapped (reviewed in Concibido et al., 2004). Two major QTLs controlling resistance to SCN that were mapped in multiple germplasm sources are located at the *rhg1* locus on linkage group G and the *Rhg4* locus on linkage group A2. Resistance to SCN in the soybean cultivar Forrest was shown to be bigenic, requiring both *Rhg1* and *Rhg4* (Meksem et al., 2001). *Rhg1* is codominant and *Rhg4* is dominant. To achieve full resistance, at least one copy of the Forrest *Rhg4* allele and two copies of the Forrest *Rhg1* allele are required. If one copy of the Forrest *Rhg1* allele is present, only partial resistance is conferred. Fine mapping of both loci is currently underway and a number of candidate genes for resistance have been identified (Meksem and Lightfoot, personal communication). Complementation and reverse genetic approaches in soybean will be required to confirm their roles in resistance to SCN. Similarly, positional cloning of other soybean QTLs for SCN resistance is underway (see Chapter 8 in this volume). The cloning and characterization of soybean genes for resistance to SCN will provide a much needed understanding of the molecular basis of soybean resistance to SCN.

The identification of the corresponding avirulence genes from nematodes, including SCN, using map-based cloning approaches has not been trivial. The inability to transform plant-parasitic nematodes has slowed progress in this area compared with other plant pathogens. In addition, the obligate nature of plant-parasitic nematodes and a lack of tools for genetic mapping have presented two major setbacks for using forward genetic strategies for the identification of SCN avirulence genes. Consequently, this approach has only been used in a few studies to date. A classical genetic approach identified several dominant and recessive *H. glycines ror* (for reproduction on a resistant host) genes in SCN inbred lines carrying fixed genes for parasitic ability on soybean (Dong and Opperman, 1997; Dong et al., 2005). However, the SCN genes controlling parasitism on resistant soybean at these loci were not cloned. As mentioned earlier, the recent development of a collection of SCN genomic tools will certainly facilitate future map-based cloning efforts (Atibalentja et al., 2005). The utility of the SCN genetic map was already demonstrated by mapping the *Hg-Cm-1* gene (Atibalentja et al., 2005). Thus, researchers will soon be equipped with both the genomic and genetic tools necessary to identify the genes controlling a variety of different traits such as nematode virulence and host range providing essential knowledge for both the development and deployment of nematode resistant soybean.

Genomics of the Soybean Response to SCN Parasitism

The complex plant response to SCN infection spans the early migratory stages during penetration and migration through roots into the later sedentary stages of syncytium induction and feeding, which ultimately facilitates nematode development and reproduction. During the early migratory phase of the nematode life cycle in either a susceptible or resistant host, SCN penetrates through the epidermis of the

root and migrates intracellularly towards the root vasculature, leaving in its wake, a path of damaged tissue. Transition from the migratory phase to the sedentary phase of the nematode life cycle occurs during syncytium induction. Syncytium formation is the result of and accompanied by drastic changes in plant gene expression giving rise to a cell type that is so far unique to the plant-nematode interaction. These localized cellular changes near the head of the nematode make up only a small fraction of the soybean root tissue. In nematode-resistant plants, syncytium development is compromised such that syncytia degenerate within just days after nematodes initiate feeding. As a result, nematode development is slowed and eventually terminated (Endo, 1965). Just the opposite occurs in susceptible host roots where well-developed syncytia provide the nutritional requirements of developing nematodes. With each molt, the nematode body expands putting stress on the surrounding cells that likely incites additional plant responses. Ultimately, adult females break through the epidermis of the root while adult males migrate back out of the root for copulation.

The complexity of the host plant response to cyst nematodes and the uniqueness of the feeding cells that are initiated have inspired numerous studies to identify plant gene expression changes in response to nematode infection. Early pioneering studies to identify genes that change their expression levels during the early compatible and incompatible interactions of cyst nematodes were conducted using differential display technology (Hermsmeier et al., 1998, 2000; Mahalingam et al., 1999; Vaghchhipawala et al., 2001). In one study, the approach successfully identified fifteen cDNA clones corresponding to mRNAs with different abundances in susceptible SCN-infected versus uninfected soybean roots (Hermsmeier et al., 1998). One of the soybean genes was identified as a phosphoglycerate mutase/biphosphoglycerate mutase enzyme, a key catalyst of glycolysis that was not previously characterized in plants (Mazarei et al., 2003). Identification of a homologous gene in *Arabidopsis* (*AtPGM*) and characterization of promoter-reporter gene fusions showed that this gene was induced in both root-knot and cyst nematode induced feeding cells (Mazarei et al., 2003). In conjunction with findings in other nematode pathosystems (Favery et al., 1998; Juergensen et al., 2003; Ithal et al., 2007b), these data suggest that increased rates of metabolism through glycolysis and the pentose phosphate pathway may elevate sugar levels within feeding cells to meet increased energy demands of the nematode. Another possible scenario presented by the authors, is that the induction of key enzymes of glycolysis and pentose phosphate pathways provides chorismate, the key substrate for the shikimate pathway, which is actively involved in producing compounds known to play important roles in plant-pathogen interactions. Chorismate would also serve as a substrate for nematode-secreted chorismate mutases described earlier (Popeijus et al., 2000; Bekal et al., 2003; Gao et al., 2003).

Other SCN-responsive soybean genes identified using differential display included two genes coding for polygalacturonases (PGs) whose transcript abundance increased during the early compatible interaction (Mahalingam et al., 1999), and a gene coding for an ethylene-responsive element-binding protein (GmEREBP), whose mRNA abundance decreased during a compatible interaction (Mazarei et al.,

2002). PGs are cell wall modifying enzymes that catalyze the hydrolysis of α -1,4 linked D-galacturons and were shown to play important roles during plant development. Although the spatial expression pattern of PGs in infected roots remains to be shown, the up-regulation of PGs in compatible interactions and down-regulation in incompatible interactions suggests a possible role for PGs in syncytium formation, possibly in conjunction with other plant CWMPs to regulate the controlled cell wall architectural changes that occur during syncytium formation (Goellner et al., 2001; Wieczorek et al., 2006). *GmEREBP* belongs to a large class of plant-specific transcription factors that were shown to play important roles in regulating plant defense responses (Gutierrez and Reuber, 2004). Further study of *GmEREBP* showed that its transcript abundance not only decreased during compatible plant-nematode interactions, but increased during SCN infection of resistant cultivars (Hermsmeier et al., 2000; Mazarei et al., 2002) suggesting that it might play a key regulatory role in SCN resistance. This hypothesis was tested by constitutive over-expression of *GmEREBP* in either Arabidopsis or a susceptible soybean cultivar (Mazarei et al., 2007). Transgenic plants displayed activated pathogenesis-related (PR) gene expression. However, plants inoculated with nematodes did not exhibit an altered susceptibility phenotype (Mazarei et al., 2007).

Attempts also were made to position SCN-responsive soybean genes on an available public linkage map of soybean to test for associations of any of these genes with known soybean SCN resistance QTLs (Vaghchhipawala et al., 2001). One soybean gene, phosphoribosylformyl-glycinamide (FGAM) synthase, was shown to be tightly linked to a major QTL for resistance, *rhg1*, on linkage group G. FGAM synthase catalyzes an important step in *de novo* purine biosynthesis and is active in rapidly dividing cells. Further studies confirmed that the promoters of two soybean FGAM synthases directed expression of GFP within syncytia induced by *Heterodera schachtii* in Arabidopsis (Vaghchhipawala et al., 2004). Nevertheless, the potential role of FGAM synthase in syncytium formation remains to be shown. Despite these findings, the early approaches identified only a handful of soybean genes responding to the nematode and little is known regarding their role, if any, in syncytium formation.

With the advent of functional genomic methodologies, plant molecular biologists and nematologists quickly set out to apply these new techniques to understand responses to cyst nematode infection. While first efforts focused on the sugar beet cyst nematode (Puthoff et al., 2003), attention quickly shifted to SCN. Initial efforts focused on generating ESTs from soybean roots infected with SCN (Alkharouf et al., 2004) and subsequently custom-made soybean cDNA microarrays were developed to examine host responses to infection (Khan et al., 2004; Alkarouf et al., 2006). In a study to examine the susceptible soybean response of whole roots at 2 days post-inoculation (2 dpi) with SCN using a soybean cDNA microarray representing 1,300 transcripts, 99 genes (8%) were found to be induced in response to nematode infection (Alkarouf et al., 2004). The majority of these genes coded for stress-responsive proteins including proline-rich glycoproteins, SAM22, β -1,3-endoglucanase, peroxidase, and others associated with plant defense. It is not surprising that SCN juveniles induce plant defense and wound-related responses at the

early timepoint of 2 dpi. During this phase of the nematode life cycle, infective J2 are still migrating intracellularly or have just completed the migratory phase of their life cycle and begun to initiate a feeding cell. Similar responses were observed at early timepoints in a time course analysis of whole soybean roots infected with SCN using a cDNA microarray representing 6,000 transcripts, (Alkharouf et al., 2006). Differentially expressed soybean genes were classified into several groups based on their expression profiles over the course of infection. A subset of wound, stress, and defense-related genes were elevated at all time points. At later time points, however, there was an observed increase in genes involved in transcription, protein synthesis, and metabolism likely reflecting alterations in plant gene expression associated with the development of highly metabolically active feeding cells in roots. With the advent of the Affymetrix Soybean Genome Array GeneChip representing 35,611 soybean transcripts and 7,431 SCN transcripts, a much more comprehensive expression profiling experiment of both soybean and SCN during a time course of the infection process was undertaken (Ithal et al., 2007a). This study provided the first global profile of gene expression changes in both soybean and SCN during different stages of nematode parasitism of roots. The simultaneous analysis of changes in both soybean and SCN mRNA levels identified 429 soybean genes with statistically significant differential expression between mock-infected and SCN-infected root tissues at three time points and 1,850 SCN genes with significant changes in expression during parasitism (Ithal et al., 2007a). Consistent with Alkharouf et al. (2006), this study observed a general activation of plant stress and defense mechanisms in response to SCN infection. Additional soybean genes differentially regulated by SCN infection included those involved in metabolism, cell wall modification, phytohormone response, and cellular signaling. The 1,850 differentially expressed SCN genes were grouped into 9 clusters based on their expression during parasitism. Of significant interest was the fact that the majority of previously identified SCN parasitism gene candidates was differentially expressed and displayed coordinated regulation during parasitism (Ithal et al., 2007a).

Despite the many efforts to profile global soybean responses to SCN infection using differential display and microarray approaches, these studies have contributed little to our understanding of gene expression changes occurring within developing feeding cells. This is due to the fact that the feeding cells make up only a small fraction of whole root tissues; thus, it is difficult to differentiate global gene expression changes from those occurring specifically within developing feeding cells. As a result, each gene identified as differentially expressed in a whole root study must then be confirmed for feeding cell expression. Alterations in plant gene expression within developing feeding cells were studied extensively in several different plant-nematode pathosystems to gain insight into the molecular mechanisms underlying their formation (reviewed in Gheysen and Fenoll, 2002). To date, however, only a handful of plant genes were shown to be expressed within developing feeding cells using promoter-reporter gene fusions, *in situ* hybridization, and immunolocalization techniques. Of these, even fewer were shown to play a role in feeding cell formation using reverse genetic approaches. Soybean is no exception. To date, only a few soybean genes were shown to be expressed in developing syncytia induced by

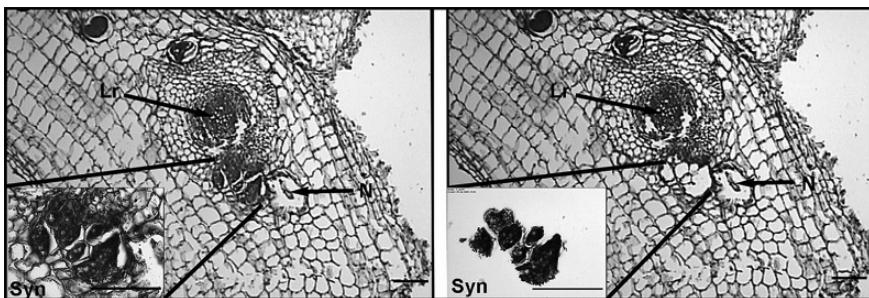


Fig. 17.4 Laser capture microdissection of a soybean cyst nematode-induced syncytium in soybean roots at 10 days post-inoculation. Reproduced with permission from Ithal et al., 2007b, Developmental transcript profiling of cyst nematode feeding cells in soybean roots, *Molecular Plant-Microbe Interactions* 20: 510–525

SCN. Nevertheless, technological advancements such as laser capture microdissection (LCM) have made cell-specific analyses feasible in plants (Day et al., 2005) and its application for isolating nematode feeding cells was recently demonstrated (Ramsay et al., 2004; Klink et al., 2005; Ithal et al., 2007b). This approach has opened the door for large-scale nematode feeding cell-specific analyses using functional genomic approaches. In a recent study, laser capture microdissection was coupled with microarray profiling to determine the first developmental transcript profile of cyst nematode feeding cells in susceptible soybean roots (Ithal et al., 2007b; Fig. 17.4). The authors identified 1,765 soybean genes expressed within syncytia that change expression within the first 2 days after syncytium induction and identified metabolic and regulatory pathways that likely play important roles in syncytium development.

Reverse Genetic Strategies to Dissect the SCN-Soybean Interaction

Until recently, a major bottleneck in interpreting large-scale SCN and soybean functional genomics data was a lack of reverse genetic approaches in both organisms. Functional analysis of gene products identified in genome-wide studies of the SCN-soybean interaction, such as those described above, is essential to advance our understanding of this plant-nematode interaction. A major breakthrough for functional biology of soybean and SCN was the application of RNA interference (RNAi) technology (Fire et al., 1998; Wesley et al., 2001) for post-transcriptional gene silencing of target genes. RNAi “soaking” methodologies to knock down genes in *C. elegans* and other nematodes were recently adapted for the plant-parasitic nematodes (Bakhetia et al., 2005; Urwin et al., 2002). This approach was used to demonstrate the roles of several SCN gland-expressed genes in nematode pathogenicity (Chen et al., 2005; Bakhetia et al., 2007). Moreover, a transgenic *in planta*-based RNAi approach (Wesley et al., 2001) to assess gene function by delivering dsRNA

or siRNA to nematodes during feeding was demonstrated for root-knot nematodes (Huang et al., 2006; Yadav et al., 2006). Similarly, *in planta*-based RNAi approaches to assess gene function in SCN are yielding promising results (Baum, T.J. and Davis, E.L., personal communication). Nematode targets have included genes required for fundamental aspects of nematode biology and essential parasitism genes, thus demonstrating the potential of RNAi not only for understanding gene function but for developing transgenic crop plant resistance to nematodes (Huang et al., 2006; Yadav et al., 2006).

In addition to *in planta*-based RNAi to assess gene function in soybean, approaches such as TILLING (Targeting Induced Local Lesions in Genomes), fast neutron mutagenesis, Virus-Induced Gene Silencing (VIGS), and transposon-tagging have been developed or are under development (see Chapter 9 in this volume). Thus, it will not be long before characterizing the role of soybean genes in various aspects of soybean-SCN interactions such as resistance and nematode feeding cell formation will be a much more straightforward process aiding in the rapid identification of plant targets for engineered resistance.

Conclusions

Advances in genomics during the last decade have contributed substantial new knowledge to our understanding of the complex soybean-SCN pathosystem. As a result, SCN is at the forefront of being developed into a model genetic system for plant-parasitic nematodes. Significant progress was made to identify the genes coding for nematode stylet secretions and progress towards elucidating the functions of secreted proteins has revealed fascinating new insight into the mechanisms of nematode parasitism of plants. Nematode transcriptome and genome sequencing projects provide unprecedented opportunities for comparative nematode biology that should shed light on the nature of the evolution of plant parasitism. Refined approaches for cell-specific analyses are enabling the discovery of the genes and pathways contributing to the formation of unique, highly specialized feeding cells induced within plant roots by parasitic nematodes, and emerging tools, such as RNA interference, hold tremendous potential not only for answering interesting biological questions related to nematode parasitism and feeding cell formation, but for identifying novel targets and mechanisms for engineering nematode resistant crop plants.

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