Molecular Insights in the Susceptible Plant Response to Nematode Infection

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Abstract Sedentary endoparasitic nematodes have evolved sophisticated strategies to form permanent feeding sites within host plant roots to ensure their survival. The process of feeding site formation entails an elaborate transformation of normal root cells into enlarged, multinucleate, and metabolically active cell types to supply the nutritional needs of the nematode. The signal-exchange that occurs between nematodes and their hosts to trigger the chain of molecular events associated with feeding cell formation has not been resolved. Presumably, the signals for the induction of feeding cells come from the nematode; thus, secretions originating in the esophageal gland cells and directly injected through the stylet into host tissues during parasitism have been implicated as key molecules. It is evident from the distinct morphological features of feeding sites that the nematode signals likely interfere with fundamental aspects of plant cell biology and differentiation. Molecular studies have shown that these changes are accompanied by extensive alterations in plant gene expression. Researchers have taken advantage of a wide array of methodologies to catalogue the genes as either up- or down-regulated in nematode feeding sites, and technological advances are now enabling feeding cell-specific analyses. As comprehensive profiles of genes expressed in feeding sites are generated, it has become increasingly important to determine which genes play essential roles in their formation. In the future, the true challenge will be to integrate our knowledge of nematode signals with host cell responses to elucidate a complete picture of feeding site formation.

1 Introduction

Plants respond to nematode infection in a variety of ways, mainly depending on the type and pathotype of the nematode. Plant-parasitic nematodes can be ecto- or endoparasites and either sedentary or roaming. In the latter case, the nematodes feed upon and quickly destroy plant cells, primarily provoking a plant stress

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response. At the other end of the spectrum, root-knot and cyst nematodes establish a complex and long-lasting relationship with their host plants, inducing the redifferentiation of selected root cells into large multinucleate nematode feeding sites (NFS). Because detailed molecular analysis of the plant response has only been performed on root-knot and cyst nematode infected roots, the data presented in this chapter has largely been derived from these systems. However, the prevalence of certain features such as enlarged nuclei and cell wall changes in very diverse feeding cells (Wyss 2002) indicates that the molecular insights gained in these well-studied systems may, to some extent, be extrapolated to other plant–nematode interactions.

This chapter will primarily deal with publications since 2002 and general insights from preceding studies. For a more thorough overview of the earlier gene expression analyses, we refer to Gheysen and Fenoll (2002). Particulars of the life cycles are presented in Bird et al. (2008) and details of the cellular changes elicited by nematode infection are reviewed by Endo (1984) and can be found in Berg et al. (2008) and Sobczak and Golinowski (2008). To facilitate the comprehension of this chapter, we will encapsulate the main features as highlighted in the following paragraphs, beginning with a brief review of the infection process.

The nematode developing inside the egg undergoes a first molt before hatching. The resulting second-stage juvenile is the infective stage that penetrates the plant root and migrates through root tissues to a suitable location near the vascular tissue. During migration, the subventral esophageal glands of the nematode are very active and secrete a mixture of cell-wall modifying enzymes to assist with burrowing. It is generally believed that secretions from the esophageal glands are also major determinants of feeding cell initiation. For more details on nematode secretions, we refer readers to several recent reviews (Davis et al. 2004; Vanholme et al. 2004; Davis et al. 2008).

Feeding cell formation is a complex process associated with dramatic changes in plant gene expression. Cyst nematodes select a single cell, often in the vascular parenchyma, to induce a feeding cell called a syncytium (Fig. 1). The selected cell responds to the nematode stimulus by gradually widening plasmodesmata to neighboring cells (Grundler et al. 1998), followed by the fusion of the protoplasts of the adjacent cells. At later stages, cell wall openings are formed de novo and the syncytium progressively expands by incorporating hundreds of adjacent cells.

Unlike cyst nematodes, root-knot nematodes induce the development of three to six giant-cells (Fig. 1) to feed from. The first sign of giant-cell induction is the formation of binucleate cells (Jones and Payne 1978). The cell plate vesicles line up between the two daughter nuclei but are then dispersed, disrupting cell plate formation. Additional mitoses uncoupled from cell division generate multinucleate, hypertrophied cells up to 100-times the size of normal root vascular parenchyma cells. In most plant hosts, the surrounding pericycle and cortical cells divide to form the typical gall or root-knot.

Feeding cells function as transfer cells to tap nutrients and solutes from the vascular tissues and provide them to the developing nematodes. Typical for transfer cells, the cell wall alongside the xylem forms finger-like projections

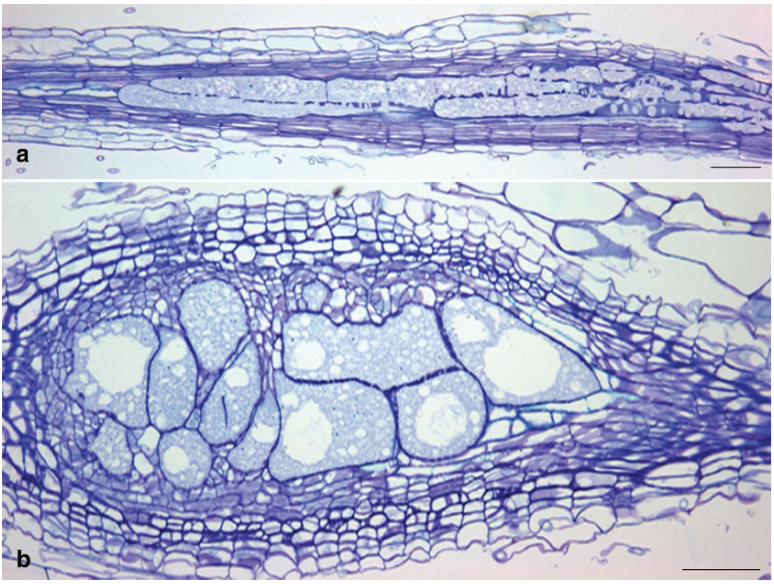

Fig. 1 Nematode feeding sites induced by cyst and root-knot nematodes. **a** A syncytium induced by *Heterodera schachtii* in Arabidopsis roots 7 days after infection. **b** Giant-cells induced by *Meloidogyne incognita* in Arabidopsis roots 10 days after infection. Bars – 100 μm

lined with plasma membrane, which facilitates water transport from the xylem into the feeding cell (Jones and Northcote 1972). The dense granular cytoplasm of feeding cells resembles that of meristematic cells: many ribosomes, organelles, and small vacuoles. High metabolic activity and constant withdrawal of cytoplasm by the nematodes convert the feeding cells into metabolic sinks for the host plant.

A variety of approaches have been used to identify gene expression changes occurring specifically within NFS including promoter–reporter assays and in situ hybridization (Fig. 2). To assess their role in NFS formation, hence gaining molecular insight into NFS development, functional analysis of these plant genes is crucial. The consequence of knocking out a gene (or over-expressing it) on NFS formation, however, has only been investigated in a few instances (Table 1). For reasons related to the amount of genomic information and the availability of mutant and marker lines, changes in gene expression of plants infected by nematodes have preferably been studied using the model plant, *Arabidopsis thaliana*. However, the use of crop plants such as soybean (*Glycine max*) and tomato (*Solanum lycopersicum*) for molecular studies on plant–nematode interactions is gaining in importance as genomic data and microarray platforms become increasingly available. Microarray analyses (Alkharouf et al. 2006; Bar-Or et al. 2005; Hammes et al. 2005; Ithal et al. 2007a,b; Jammes et al. 2005; Khan et al. 2004; Klink et al. 2005; Puthoff et al. 2003; Ramsay et al.

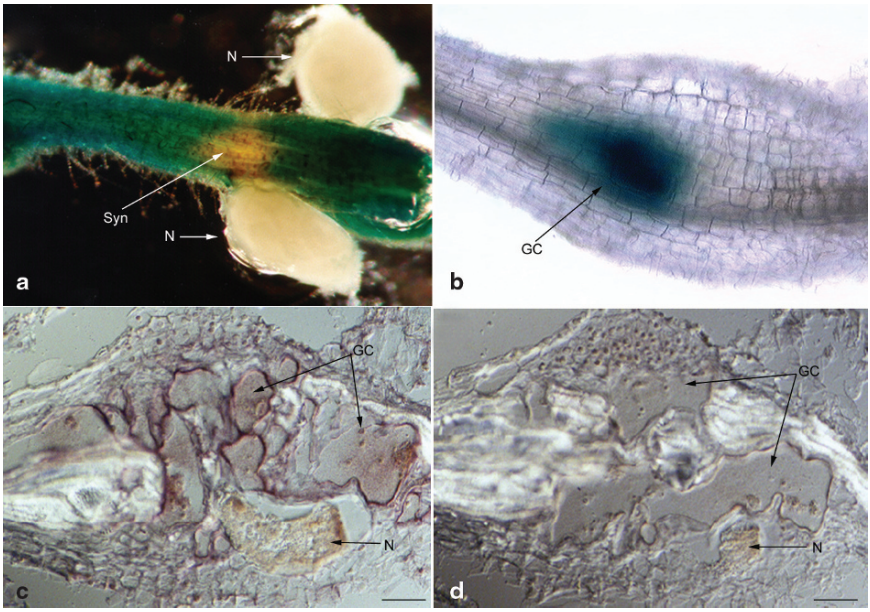

Fig. 2 Gene expression changes occurring specifically within nematode feeding sites detected using promoter–reporter assays and *in situ* hybridization. **a** Activity of the *NOS* promoter (pNOS) in nematode-infected sugarbeet hairy roots. A sugarbeet hairy root with the *pNOS-GUS* construct at 20 days after inoculation with *H. schachtii*. The blue color demonstrates very strong promoter activity outside the syncytium, but down-regulation of the *NOS* promoter inside the syncytium. In sections this down-regulation is visible as early as 4 days after inoculation. **b** Activity of the *AtCel1* promoter in nematode-infected tobacco roots. Transgenic tobacco with the *Cel1-GUS* construct at 7 days after inoculation with *Meloidogyne incognita*. The blue color demonstrates very strong promoter activity within the developing galls. In sections, *Cel1-GUS* expression was localized to developing giant-cells (Mitchum et al. 2004). **c**, **d** Sections of nematode-infected tobacco roots. Two-week-old galls induced by *Meloidogyne incognita* on tobacco roots were sectioned and probed with a digoxigenin-labeled antisense (**c**) or sense (**d**) probe corresponding to the tobacco endo-β-1,4-glucanase 7 (*Cel7*) gene (Goellner et al. 2001). The purple color demonstrates very strong expression of *Cel7* within giant-cells. No staining was observed with the sense probe control. GC, giant-cell; N, nematode; Syn, syncytium; *NOS,* nopaline synthase. Bars – 100 μm

2004; Wang et al. 2003) have significantly enlarged the list of nematode-responsive genes (Li et al. 2008) and confirmed previous analyses that the down-regulation of genes is as relevant to NFS formation as gene induction (Gheysen and Fenoll 2002). In the following sections, we highlight recent molecular insights into the susceptible plant response to nematode infection, particularly with regard to the plant genes involved, and how these genes may be contributing to the induction and function of NFS.

Table 1 Changes in nematode infections on transgenic and mutant plants compared to the control

Gene name	Characteristics	Plant	Root-knot	Cyst	Reference
dgt	Auxin-insensitive mutant	*Solanum lycopersicum*	↘	↘	Richardson and Price (1984), Goverse et al. (2000)
axr2	Auxin-insensitive mutant	*Arabidopsis thaliana*	n.t.	↘	Goverse et al. (2000), Wubben et al. (2001)
pin1/1-ttg1	Auxin transport mutant	*Arabidopsis thaliana*	n.t.	↘	Goverse et al. (2000)
pin2/eir1-1	Auxin transport mutant	*Arabidopsis thaliana*	n.t.	↘	Goverse et al. (2000), Wubben et al. (2001)
rhd1	Hypersensitive ethylene mutant	*Arabidopsis thaliana*	n.t.	↗	Wubben et al. (2004)
eto1, eto2, eto3	Ethylene over-producing mutants	*Arabidopsis thaliana*	n.t.	↗	Goverse et al. (2000), Wubben et al. (2001)
etr1	Ethylene receptor mutant	*Arabidopsis thaliana*	n.t.	↘	Wubben et al. (2001)
ein2, ein3	Ethylene insensitive mutants	*Arabidopsis thaliana*	n.t.	↘	Wubben et al. (2001)
har1	CLAVATA1-like receptor kinase mutant	*Lotus japonicus*	↗	n.t.	Lohar and Bird (2003)
AtCKX3, ZmCKX1	Transgenics with lower sensitivity to cytokinin	*Lotus japonicus*	↘	n.t.	Lohar et al. (2004)
sid2-1, pad4-1	Salicylic acid deficient mutants	*Arabidopsis thaliana*	n.t.	↗	Wubben et al. (2008)
NAHG	Salicylic acid deficient transgenic	*Arabidopsis thaliana*	n.t.	↗	Wubben et al. (2008)
npr1-2, npr1-3	Salicylic acid insensitive mutants	*Arabidopsis thaliana*	n.t.	↗	Wubben et al. (2008)
sni1	*NPR1*-suppressor mutant	*Arabidopsis thaliana*	n.t.	↘	Wubben et al. (2008)
NAHG	Salicylic acid deficient transgenic	*Solanum lycopersicum*	n.s.d.	n.t.	Bhattarai et al. (2008)
jai1	JA insensitive mutant	*Arabidopsis thaliana*	↘	n.t.	Bhattarai et al. (2008)
def1	JA deficient mutant	*Arabidopsis thaliana*	n.s.d.	n.t.	Bhattarai et al. (2008)

(continued)

Table 1 (continued)

Gene name	Characteristics	Plant	Root-knot	Cyst	Reference
TOBRB7 antisense	Aquaporin down-regulated	*Nicotiana tabacum*	↘	n.t.	Opperman and Conkling (1996)
rpe	T-DNA tagged mutant in *RPE*	*Arabidopsis thaliana*	↘	n.s.d.	Favery et al. (1998)
PHAN antisense	Phantastica down-regulated	Unpubl.	↘	n.t.	Koltai et al. (2001)
ENOD40 overexpression	Early nodulin gene up-regulated	*Medicago truncatula*	↗	n.t.	Favery et al. (2002)
nfr1 and *nfr5*	Nod factor receptor mutants	*Lotus japonicus*	↘	n.t.	Weerasinghe et al. (2005)
WRKY23 RNAi	*WRKY23* down-regulated	*Arabidopsis thaliana*	n.t.	↘	Grunewald et al. (2008) and Gheysen (unpublished)
CDKA;1 cosuppression	*CDKA;1* down-regulated	*Arabidopsis thaliana*	↘	↘	Van de Cappelle et al. (2008)
CCS52A RNAi	*CCS52A2* down-regulated	*Arabidopsis thaliana*	n.t.	↘	Van de Cappelle, de Almeida-Engler and Gheysen (unpublished)
EXPA5 antisense	Hairy roots with expansin down-regulated	*Solanum lycopersicum*	↘	n.t.	Gal et al. (2005)
map65-3	T-DNA tagged mutant in *MAP65-3*	*Arabidopsis thaliana*	↘	n.t.	Caillaud et al. (2008)
cel2	Endo-1,4-β-glucanase mutant	*Arabidopsis thaliana*	n.t	↘	Wieczorek et al. (2008)
kor3	Endo-1,4-β-glucanase mutant	*Arabidopsis thaliana*	n.t	↘	Wieczorek et al. (2008)
CEL7 RNAi	*CEL7* endo-1,4-β-glucanase silenced	*Solanum tuberosum*	n.t	↘	Karczmarek et al. (2008)
CEL9C1 RNAi	*CEL9C1* endo-1, 4-β-glucanase silenced	*Solanum tuberosum*	n.t	↘	Karczmarek et al. (2008)

Direction of arrow indicates if infection is higher or lower on this plant line than on the corresponding wild type. n.t. not tested; n.s.d. not significantly different

2 Activation of Plant Defense Responses During a Compatible Interaction

From the moment a nematode penetrates the plant root, a molecular dialogue is set up resulting in chemical warfare. Defense responses include the production of harmful oxygen radicals and systemic signaling compounds as well as the activation of defense genes that lead to the generation of structural barriers, pathogenesis-related proteins and toxins (such as phytoalexins) that debilitate the invader. It is not surprising that this also happens in the compatible interaction, but then the nematode can either evade or overcome the plant defense. The protein surface coat of plant-parasitic nematodes contains peroxiredoxin and lipid-binding proteins that might respectively break down hydrogen peroxide and inhibit lipoxygenase activity, an important enzyme in the jasmonic acid signaling pathway (reviewed in Vanholme et al. 2004).

Plant genes known to be involved in wound and defense response are up-regulated during infection by nematodes (reviewed in Gheysen and Fenoll 2002). Quite a few of the up-regulated genes are also involved in cell wall modifications or in the biosynthesis of secondary metabolites that could be crucial in establishing the parasitic interaction. Cell wall fortification is an important induced plant defense response for confining the pathogen, and many of the genes implicated in this process such as extensin, lignin biosynthesis genes, and peroxidase are up-regulated upon nematode infection. However, extensive cell wall modifications are also fundamental to feeding site structure (for details, see Sect. 5). Similarly, the accumulation of flavonoids, isoflavonoids, and other phenolics is a typical plant defense response. On the other hand, flavonoids inhibit auxin transport and their local production could trigger the accumulation of auxin, an outstanding candidate implicated in early feeding site development (see Sect. 9). There are also indications that specific plant defense pathways may be locally down-regulated within the feeding sites. For example, several genes encoding key enzymes in jasmonic acid biosynthesis, an important defense signaling molecule, were found to be down-regulated in syncytia of soybean induced by *H. glycines* (Ithal et al. 2007b).

3 Polyploidy in NFS Is Correlated with Activation of Cell-Cycle Genes

From initial microscopy analyses (Endo 1984), it was evident that characteristic cytological changes take place in the nuclei of developing feeding cells (Berg et al. 2008; Sobczak and Golinowski 2008). Syncytia, as well as giant-cells, contain multiple large and amoeboid nuclei. These nuclear responses are among the first visible changes in developing feeding cells. Cytological observations suggest that nuclei in giant-cells divide through successive mitoses without intermittent cytokinesis (acytokinetic mitosis) while the multinucleate state in syncytia is attained by amalgamation of

many uninucleate root cells (Gheysen et al. 1997). Although mitosis takes place in the surrounding dividing cells, it has never been observed inside the syncytium. Enlargement of nuclei is generally caused by endoreduplication, a term used for an abnormal cell cycle that repeatedly goes through the DNA synthesis-phase but skips mitosis (Gheysen and Fenoll 2002). There is little doubt that endoreduplication occurs in syncytia; in contrast, the enlargement of giant-cell nuclei could also be explained by the fusion of nuclei, possibly due to entanglement of the abundant cellular mitotic apparatus.

More recently, the molecular mechanisms underlying the cell cycle events in syncytia and giant-cells have been explored in detail. Initial studies on the expression of a few core cell cycle genes in *A. thaliana* revealed their up-regulation within the first hours after the nematode has selected a cell to initiate a NFS. Two cyclin-dependent kinases (*CDKA;1* and *CDKB1;1*), an A-type cyclin (*Arath;CycA2;1*) and a mitotic cyclin (*Arath;CycB1;1*), typical for the late G2 until M phase, were found to be expressed in giant-cells as well as syncytia (Niebel et al. 1996). It was therefore concluded that the cell cycle proceeds at least until the late G2 phase in both types of feeding cells. This has also been recently reported for endoreplicating trichome-neighboring cells (Weinl et al. 2005). During later stages of syncytium development [around 10–14 days after infection (dai)], the cell cycle genes were still expressed at the growing edges to probably allow syncytia to reach a critical size necessary for the cyst nematodes to properly complete their life cycle.

To analyze the importance of cell cycle activation for the development of feeding cells, cell cycle-inhibiting drugs have been used (de Almeida-Engler et al. 1999). Upon hydroxyurea treatment (which prevents DNA synthesis), early giant-cell and syncytium development was blocked in Arabidopsis. This demonstrates that genome multiplication is essential for the formation of both types of feeding cells. However, the application of hydroxyurea at later stages resulted in normal development of the feeding sites. Upon early application (1 and 3 dai) of oryzalin (which arrests cells in mitosis), root-knot nematode development in Arabidopsis was completely inhibited (de Almeida-Engler et al. 1999; Wiggers et al. 2002). The formation of giant-cells was initiated but their development severely hampered. In contrast, when oryzalin was applied at later stages (9 dai), the majority of the root-knot nematodes could complete their life cycle. This indicates that mitosis is mainly required for early giant-cell differentiation and initial expansion. Mitosis can occur up to 21 dai in *Pisum sativum* infected by *M. incognita*, probably supporting an optimal but nonessential expansion of the feeding cell (Wiggers et al. 2002). Syncytia arise by fusion of adjacent cells; therefore, one would assume that mitosis is not involved in the formation of the multinucleate syncytium, and thus oryzalin should not affect cyst nematode development. Application of oryzalin at 1 dai, however, resulted in the complete inhibition of syncytium development and no cysts were formed on these plants. When oryzalin was applied at later stages (3 and 9 dai), an increasing number of the infective juveniles developed into cysts. These data indicate that mitotic activity is also required for proper syncytium development. It was observed that oryzalin inhibits the mitotic activity in cells prior to syncytium incorporation and, as a consequence, syncytium expansion is restricted

(de Almeida-Engler et al. 1999). Recently, it was demonstrated that silencing *AtCDKA;1*, an essential cell cycle gene, using a NFS-promoter results in significant reduction of nematode development on *A. thaliana* (Van de Cappelle et al. 2008).

These studies do not distinguish cell cycle events in giant-cells versus syncytia, particularly with regard to the importance of endoreduplication. Endoreduplication is a very important process in plant cell differentiation and therefore has received a substantial amount of scientific attention. The control of endoreduplication is not yet fully understood, but several key proteins have been identified, mainly in *A. thaliana* (Fig. 3). The heterodimeric transcription factor E2Fa-DPa activates the G1/S transition in both the mitotic and endoreduplication cycles. The transition into the mitotic cycle was postulated to depend on the presence of the MIF (mitosis-inducing factor) (De Veylder et al. 2002) and the activity of specific inhibitors of the endocycle (DEL1) (Vlieghe et al. 2005). MIF might be a complex of a cyclin-dependent-kinase B (CDKB1;1) (Boudolf et al. 2004) and an A2 class cyclin (Imai et al. 2006). The switch between mitosis and the endocycle almost certainly occurs as a result of specific premature destruction of mitotic cyclins by a CCS52 (cell cycle switch) complex (Cebolla et al. 1999). *A. thaliana* contains three *CCS52* genes. CCS52 defines the activity and substrate specificity of the anaphase-promoting complex (APC), a multi-component ubiquitin ligase that plays a fundamental role in the metaphase–anaphase transition and exit from mitosis by targeting specific cell cycle proteins for degradation by the ubiquitin-proteasome pathway (Kondorosi and Kondorosi 2004).

If feeding cells endoreduplicate, one would expect low expression of *CDKB1;1*, *Cyclin A2*, and *DEL1* and a high level of expression of *CCS52*. *CCS52* was first discovered in *Medicago truncatula*, where it was highly expressed in endoreduplicating nodule tissues (Cebolla et al. 1999), and its expression was also analyzed in galls (Favery et al. 2002; Koltai et al. 2001). *CCS52* expression was found inside the giant-cells and in the surrounding cells, indicating the occurrence of endoreduplication. In the same experiments however, high *CCS52*

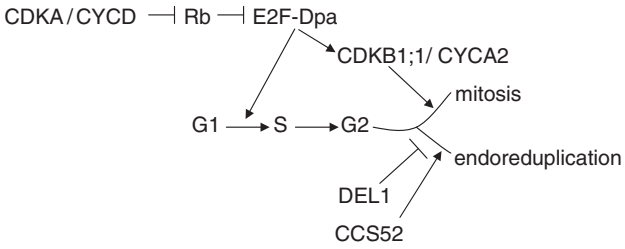

Fig. 3 E2Fa-DPa activates the G1/S transition in both the mitotic and endoreduplication cycles. The transition into the mitotic cycle was postulated to depend on the presence of MIF (mitosis-inducing factor) and the activity of DEL1. MIF might be a complex of a cyclin-dependent-kinase B (CDKB1;1) and an A2 class cyclin. The switch between mitosis and the endocycle almost certainly occurs as a result of specific premature destruction of mitotic cyclins by a CCS52 (cell cycle switch) complex. Scheme modified from Boudolf et al. (2004), with information incorporated from Vlieghe et al. (2005) and Imai et al. (2006)

expression was also observed in lateral root meristems, challenging the model that *CCS52* is typically expressed in endoreduplicating tissues. Nevertheless, up-regulation of *CCS52* clearly results in higher ploidy (Cebolla et al. 1999; Van de Cappelle, de Almeida-Engler and Gheysen, unpublished results) and down-regulation of *CCS52* in lower ploidy (Vinardell et al. 2003). Transgenic *M. truncatula* plants with lower *CCS52* expression showed severe inhibition of nodule development (Vinardell et al. 2003), and preliminary experiments also indicate inhibition of nematode development (Van de Cappelle, de Almeida-Engler and Gheysen, unpublished results).

According to Roudier et al. (2003) *MtCycA2;2*, shown to exclusively play a role in mitotic cycles and not be expressed during endoreduplication, is repressed during nodule and gall development in *M. truncatula*, indicating endoreduplication in both systems (symbiosis and nematode parasitism). Unfortunately, no information is available on the expression of this gene in syncytia.

These results so far would indicate the occurrence of endoreduplication in giant-cells as well as syncytia. In giant-cells, endoreduplication is probably preceded by or even alternates with mitosis. Indeed, it was recently shown that endoreduplicated cells can re-enter the mitotic cell cycle (Weinl et al. 2005). Another study by Huang et al. (2003b) renders the story even more complicated. The *A. thaliana Prolifera* gene (*PRL*), which is normally expressed in dividing cells and not in endoreduplicating tissues, is up-regulated in giant-cells and syncytia but not in the surrounding, dividing cells. This indicates that our assessment of cell cycle expression is not yet precise enough to predict or explain how the aberrant cycles in NFS are controlled. It has been shown that a particular expression level often determines the outcome of the cell cycle for certain genes. For example, moderate over-expression of *DEL1* inhibits the endocycle whereas higher levels also block mitosis (Vlieghe et al. 2005). Similarly, a moderate level of *KRP2* (a cell cycle inhibitor) over-expression increases endoreduplication whereas higher levels decrease endoreduplication (Verkest et al. 2005). On top of all of this, the importance of posttranscriptional regulation and protein activity changes makes it clear that the more we learn about cell cycle, the more complicated the picture becomes.

4 Cytoskeletal Rearrangements Within NFS

Numerous studies have demonstrated the importance of plant cytoskeletal rearrangements upon pathogen attack (Takemoto et al. 2003 and references therein). The actin filaments are focally reorganized towards sites of penetration and, in the case of a biotrophic fungus, actin cables are targeted to the fungal feeding organs (haustoria). Sedentary nematodes also induce long-term rearrangements of the cytoskeleton. Actin genes are highly expressed in NFS as would be expected in large expanding cells that need extensive internal transport (de Almeida-Engler et al. 2004). In *A. thaliana*, tubulin genes are moderately up-regulated in syncytia and at higher levels in giant-cells, probably reflecting the need of a mitotic

cytoskeleton for the rapidly dividing nuclei in the latter. RT-PCR experiments using RNA extracted from laser-captured soybean syncytia induced by *H. glycines* revealed a strong induction of β-tubulin and a weaker up-regulation of α-tubulin as compared to samples extracted from uninfected roots (Klink et al. 2005). Analysis of the cytoskeleton in *A. thaliana* by immunolocalization of the actin and tubulin proteins and by the use of *GFP*-fusions showed that the cytoskeleton was strongly disrupted in syncytia (de Almeida-Engler et al. 2004). The same diffuse or clumped tubulin labeling was seen in soybean syncytia (Klink et al. 2005). In giant-cells, although disturbed compared to normal root cells, actin and microtubular fibers are visible with abnormally thick actin cables present in the cell cortex. A functional mitotic apparatus, consisting of microtubule-forming spindles (for separating the chromosomes during nuclear division) and phragmoplasts (initiating the cell plate), is present in the developing giant-cells (de Almeida-Engler et al. 2004).

Depolymerization of the actin and microtubular cytoskeleton with cytochalasin D or oryzalin, respectively, only inhibited feeding cell and nematode development when applied at early stages of infection (3 dai). In contrast, stabilization of the microtubular cytoskeleton with taxol at later stages (14 dai) resulted in normally developed feeding cells but interfered with nematode development. As nematode feeding involves the retrieval of large volumes of cytoplasm, a degree of cytoskeleton fragmentation may decrease cytoplasm viscosity and therefore facilitate uptake during nematode feeding.

It is not known how nematode infection brings about the observed cytoskeletal changes. A possible player from the plant side is the actin-nucleating protein, formin. Formins are cytoskeleton-organizing proteins involved in the establishment of cell polarity and growth. From the 21 predicted *A. thaliana* genes, three (*AtFH1, AtFH6*, and *AtFH10*) were shown to be up-regulated in giant-cells at 7 and 14 dai (Favery et al. 2004). *AtFH6* was expressed as early as 2 dai and the protein was uniformly distributed throughout the plasma membrane. It was therefore suggested that AtFH6 distribution could be involved with the isotropic growth of the giant-cells.

Another cytoskeletal protein, microtubule-associated protein 65-3 (MAP-65-3), that is essential for cytokinesis in somatic plant cells, is also required for giant-cell ontogenesis (Caillaud et al. 2008). The protein colocalizes to the mitotic microtubular arrays and the cell plate in mitotic cells. The corresponding gene is up-regulated in giant-cells and the protein can be found on the aborted cell plates. Knocking-out the gene results in dwarf plants with defects in cell division and upon nematode infection, giant-cells are initiated but cannot develop normally, resulting in the death of the nematode (Caillaud et al. 2008).

5 Cell Wall Architectural Modifications in NFS

Plant cell walls are composed of cellulose microfibrils embedded in a matrix of hemicellulose and pectin polymers. The cell wall undergoes pronounced changes throughout plant development by recruiting several different classes of cell-wall

modifying proteins (CWMPs) and enzymes to the extracellular space. The plant cell wall also represents a structural barrier to infection by a wide range of plant pathogens. Sedentary endoparasitic cyst and root-knot nematodes have evolved sophisticated mechanisms to breach the structural barrier of the plant cell wall during the penetration and migration phase of the life cycle yet fine-tuned their manipulation of the cell wall for the establishment of feeding sites.

Cyst and root-knot nematodes secrete a battery of cell-wall modifying proteins to facilitate penetration and migration, albeit by different modes (i.e., intracellular vs. intercellular, respectively) through host root tissues. CWMPs are encoded by nematode parasitism genes, the details of which are covered in Davis et al. (2008). A large number of genes encoding CWMPs have been identified in cyst and root-knot nematodes, yet there is very little evidence to support a role during the induction and formation of syncytia and giant-cells. Accumulating evidence suggests that the CWMPs involved in the extensive wall modifications within feeding cells are of plant origin (Goellner et al. 2001; Karczmarek et al. 2008; Mitchum et al. 2004; Vercauteren et al. 2002; Wang et al. 2007; Wieczorek et al. 2006, 2008).

Despite the conserved function of syncytia and giant-cells as nutrients sinks, their ontogeny particularly differs with regard to alterations in cell wall architecture. Giant-cells are formed by hypertrophy of single vascular parenchyma cells up to 100x their normal size. In contrast, syncytia form by progressive cell wall dissolution and fusion of adjacent cells. Although giant-cells and syncytia differ in the extent of cell wall degradation, the walls of both types of feeding structures require loosening for expansion and elongation. Additional characteristic cell wall modifications shared by both giant-cells and outer walls of syncytia include thickening and formation of finger-like ingrowths adjacent to xylem vessels which increase the plasmalemma surface area to support increased solute uptake. Although the nature of the thickened walls is not clear, it may involve callose or lignin deposition (Grundler et al. 1998; Jones and Northcote 1972) to strengthen the walls so that the NFS can withstand the increasing turgor pressure. The extensive cell wall architectural modifications, namely loosening, dissolution, growth, and thickening, appear to be mediated by a tightly regulated cohort of CWMPs.

Detailed molecular studies to characterize host gene expression changes specifically occurring within syncytia and giant-cells have implicated an important role for several different classes of plant CWMPs in the observed cell-wall architectural modifications (Table 2). The first molecular study to characterize cell wall changes induced in nematode feeding cells was conducted on a tobacco extensin (EXT) (Niebel et al. 1993). Extensins are hydroxyproline-rich glycoproteins (HRGPs) and the most abundant structural protein family of the plant cell wall. Expression of a tobacco extensin was shown to increase in roots infected with root-knot nematodes but not cyst nematodes. The extensin promoter was active in the center of initiating galls as early as 2 dai and peaked at 7 dai. After one week, expression was restricted to the cortex, endodermal and pericycle layers that divide to form the gall. It remains to be shown whether the expression of extensin during the early stages of the root-knot nematode host interaction is important for giant-cell formation or whether this is a reflection of a general defense response elicited by the plant in an

Table 2 Nematode-responsive plant genes encoding cell wall modifying proteins

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
Endo-β-1,4-glucanases (EGases)					
NtCEL2	Endo-β-1,4-glucanase	AF362948	Up-regulated 7-9 dai IR[a,e]	Up-regulated 7-14 dai NFS[e]	Goellner et al. (2001)
NtCEL4	Endo-β-1,4-glucanase	AF362950	Up-regulated 7-9 dai IR[e]	Up-regulated 7-9 dai IR[e]	Goellner et al. (2001)
NtCEL5	Endo-β-1,4-glucanase	AF362951	Up-regulated 7-9 dai IR[e]	Up-regulated 7-9 dai IR[e]	Goellner et al. (2001)
NtCEL7	Endo-β-1,4-glucanase	AF362947; DQ156498 promoter	Up-regulated 7 dai NFS[a,b,e]	Up-regulated 7-14 dai NFS[a,b,e]	Goellner et al. (2001), Wang et al. (2007)
NtCEL8	Endo-β-1,4-glucanase	AF362949	Up-regulated 7 dai NFS[a,b,e]	Up-regulated 7-14 dai NFS[a,e]	Goellner et al. (2001)
AtCEL1	Endo-β-1,4-glucanase	At1g70710; X98543 promoter	ND NFS[b,d]	Up-regulated 3-13 dai NFS[b]	Mitchum et al. (2004), Wieczorek et al. (2008)
AtCEL2	Endo-β-1,4-glucanase	At1g02800	Up-regulated 5-7 dai NFS[d] and 10 dai NFS[a]	N.I.	Wieczorek et al. (2008)
AtCEL3	Endo-β-1,4-glucanase	At1g71380	Up-regulated 5-7 dai NFS[d], up-regulated 5-15 dai IR[e]	N.I.	Wieczorek et al. (2008)
AtCEL5	Endo-β-1,4-glucanase	At1g22880	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
KOR	Endo-β-1,4-glucanase	At5g49720	Up-regulated 5-7 dai NFS[d]	N.I.	Wieczorek et al. (2008)
KOR2	Endo-β-1,4-glucanase	At1g65610	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
KOR3	Endo-β-1,4-glucanase	At4g24260	Up-regulated 5-7 dai NFS[d] and 10 dai NFS[a]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At1g19940	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At1g23210	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At1g48930	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
AtCEL	Endo-β-1,4-glucanase	At1g64390	Up-regulated 5-7 dai NFS[d], up-regulated 5-15 dai IR[e]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At1g75680	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At2g32990	Up-regulated 5-7 dai NFS[d], up-regulated 5-15 dai IR[e]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At2g44540	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At2g44550	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At2g45560	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At2g44570	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At3g43860	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g02290	Up-regulated 5-7 dai NFS[d], up-regulated 5-15 dai IR[e]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g09740	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g11050	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g38990	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β–1,4-glucanase	At4g39000	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g39010	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
AtCEL	Endo-β-1,4-glucanase	At4g23560	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. (2008)
SlCel1	Endo-β-1,4-glucanase	U13054	ND 7 dai IR[e]	N.I.	Karczmarek et al. (2008)
SlCel2	Endo-β-1,4-glucanase	U13055	ND 7 dai IR[e]	N.I.	Karczmarek et al. (2008)
SlCel3	Endo-β-1,4-glucanase	U78526	ND 7 dai IR[e]	N.I.	Karczmarek et al. (2008)
SlCel4	Endo-β-1,4-glucanase	U20590	ND 7 dai IR[e]	N.I.	Karczmarek et al. (2008)

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
SlCel5	Endo-β-1,4-glucanase	AF077339	ND 7 dai IR[e]	N.I.	Karczmarek et al. (2008)
SlCel7	Endo-β-1,4-glucanase	Y11268	Up-regulated 7 dai IR[e], up-regulated 2-10 dai NFS[a,c]	N.I.	Karczmarek et al. (2008)
SlCel9C1	Endo-β-1,4-glucanase	AF098292	Up-regulated 7 dai IR[e], up-regulated 5-10 dai NFS[a,c]	N.I.	Karczmarek et al. (2008)
Extensins (EXT)					
NpEXT	Extensin	M34371	Up-regulated during migration[a,b]	Up-regulated 2-7 dai NFS; 7+ dai galls[a,b,c]	Niebel et al. (1993)
GmEXTL	Extensin-like	BU577532	Up-regulated 2-10 dai IR[h]	N.I.	Ithal et al. (2007a)
GmEXTL	Extensin-like	BI971744	Up-regulated 2-10 dai IR; up-regulated 2dai NFS[g,h]	N.I.	Ithal et al. (2007a)
GmEXTL	Extensin-like	CK606454	Up-regulated 2-10 dai IR[h]	N.I.	Ithal et al. (2007a)
GmEXTL	Extensin-like	CF807746	Up-regulated 2-10 dai IR; up-regulated 2dai NFS[g,h]	N.I.	Ithal et al. (2007a)
GmEXT	Extensin	BM139486	Up-regulated 2 dai IR[h]	N.I.	Khan et al. (2004)
GmEXTL	Extensin-like	BU577532	Up-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	AW348859	Up-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	AW307368	Up-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	BQ454193	Up-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXT	Extensin	AW757140	Down-regulated 2-10 dai IR[h]	N.I.	Ithal et al. (2007a)
GmEXTL	Extensin-like	AW185750	Down-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	BQ453262	Down-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	BI497973	Down-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
GmEXTL	Extensin-like	CF807748	Down-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
GmEXTL	Extensin-like	BI974583	Down-regulated 2 dai NFS[g]	N.I.	Ithal et al. (2007b)
Expansins (EXP)					
LeEXPA5	Expansin	AF059489	N.I.	ND 4 or 10 dai NFS[a,h]	Gal et al. 2005
AtEXPA1	Expansin	At1g69530	upregulated 5-7 dai NFS[b,d]	upregulated 7-14dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA2	Expansin	At5g05290	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA3	Expansin	At2g37640	upregulated 5-7 dai NFS[a,b,d]	N.I.	Wieczorek et al. 2006
AtEXPA4	Expansin	At2g39700	upregulated 5-7 dai NFS[b,d]	N.I.	Wieczorek et al. 2006
AtEXPA5	Expansin	At3g29030	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA6	Expansin	At2g28950	upregulated 3-15 dai NFS[a,b,d]	upregulated 14-21 dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA7	Expansin	At1g12560	ND NFS 5-7 dai[d]	upregulated 14-21 dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA8	Expansin	At2g40610	upregulated 5-7 dai NFS[a,d]	N.I.	Wieczorek et al. 2006
AtEXPA9	Expansin	At5g02260	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA10	Expansin	At1g26770	upregulated 5-7 dai NFS[a,b,d]	upregulated 14-21 dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA11	Expansin	At1g20190	ND NFS 5-7 dai[d]	upregulated 14-21 dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA12	Expansin	At3g15370	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA13	Expansin	At3g03220	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA14	Expansin	At5g56320	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA15	Expansin	At2g03090	upregulated 5-7 dai NFS[b,d]	upregulated 7-14dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
AtEXPA16	Expansin	At3g55500	upregulated 5-7 dai NFS[a,b,d]	upregulated 14-21 dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
AtEXPA17	Expansin	At4g01630	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA18	Expansin	At1g62980	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA19	Expansin	At3g29365	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA20	Expansin	At4g38210	upregulated 5-7 dai NFS[d]	N.I.	Wieczorek et al. 2006
AtEXPA21	Expansin	At5g39260	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA22	Expansin	At5g39270	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA23	Expansin	At5g39280	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA24	Expansin	Atg39310	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA25	Expansin	At5g39300	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPA26	Expansin	At5g39290	ND NFS 5-7 dai[d]	N.I.	Wieczorek et al. 2006
AtEXPB1	Expansin	At2g20750	ND NFS 5-7 dai[d]; down-regulated 3 dai IR[h]	upregulated 7-14dai galls[h]	Puthoff et al. 2003; Jammes et al. 2005; Wieczorek et al. 2006
AtEXPB2	Expansin	At1g65680	ND NFS 5-7 dai[d]; upregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003; Wieczorek et al. 2006
AtEXPB3	Expansin	At4g28250	upregulated 5-7 dai NFS[d]	upregulated 7-14dai galls[h]	Jammes et al. 2005; Wieczorek et al. 2006
GmEXPA3	Expansin	BM091956	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmEXPA4	Expansin	AF516880, CF805822	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmEXPA5	Expansin	AW509184	upregulated 2-10 dai IR[h]; upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007a,b
GmEXPA6	Expansin	CA785167	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmEXPA8	Expansin	AI759701, CF805734	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
GmEXPA10	Expansin	CD394837	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmEXPA15	Expansin	CD417217	upregulated 2-10 dai IR[h]; upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007a,b
GmEXPL	Expansin-related	BQ742395	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
Xyloglucan Endotransglycosylase (XET)					
AtXET	Xyloglucan endotransglycosylase	At4g14130	downregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003
AtXET	Xyloglucan endotransglycosylase	At4g30290	N.I.	downregulated 7-21 dai galls[h]	Jammes et al. 2005
AtXET	Xyloglucan endotransglycosylase	At3g48580	N.I.	upregulated 7 dai galls[h]; downregulated 21 dai galls[h]	Jammes et al. 2005
GmXET	Xyloglucan endotransglycosylase	AW310549	downregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmXET	Xyloglucan endotransglycosylase	BM568229	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmXET	Xyloglucan endotransglycosylase	BQ298739	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmXET	Xyloglucan endotransglycosylase	AW707175	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmXET	Xyloglucan endotransglycosylase	CD414740	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmXET	Xyloglucan endotransglycosylase	BG363116	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmXET	Xyloglucan endotransglycosylase	CK605938	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
Polygalacturonase (PG)					
GmPG1	Polygalacturonase	AF128266	upregulated 1-3 dai IR[i,j]	N.I.	Malalingam et al. 1999
GmPG2	Polygalacturonase	AF128267	upregulated 1-3 dai IR[i,j]	N.I.	Malalingam et al. 1999
AtPG	Polygalacturonase	At2g41850	upregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
AtPG	Polygalacturonase	At1g05660	downregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003
Pectinacetylesterase (PAE)					
AtPAE	Pectinacetylesterase	AY050847	upregulated 3 dai NFS; ND NFS 7 dai[a,c]	upregulated 1-5 dai NFS[a,c]	Vercauteren et al. 2002
AtPE	Pectinesterase	At2g45220	downregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003
AtPE	Pectinesterase	At1g53830	downregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003
GmPE	Pectinesterase	BE822184	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmPE	Pectinesterase	BI967224	upregulated 2-5 dai IR[h]	N.I.	Ithal et al. 2007a
GmPE	Pectinesterase	BF066411	upregulated 2-5 dai IR[h]	N.I.	Ithal et al. 2007a
GmPE	Pectinesterase	BI970277	downregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmPE	Pectinesterase	BE821923	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmPE	Pectinesterase	AW309342	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmPE	Pectinesterase	BE475550	downregulated 2dai NFS[g]	N.I.	Ithal et al. 2007b
Pectate Lyase (PEL)					
AtPEL	Pectate lyase	At1g67750	N.I.	upregulated 21 dai galls[h]	Jammes et al. 2005
AtPEL	Pectate lyase	At4g24780	N.I.	upregulated 7-14 dai galls[h]	Jammes et al. 2005
AtPEL	Pectate lyase	At1g04680	N.I.	upregulated 14-21 dai galls[h]	Jammes et al. 2005
AtPEL	Pectate lyase	At3g27400	N.I.	upregulated 14-21 dai galls[h]	Jammes et al. 2005
AtPEL	Pectate lyase	At3g09540	N.I.	upregulated 14-21 dai galls[h]	Jammes et al. 2005
Glycosyl Hydrolase					
AtGH	Glycosyl hydrolase family 9 protein	At1g71380	upregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003
AtGH	Glycosyl hydrolase family 1 protein	At3g60140	upregulated 3 dai IR[h]	N.I.	Puthoff et al. 2003

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
AtGH	Glycosyl hydrolase family 3 protein	At5g49360	N.I.	downregulated 7-21 dai galls[h]	Jammes et al. 2005
AtGH	Glycosyl hydrolase family 28 protein	At3g15720	N.I.	upregulated 7-21 dai galls[h]	Jammes et al. 2005
GmGH	Glycosyl hydrolase family 28 protein	BQ612383	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmGH	Glycosyl hydrolase family 1 protein	BQ080473	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmGH	Glycosyl hydrolase family 1 protein	BG507836	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmGH	Glycosyl hydrolase family 17 protein	CA819291	upregulated 2-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmGH	Glycosyl hydrolase family 1 protein	BU926932	upregulated 5-10 dai IR[h]	N.I.	Ithal et al. 2007a
GmGH	Glycosyl hydrolase family 8 protein	CD407063	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 8 protein	AI416659	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 8 protein	BE658250	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 3 protein	BI971040	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 17 protein	BI969847	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b

(continued)

Table 1.2 (continued)

Gene name	Putative function	AGI or accession #	Cyst	RKN	References
GmGH	Glycosyl hydrolase family 17 protein	CD396678	upregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 1 protein	AW309536	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 1 protein	BI785739	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 31 protein	CA799910	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 3 protein	AW705239	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 1 protein	BU551231	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 1 protein	BQ612438	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 3 protein	BU549884	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b
GmGH	Glycosyl hydrolase family 20 protein	CD416483	downregulated 2 dai NFS[g]	N.I.	Ithal et al. 2007b

Abbreviations: At = Arabidopsis thaliana; dai = days after infection; Gm = Glycine max; IR = infected roots; ND = not detected; NFhS = nematode feeding site; N.I. = no information; Np = Nicotiana plumbaginifolia; Nt = Nicotiana tabacum; Sl= Solanum lycopersicum

[a] *in situ* hybridization
[b] promoter-GUS
[c] antibody
[d] feeding cell specific cDNA library
[e] RT-PCR infected root segments
[f] RT-PCR LCM feeding cells
[g] microarray LCM feeding cells
[h] microarray; infected root segments
[i] differential display; infected root segments
[j] Northern blot; infected root segments

attempt to ward off an invading pathogen by strengthening the cell walls. It has been suggested that extensin expression in the gall pericycle may be contributing to a need for new structural cell wall components in dividing cells. Furthermore, expression in the gall cortex may be required to increase the rigidity of the walls in response to mechanical pressure created by the swelling of the central cylinder (Niebel et al. 1993). More recent microarray studies have shown that extensins are also up-regulated during cyst-nematode plant interactions (Ithal et al. 2007b; Khan et al. 2004; Puthoff et al. 2003). Similarly, up-regulation of peroxidases, enzymes that play an important role in strengthening of the cell wall through rapid cross-linking of cellulose, HRGPs, and lignin, has been observed. Increased expression of structural cell wall proteins and peroxidases in nematode-infected roots (Puthoff et al. 2003; Ithal et al. 2007b) may be one mechanism used to maintain a balance between cell wall strengthening and loosening during feeding cell formation.

In contrast to cell wall strengthening, cell wall loosening for expansion, elongation, and dissolution requires the activity of different classes of hydrolytic enzymes, and several have been implicated in NFS formation (Goellner et al. 2001; Mitchum et al. 2004; Vercauteren et al. 2002). Beta-1,4-endoglucanase (EGases) are hydrolytic enzymes that cleave beta-1,4-glucosidic linkages, and there is considerable evidence supporting a role for these enzymes in a wide range of plant developmental processes involving changes in cell wall architecture. EGase genes are up-regulated in both root-knot and cyst nematode infected root tissues compared to uninfected roots, and their expression has been localized within developing giant-cells and syncytia (Goellner et al. 2001; Karczmarek et al. 2008; Wieczorek et al. 2008). Five tobacco EGases and two of at least eight tomato EGases were found to be up-regulated by the tobacco cyst nematode and potato cyst nematode, respectively, in nematode-infected roots (Goellner et al. 2001; Karczmarek et al. 2008). Seven of the 25 members of the Arabidopsis EGase gene family are up-regulated in syncytia induced by the beet cyst nematode. Two of these family members, *AtCEL2* and *AtKOR3*, are exclusively expressed in shoot tissues during normal plant growth and development but are strongly up-regulated in developing syncytia. A reduction in female development on *cel2* and *kor3* mutants indicates that these two EGases play an important role in nematode feeding site formation. Similarly, growth and development of potato cyst nematodes was severely hampered on potato (*Solanum tuberosum*) plants silenced for *StCEL7* or *StCEL9C1* (Karczmarek et al. 2008). An Arabidopsis pectin acetylesterase (*PAE*) gene was also shown to be up-regulated in initiating giant-cells, syncytia, and cells surrounding the nematode (Vercauteren et al. 2002). The level of *PAE* declined in giant-cells at later time-points but was still detectable in parenchyma, endodermis, and pericycle cells of the root gall. PAEs catalyze the deacetylation of esterified pectin thereby increasing the accessibility of pectin to degrading enzymes. The expression of the same EGase and PAE genes in both types of feeding cells suggests that similar mechanisms for cell growth involving loosening of the primary cell wall and middle lamella contributes to their formation. On the other hand, distinct expression of individual gene family members in either giant-cells or syncytia may contribute to observed differences in the extent of cell wall dissolution for each cell type. This is true for the promoter

of the Arabidopsis elongation-specific EGase gene, *AtCel1*, which was shown to be expressed in developing giant-cells but not syncytia (Mitchum et al. 2004; Wieczorek et al. 2008), suggesting that this EGase plays a unique role in giant-cell formation.

More recently, the differential expression of plant expansins has been observed in cyst and root-knot nematode infected roots (Gal et al. 2005; Jammes et al. 2005; Puthoff et al. 2003; Wieczorek et al. 2006). Expansins are cell-wall loosening proteins that function by disrupting noncovalent bonds between cellulose and hemicellulose polymers to assist in the elongation and expansion of cell walls. In galls induced by root-knot nematodes on tomato roots, *LeEXPA5* transcript was detected in gall cells surrounding the giant-cells, but not within giant-cells at either 4 or 10 dai (Gal et al. 2005). The use of an antisense construct to disrupt expansin expression in tomato roots resulted in reduced galling upon infection by root-knot nematodes suggesting that expansins play an important role in gall formation. Similarly, an Arabidopsis gene expression profiling experiment comparing gall tissue to uninfected root tissues detected up-regulation of seven *AtEXPA* genes, two *AtEXPB* genes, and two expansin-like (*AtEXL*) genes in developing galls (Jammes et al. 2005). It remains to be shown whether these expansins are specifically expressed within developing giant-cells to contribute to their formation. On the contrary, Wieczorek et al. (2006) have characterized the expression of the Arabidopsis α- and β-expansin gene families in cyst nematode-infected roots and demonstrated the expression of several different expansins within developing syncytia. Of the 26 member Arabidopsis α-expansin gene family, nine members were up-regulated and two members were down-regulated within developing syncytia. Of the three β-expansin members tested, only *AtEXPB3* was shown to be up-regulated in the syncytium. Interestingly, two of the α-expansins (*AtEXPA3* and *AtEXPA16*) up-regulated in the syncytium were found to be shoot-specific. Similarly, laser-capture microdissection coupled with microarray profiling has also identified the specific up-regulation of seven soybean α-expansins (*GmEXPA*) in syncytia induced by soybean-cyst nematode (Ithal et al. 2007b).

More recent microarray analyses in Arabidopsis and soybean (Jammes et al. 2005; Puthoff et al. 2003; Ithal et al. 2007a,b) have identified the differential regulation of several additional classes of CWMPs, including xyloglucan endotransglycosylases (XETs), β-xylosidases, reversibly glycosylated polypeptides (RGPs), and pectate lyases (see Li et al. 2008). From these studies it is evident that feeding cell formation is a complex process that likely requires the synergistic activity and coordinated regulation of several different types of plant CWMPs, as well as the differential expression of individual gene family members. Moreover, the processes that give rise to feeding cells involve recruitment of plant genes that are not necessarily expressed in roots during their growth and development. The regulation of genes encoding CWMPs within developing feeding cells also appears to be conserved among diverse plant species which is consistent with the idea that nematodes interfere with fundamental aspects of plant development. Conserved activity of tobacco EGase promoters in nematode feeding sites induced in Arabidopsis, tomato, and soybean supports this idea (Mitchum et al. 2004; Wang et al. 2007). Phytohormones function as key regulators

of plant hydrolytic enzymes and the altered phytohormone balance within developing feeding cells (see Sect. 9) may trigger a developmental cascade leading to the controlled regulation of genes encoding CWMPs. Future exploitation of genomic information for the detailed characterization of entire gene families will aid in determining subsets of gene family members that are either specifically expressed or suppressed within feeding cells. This information can be compared to gene expression in other tissues and developmental processes to provide important insight into their formation. In addition, gene knock-out approaches will allow the role of individual family members in feeding cell formation to be assessed.

6 Transport Routes of Nutrients and Water into NFS

Sedentary nematodes continuously withdraw nutrients from metabolically active feeding sites as they develop. Therefore, these NFS represent major sinks for metabolites such as sugars and amino acids imported from the phloem. This occurs symplastically through plasmodesmata or apoplastically via transmembrane transporter proteins. Plasmodesmata connecting the syncytium with neighboring cells were assumed to be nonfunctional because of cell wall deposits, and this was supported by the fact that fluorescent dyes injected into syncytia were unable to move into neighboring cells (Bockenhoff and Grundler 1994; Bockenhoff et al. 1996). These data led to the widely accepted concept that transport from the phloem into *H. schachtii*-induced syncytia is apoplastic, involving transmembrane transporters. However, Hoth et al. (2005) challenged this concept by demonstrating the trafficking of GFP from unloading phloem into syncytia. Recent grafting experiments, using transgenic *A. thaliana* scions expressing GFP in the phloem along with wild-type rootstocks, unequivocally confirmed the movement of GFP from the phloem into syncytia and corroborated the existence of a symplastic route (Hofmann and Grundler 2006; Hofmann et al. 2007). In tomato, nematode-induced galls are also connected to the phloem by plasmodesmata (Dorhout et al. 1993).

Nevertheless, several studies have shown up-regulation of transporter proteins in NFS which would indicate the importance of an apoplastic route. *A. thaliana* genes encoding sucrose transporters were expressed higher in NFS than in uninfected roots [shown for *AtSUC2* and *AtSUC4* in syncytia (Hofmann et al. 2007) and for *AtSUC1* in giant-cells (Hammes et al. 2005)]. The role of these transporters was validated via competitive inhibition using maltose in the plant growth medium which inhibits SUC activity. Nematode development was significantly reduced in comparison to sucrose or glucose although root growth was not affected (Hofmann et al. 2007). A comprehensive microarray analysis was performed on the expression of 634 transport proteins represented in the Arabidopsis Membrane Protein Library; 30 were found to be up-regulated and 20 down-regulated at several time-points after infection with *Meloidogyne* (Hammes et al. 2005). Expression of different members of a gene family is often divergently affected. Functional and protein localization studies will be needed to determine the potential role of these transporters in plant–nematode interactions.

The current model for solute transport from the phloem into NFS (based on syncytium research) takes into account both apoplastic and symplastic transport. In initial feeding cells, plasmodesmatal connections with surrounding cells close in response to the infecting nematode, except at sites of cell fusion. In the following days, the turgor pressure increases in the syncytium, and increased expression of transporter proteins facilitates nutrient supply from the phloem (at 7 dai 80% of the studied syncytia are still symplastically isolated and thus dependent on transporter-based solute import) (Hofmann et al. 2007). As syncytia approach their final stage of expansion, plasmodesmata reopen (around 12 dai).

Sijmons et al. (1991) estimated that developing juveniles of *H. schachtii* withdraw an amount of solute equivalent to four times the syncytial volume every 24 h (Sijmons et al. 1991). The water necessary for replenishing this comes from the xylem, with the invaginations of cell wall and plasma membrane facilitating this drain. Genes encoding water channel proteins (aquaporins) are highly up-regulated in NFS to support this massive water transport. For example, the *TobRB7* gene from tobacco is induced in giant-cells from 4 dai until 35 dai (Opperman et al. 1994), and the corresponding gene in tomato was also shown to be highly up-regulated in giant-cells (He et al. 2005). *TobRB7* was not up-regulated in tobacco syncytia, but the soybean aquaporin *GmPIP2.2* showed a sevenfold enhanced expression in soybean syncytia formed by *H. glycines* (Klink et al. 2005). This apparent contradiction can again be explained by dissimilar expression patterns of different genes from the same family as clearly illustrated by two *A. thaliana* aquaporin genes. The expression of *AtPIP2.5* was induced 17-fold in galls compared to control root tissue while *AtPIP2.6* was expressed at similar levels in both tissues (Hammes et al. 2005).

7 Increased Metabolic Activity of NFS

Metabolic reprogramming is a key feature of nematode feeding cells. Consistent with their role as nutrient sinks, elevated sugar levels and increased rates of metabolism through the glycolytic and pentose phosphate pathways have been observed in both syncytia and giant-cells. Early physiological studies detected high levels of glucose-6-phosphate and 6-phosphogluconate dehydrogenase (G6PDH) activity in histochemical preparations of nematode feeding cells (Endo and Veech 1968), hinting at an increased flux through these pathways and the simultaneous production of a wide array of intermediates for use in other metabolic processes. Glucose-6-phosphate serves as a precursor for glycolysis and the pentose phosphate pathway (PPP). In glycolysis, glucose is oxidized to pyruvic acid, a process that generates energy in the form of ATP, NADH, and three-carbon and six-carbon intermediates for other biosynthetic pathways. G6PDH is the first step in the oxidative pentose phosphate pathway catalyzing the conversion of glucose-6-phosphate to 6-phosphogluconate. More recent molecular studies are beginning to confirm some of these earlier observations. For example, an Arabidopsis phosphoglycerate mutase/biphosphoglycerate mutase (*PGM/bPGM*) gene was up-regulated early in both giant-cells and syncytia, and its expression

increased during feeding cell development (Mazarei et al. 2003). PGM catalyzes the reversible interconversion of 3-phosphoglycerate and 2-phosphoglycerate, a key step in sugar metabolism during glycolysis. In addition, microarray analysis of laser-captured syncytia induced in soybean identified the up-regulation of genes encoding enzymes at each step of the glycolytic and PP pathways (Ithal et al. 2007b). The PPP plays several roles in plant metabolism. Cells utilize the PPP to maintain a pool of reducing molecules in the form of NADPH to protect against oxidative stress. During the nonoxidative phase of the PPP, carbohydrate intermediates are produced. Carbohydrate intermediates such as ribose-5-phosphate serve as the building blocks of ribose and deoxyribose for the synthesis of nucleic acids. Consistent with this, the promoter of a soybean phosphoribosylformyl-glycinamidine (FGAM) synthase, an enzyme of the de novo purine biosynthetic pathway, was shown to be activated during syncytia formation (Vaghchhipawala et al. 2004). Erythrose-4-phosphate, another product of the PPP, serves as a precursor to the shikimate pathway for the production of plant phenolic compounds including aromatic amino acids for protein and IAA synthesis, flavonoids, alkaloids, lignin, and other secondary metabolites. An essential role for the pentose phosphate pathway in feeding cell maintenance is supported by a study of the Arabidopsis *RPE* gene. *RPE* encodes ribulose-phosphate 3-epimerase (*RPE*), an enzyme that catalyzes the reversible conversion of ribulose-5-phosphate and xylulose-5-phosphate. *RPE* is up-regulated in giant-cells and during the late stages of syncytium development, suggesting increased flux through the PPP (Favery et al. 1998). Mutations in *RPE* result in a lethal phenotype due to the inability of the plant to maintain sugar phosphate levels; however, partial rescue can be established with exogenous sucrose to produce dwarf plants with light green leaves and reduced root systems that eventually die. Upon root-knot infection, rescued *rpe* dwarfs do not support gall development, highlighting the importance of the PPP in this process. In contrast, cyst nematode development was not impaired on *rpe* mutants (Favery et al. 1998). Currently, there is not enough data to explain the observed differences in the requirement of the PPP pathway for giant-cell but not syncytia formation.

8 Signal Transduction Pathways and Transcription Factors

Transcription factors and protein kinases involved in signal transduction pathways are crucial in cell differentiation processes. Those found to be up- or down-regulated in NFS, therefore, merit particular attention. For example, Puthoff et al. (2003) found genes encoding serine-threonine kinases, a calmodulin-related protein and a calcium-dependent protein kinase to be down-regulated in cyst nematode-infected roots of *Arabidopsis thaliana*. More recent microarray analyses have added several genes potentially important in NFS signaling (Jammes et al. 2005; Khan et al. 2004; Ithal et al. 2007b). Proteins encoded by gene families are often involved in complex pathways; therefore, their specific

roles in NFS formation are difficult to grasp without detailed functional studies. Some transcription factors, however, have been analyzed more thoroughly. For example, the Mt-KNOX transcription factor is highly expressed in giant-cells while its role in uninfected plants is to maintain meristem function and develop lateral organs (Koltai et al. 2001). Disruption of auxin transport was found to mimic KNOX over-expression, and a strict correlation between KNOX expression and elevated cytokinin levels has been observed. The importance of auxin and cytokinin in NFS development is discussed below. KNOX expression is in turn regulated by the transcription factor PHAN, which is also highly expressed in giant-cells (Koltai et al. 2001). Another transcription factor highly expressed in both early giant-cells and syncytia is *AtWRKY23*. This gene is induced by auxin and is important for primary root development and nematode infection (Grunewald et al. 2008; Gheysen, unpublished results). Another transcription factor, AtABI3, is up-regulated in early syncytia but not giant-cells (De Meutter et al., 2005). ABI3 was originally identified as a central signaling factor in desiccation during embryogenesis, but further analyses have indicated an important role for this protein in cell differentiation and meristem quiescence (Rohde et al. 2000). An EREBP-domain protein (EREBP; ethylene-responsive element-binding protein) is a transcription factor down-regulated in the compatible soybean–*H. glycines* interaction but up-regulated in the incompatible interaction (Mazarei et al. 2002). Overexpression of this transcription factor results in the up-regulation of several PR-proteins suggesting that its down-regulation in the compatible interaction may interfere with the plant defense response to facilitate successful nematode infection (Mazarei et al. 2007).

The only plant receptor kinases in the plant–nematode interaction that have been studied in some detail are part of a remarkable common signal transduction pathway for root-knot nematodes and rhizobial Nod factors in *Lotus japonicus* (Weerasinghe et al. 2005). Root-knot nematodes invoke root-hair waviness and branching in Lotus root hairs similar to the effect of Nod factors. Furthermore, the ability of root-knot nematodes to establish feeding sites and reproduce was notably reduced in mutant lines defective in the Nod factor receptor genes *NFR1, NFR5*, and *SYMRK* (Table 1) (Weerasinghe et al. 2005).

9 Developmental Reprogramming for NFS Formation

Comparisons with better understood systems of plant cell differentiation and organ formation may be helpful for our understanding of NFS development. It was postulated by Nutman (1948) that microbes can tap into plant developmental pathways and, more specifically, that nodulation was related to lateral root formation. In addition, root-knot nematodes and endosymbiotic rhizobia induce similar structures within plant roots (galls and nodules, respectively) and this has inspired studies comparing gene expression between these two processes (reviewed in Davis and

Mitchum 2005). The genes encoding the transcription factors PHAN and KNOX, the early nodulin gene *ENOD40*, and the cell cycle gene *CCS52* are highly expressed in nodules, giant-cells, and lateral root initials (Koltai et al. 2001). Similarly, *MtENOD11*, encoding a cell wall proline-rich protein, is up-regulated by *Rhizobium* and *Meloidogyne* infection (Boisson-Dernier et al. 2005). However, a larger-scale comparison (Favery et al. 2002) revealed that similarities in gene expression between nodules and galls may be limited. Only 2 of 192 genes expressed in nodules in *Medicago truncatula* were up-regulated upon root-knot nematode infection in their experimental set-up.

The correlation of genes expressed in NFS and initiation sites of lateral roots is striking. In a tagging experiment where 103 lines were first selected for their expression in NFS, 38% of these lines also displayed expression at the base of developing lateral roots (Barthels et al. 1997). Among those studied in more detail were Att0001 and Att0728 (Barthels et al. 1997) in which the *AtWRKY23* and *TIFY10a* genes were tagged, respectively. This former gene encodes a WRKY transcription factor involved in root development (Grunewald and Gheysen, unpublished results) while the latter is a member of the JAZ protein family. The similarity in the expression pattern of these genes is not only in their early expression in NFS (giant-cells and syncytia) but also their up-regulation upon auxin treatment (Grunewald and Gheysen, unpublished results). Other auxin-regulated genes involved in feeding cell formation include cell cycle genes, expansins, and cell-wall hydrolyzing enzymes.

Auxin has long been suspected to play a central role in NFS formation. Auxin also seems to be a common factor in root developmental events such as lateral root initiation, symbiotic nodules, root-knot galls, and syncytia (Mathesius 2003). Many of the cellular changes occurring when nematodes become sedentary can be partially mimicked by application of indole-3-acetic acid (IAA), i.e., endoreduplication (Valente et al. 1998), acytokinetic mitosis (Naylor et al. 1954), cell enlargement (Jones et al. 1998), cell-wall breakdown (Fan and Maclachlan 1967), and lateral root formation (Torrey 1950). Resistant peach rootstocks are susceptible to *M. javanica* following auxin treatment (Kochba and Samish 1971), and the auxin-insensitive tomato mutant *diageotropica* is resistant to *M. incognita* (Richardson and Price 1984). Ever since, evidence for the role of auxin in NFS formation has continuously accumulated. The auxin-responsive promoter *GH3* is rapidly and transiently activated in initiating rhizobial nodules and during root gall initiation by *Meloidogyne* in white clover (Hutangura et al. 1999). Auxin appeared to accumulate basipetal (above) and was reduced acropetal (below) to the forming gall, indicating a block in auxin transport at the infection site. *DR5* is an artificial promoter with auxin-responsive elements derived from the *GH3* promoter and it is often used as a sensitive and specific indicator of the presence of auxin in plant tissues. Strong and local DR5::GUS activation was visible in *A. thaliana* roots infected by *M. incognita* or *H. schachtii* (Karczmarek et al. 2004) at 18 h after infection (the earliest tested time-point). Although the possible secretion of auxin-like compounds by the nematode or increased plant auxin biosynthesis or sensitivity cannot be excluded, the most likely explanation is an accumulation of auxin due to disruption of polar auxin transport. Hutangura et al. (1999) demonstrated a

local accumulation of intracellular flavonoids in initiating root galls of white clover roots infected by *M. javanica*. Several flavonoids are known to be potent auxin transport inhibitors. Disturbance of auxin gradients in the plant medium by addition of N-(1-naphtyl)phtalamic acid (NPA), a polar auxin transport inhibitor, results in abnormal feeding cells, and auxin-insensitive mutants hardly support cyst nematode reproduction (Goverse et al. 2000) (Table 1). These experiments demonstrate that auxin is an essential plant hormone for successful root-knot and cyst nematode infection.

For cytokinins, on the other hand, there is only support for a role in root-knot nematode infection. High cytokinin levels, either endogenous or applied, correlate with plant susceptibility to root-knot nematodes, and biologically active cytokinins have been shown to be produced by *Meloidogyne* spp. (Bird and Loveys 1980; De Meutter et al. 2003; Dropkin et al. 1969; Kochba and Samish 1971; Kochba and Samish 1972). The cytokinin-responsive promoter *ARR5* is up-regulated in young galls induced by *M. incognita*, at the base of lateral root primordia and in rhizobial-induced nodule primordia (Lohar et al. 2004), again illustrating similarities between these processes. Nevertheless, in roots expressing cytokinin oxidase (causing a decrease in cytokinin levels), lateral root formation is greater while the numbers of both nodules and galls are decreased compared to wild-type roots (Lohar et al. 2004).

A third plant hormone which has emerged as a player in plant–cyst nematode interactions is ethylene. *A. thaliana* ethylene-insensitive mutants are less susceptible to *H. schachtii* compared to wild-type plants (Table 1) (Wubben et al. 2001), while ethylene-overproducing *A. thaliana* mutants attract more juveniles and result in larger syncytia and females (Table 1) (Goverse et al. 2000). Enhanced syncytial cell wall dissolution and expansion in the latter mutants suggests that ethylene-induced cell wall degradation is involved in syncytium formation (Goverse et al. 2000). More recent experiments on a different mutant with increased ethylene susceptibility and hypersusceptibility to *H. schachtii* confirms the importance of ethylene for cyst nematode infection (Table 1) (Wubben et al. 2004).

Concerning the hormones that have a distinguished role in plant defense signaling after pathogen or insect attack, namely salicylic acid (SA) and jasmonic acid (JA), conflicting results have apparently been obtained (Bhattarai et al. 2008; Cooper et al. 2005; Wubben et al. 2008). More research is certainly needed to understand the role of these hormones in plant–nematode interactions.

10 Initiation of NFS

The identification of the nematode signal(s) triggering NFS development also promises to provide new opportunities for unraveling the infection process. In contrast to nodulation by Rhizobia, where Nod-factors are the key molecules in nodule initiation, the nematode almost certainly uses a medley of different enzymatic and regulatory molecules for establishing a successful parasitic interaction. At least,

this is what can be deduced from the variety of proteins that are secreted from the esophageal glands into the plant roots (De Meutter et al. 2001; Gao et al. 2003; Huang et al. 2003a; Jaubert et al. 2002). For more details, see Davis et al. (2008); however, we will discuss several "parasitism proteins" to illustrate the range of possibilities for their role in NFS formation.

Cyst nematodes and root-knot nematodes both secrete chorismate mutases (Jones et al. 2003; Lambert et al. 1999). Chorismate mutase converts chorismate to prephenate in the shikimate pathway. Compounds derived from chorismate include auxin and salicylic acid, while a variety of secondary metabolites are derived from prephenate, including flavonoids which can act as auxin transport inhibitors. It is not known whether this nematode-secreted enzyme interferes with auxin biosynthesis or transport, plant defense, or other metabolic processes of host cells.

Nematode-secreted peptides increasingly demand attention as possible actors in NFS establishment. A secreted peptide from *H. glycines* containing a conserved CLAVATA3/ESR (CLE) motif characteristic of plant CLE peptides (Olsen and Skriver 2003) was shown to partially rescue the *A. thaliana clv3-1* mutant phenotype, and its expression in wild-type plants caused a wuschel-like phenotype typical for CLV3 overexpression (Wang et al. 2005). These results suggest that cyst nematode-secreted CLE peptides may function as ligand mimics of host plant CLEs for the redifferentiation of root cells for syncytium formation (reviewed in Mitchum et al. 2008). A secreted peptide from *Meloidogyne incognita* that is essential for parasitism (Huang et al. 2006b) may also interfere with root cell differentiation for giant-cell formation because it has been shown to interact with the SAW domain of SCL6 and SCL21, two Scarecrow-Like (SCL) transcription factors (Huang et al. 2006a) which belong to the GRAS protein family (Bolle 2004). Although the functions of SCL6 and SCL21 are unknown, several other members of the GRAS protein family have been shown to play important roles in plant development and signaling (Bolle 2004).

The ubiquitination pathway plays a prominent role in many plant regulatory pathways: as part of hormone signaling, for cell cycle regulation, during rhizobial nodulation, and pathogen infection (Zeng et al. 2006). There is mounting evidence that cyst nematodes exploit the host ubiquitination pathway in order to manipulate the plant response. Several cyst nematodes secrete an ubiquitin-extension protein from their dorsal gland cells (Tytgat et al. 2004). Other proteins similar to those involved in ubiquitination pathways are also expressed in their dorsal gland including S-phase kinase associated proteins (Skp-1) and RING-H2 proteins, which are subunits of the complex that transfers ubiquitin tags to target proteins (Gao et al. 2003). Although understanding the role of the ubiquitination pathway in syncytium development still requires more research, it is intriguing to note that changes in ubiquitination pathways can affect cell cycle regulation in some organisms and give rise to cells that go through multiple S phases with no intervening mitosis (Lammer et al. 1998). This results in hypertrophic nuclei similar to those found in syncytia.

11 Conclusions

The mechanisms used by phytonematodes to redifferentiate plant cells into morphologically distinct cell types with ontogenies unique to each plant–nematode combination have long fascinated researchers. Although our knowledge of the complex molecular mechanisms underlying feeding site formation still remains fragmentary, substantial progress to characterize gene expression in these cells over the last decade has established the framework necessary to begin to unravel the genetic networks directly involved in their formation. Despite the fact that significant progress has been made in cataloguing plant and nematode genes involved in the compatible interaction, the ultimate question of how sedentary nematodes trigger feeding site development remains a puzzle with many missing pieces. Thus, an exciting and challenging road lies ahead and promises to unveil new molecular insights into our understanding of nematode feeding sites.

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