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MANIPULATION OF HOST GENE EXPRESSION BY ROOT-KNOT NEMATODES

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ABSTRACT: Root-knot nematodes (*Meloidogyne* spp.) establish elaborate feeding sites in their host. Unique patterns of gene expression are induced in root cells, resulting in formation of a novel cell type called a giant cell. Based on analysis of approximately 220 giant cell expressed genes, key elements of giant cell function and regulation have been identified; examples are discussed in the context of giant cell biology and ontogeny. The potential to effect nematode control by manipulating these genes in transgenic host plants is considered, and models for giant cell induction are presented.

Nematodes are a ubiquitous life form. Numerically they account for approximately 80% of all animals (Platt, 1994). Most species are, like *Caenorhabditis elegans*, free-living microbivores, although a significant number have adopted a parasitic lifestyle. Those of medical and veterinary importance are well known to parasitologists, and many are the subject of extensive research. However, although it is likely that all vascular plants serve as hosts for at least one parasitic nematode species, most plant-parasitic forms remain poorly understood. This is not because they don't cause damage to world agriculture. Based on an international survey (Sasser and Freckman, 1987) it has been calculated that plant-parasitic nematodes reduce the yield of the world's forty major food staples and cash crops by an average of 12.3%, with the losses being substantially higher for some commodities (e.g., 20.6% for tomato). Rather, the barriers to research have been the technical challenges familiar to parasitologists. Most plant-parasitic nematodes are microscopic, obligate root-parasites. Many are endo-parasites, with complex host associations and, in many instances exhibit obligate, or facultative parthenogenetic or hermaphroditic reproduction. Additionally, the availability for many years of effective agricultural nematicides served to reduce the practical necessity to study the basic biology of plant-parasitic nematodes. However, increasing restrictions to the use of various nematicides for environmental and/or health reasons (Thomason, 1987), particularly the imminent suspension of the widely used soil fumigant methyl bromide (in 2000), has introduced a sense of urgency to the search for alternative control measures.

The most environmentally benign and also most cost-effective control approach is the deployment of nematode-resistant cultivars (Trudgill, 1991). Introduction of the soybean cyst nematode (*Heterodera glycines*)-resistant cultivar "Forrest" for example, has reportedly saved soybean growers in the southern US over $400 million during a 5 year period (Bradley and Duffy, 1982). However, in contrast to their response to other pathogens, most plants are probably not resistant to most nematodes (Trudgill, 1991). Root-knot nematodes (*Meloidogyne* spp.) for example have a host range in excess of 2,000 plant species (Sasser, 1980). Consequently, nematode resistance is available for only a few crops. Furthermore, many of the currently available resistance (*R*) genes are highly specific for particular nematode biotypes. For example, tomato plants carrying the *Mi* gene may be resistant to *M. arenaria*, *M. incognita*, and *M. javanica*, but not to *M. hapla* (Gilbert and McGuire, 1956). Some *R* genes, including Mi which fails when the temperature is at or above 28°C (Gilbert and McGuire, 1956), have conditional phenotypes. The utility of *R* genes is further compromised by field selection of nematode populations able to reproduce on resistant germplasm.

An alternative to the use of natural *R* genes is the rational design of resistance based on a thorough understanding of the biology of the parasitic interaction, in particular, the nature of the host response. Although regeneration of many transformed crop plants remains a technical challenge (Walden and Wingender, 1995), the ability to construct transgenic hosts is an avenue more readily available to plant parasitologists than to animal parasitologists. One rational-resistance approach, based on transgenic expression of a specifically nematode-responsive, plant gene promoter (Opperman et al., 1994) driving either a cell-autonomous toxin gene or a gene disruption cassette, already has been validated by several years of greenhouse and field trials (Opperman et al., 1994; C. H. Opperman and M. A. Conkling, pers. comm.). Refinements to this strategy will likely see this approach available for commercial use in a range of crops in the next few years. Equally important to the design of new control strategies will be a thorough understanding of parasite biology. Construction of a linkage map for *H. glycines* (K. Dong and C. H. Opperman, pers. comm.), coupled with recent development of transformation technology for this parasite (Pedersen, Bird and Opperman, unpublished) will permit identification and functional testing of genes central to the parasitic interaction. However, this research is for the future. In this article I will discuss the molecular basis of host responses to plant-parasitic nematodes, particularly the root-knot nematode (*Meloidogyne* spp.). This is not intended to be a general review of the topic. Rather I will focus on results from my group that give both a "global" picture of molecular events at the *Meloidogyne* -induced feeding site, and also results of detailed analyses of selected genes and their products. I will emphasize the host-parasite interaction in the context of host biology and development, and present the argument that the parasite exploits normal host biology in very subtle ways, and that the apparently highly modified feeding structures might not differ much from normal plant cells. Being based in some instances on preliminary data, aspects of this discussion will necessarily be speculative, and doubtlessly will require modification in light of future results. Never-the-less, I anticipate these models will be a stimulus to further nematology research, and a useful adjunct to other studies of root biology. It also is likely that the paradigms presented here will be pertinent to other host-parasite systems, and vice versa.

THE *MELOIDOGYNE* -HOST INTERACTION

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Although there are hundreds of agriculturally important nematode species (Nickle, 1991), the bulk of the damage is caused by sedentary endoparasitic forms, particularly the root-knot (*Meloidogyne* spp.) and cyst (*Heterodera* and *Globodera* spp.) nematodes. Because of the apparent complexity of the feeding sites they elaborate in host roots, they are widely considered to be the most evolutionarily advanced and have been described as being "among nature's most successful parasites" (Hussey et al., 1994).

Root-knot nematodes are complex metazoans, and exhibit the striking morphological conservatism that is a feature of their phylum (Bird and Bird, 1991). They hatch in the soil from an environmentally resistant egg as motile, vermiform second stage larvae (L_2) and actively seek host tissue, responding to as yet unknown cues. The preferred invasion sites are behind the root tip in the zone of elongation, and at the site of lateral root rupture (Krusberg and Nielsen, 1958). An enhanced-video-microscopy analysis (Wyss et al., 1992) showed that, on cultured *Arabidopsis thaliana*, the parasite first uses its stomatostyle (hollow, retractable feeding stylet) to rupture epidermal cells to gain access to the host, and then migrates apoplastically (i.e., between cells) into the developing vascular cylinder.

Following root invasion, a feeding site is selected and feeding initiated. Induction of a novel cell type (termed a giant cell) in the roots of infected plants supervenes feeding. During induction, cells in the stele accessible by the parasite's feeding-stylet increase greatly in size, become metabolically active and undergo rounds of synchronous nuclear division uncoupled from cytokinesis. Individual nuclei become highly polyploid (Huang and Maggenti, 1969) and appear irregularly lobed. These multinucleate cells become large and avacuolate, and undergo extensive remodeling of their cell wall, including development of extensive wall ingrowths (Bird, 1961). Giant cell formation, coupled with limited proliferation of nearby pericycle and cortical cells results in the root-knot gall. The large numbers of galls that arise following a heavy infestation produce grotesque roots with impaired function, in particular with regards water uptake.

Giant cells serve as the obligate nutritive source for the nematode, and efficiently unload photosynthate from the phloem stream for transfer to the parasite (Bird and Loveys, 1975) which develops through three additional, superimposed molts to a sedentary, pear-shaped, reproductive adult female. The metabolic status of the giant cells is coupled to the nutritive demand of the parasite (Bird, 1971), with giant cell development reaching a peak at the onset of egg laying.

Although microscopy has provided a description of *Meloidogyne* parasitism, many unanswered questions remain. For example, although it seems reasonable to assume that the parasite exploits host-derived cues for root location, invasion, migration to the site of giant cell induction, and perhaps feeding initiation, the nature of such signals is unknown. Similarly, although it is widely believed that proteins synthesized in the dorsal and/or subventral pharyngeal glands and secreted through the feeding stylet into host cells include the signal(s) "responsible" for giant cell induction (for example, see reviews by Bird, 1974; Hussey, 1989; Hussey et al., 1994; Sijmons et al., 1994) there is, as yet, no direct evidence to validate this hypothesis. In fact, there remains no formal demonstration that the *Meloidogyne* stylet even crosses the host plasmalemma. However, a plausible paradigm for root-knot nematode access to host cytoplasm is provided by *Criconemella xenoplax* where the stylet orifice contacts host cytoplasm in a manner resembling a "patch-clamp" electrode (Hussey et al., 1992). Alternative models for giant cell induction have been considered (Bird, 1992); some are discussed below.

Several researchers (for example, Dropkin and Boone, 1966; Bird, 1974) have proposed that the question of giant cell induction be considered in terms of regulation of host differentiation. This is attractive because it permits tractable questions to be conceived, and is the basis of the approach taken by my group.

GIANT CELL ONTOGENY

If one considers giant cell induction in terms of plant development, then identifying the giant cell progenitors (initials) might be informative. Careful microscopic examination (Krusberg and Nielsen, 1958; Jones and Payne, 1978) implicate the parenchyma adjacent to xylem elements as the preferred site for *Meloidogyne* feeding initiation, although induction of giant cells from pericycle, cortex and even epidermal cells has been reported. Thus, the events that give rise to giant cells are not strictly predicated on having a particular cell type as an initial, although the degree of differentiation might not be much different between the cell types exploited by *Meloidogyne*. If differences exist between giant cells that arise from different cell types, they are yet to be reported.

A similar situation exists for the feeding sites (called syncytia) induced following feeding by the cyst nematodes. Syncytia arise from the coalescence of adjacent cells. The origin of potato cyst-nematode syncytia appears to be in the cortex (Jones and Northcote, 1972), whereas the *Heterodera*-induced syncytia of *Brassica napus* seem to be initiated in the pericycle, with xylem parenchyma cells subsequently recruited into the syncytium (Magnusson and Golinowski, 1991). In *A. thaliana*, *Heterodera schachtii* initiates the syncytium from a procambial cell; cells of this type are subsequently incorporated into the developing syncytium. Syncytial formation was reported to suppress development of primary xylem (Grundler et al., 1994), an observation consistent with the notion that the procambial cells recruited into the syncytium were in fact xylem initials. Perhaps rather than "suppressing" xylem formation, syncytial formation depletes the supply of normal xylem precursors. It is significant that the final stages of metaxylem differentiation (in tracheid formation) involve cell wall dissolution, effectively forming what would be a syncytium if the cells remained alive. The outcome of precocious expression of this normally programmed cell-wall dissolution might well be the formation of a cell-type strongly resembling a *Heterodera*-induced syncytium.

Based on morphology, parallels between giant cells and developing xylem also can be seen, including elevated cytoplasmic density with an increased number of organelles, reduction of the tonoplast, chromosome endoreduplication, and secondary cell wall deposition. Indeed, during the first days of giant cell induction, giant cells can bear a striking resemblance to developing xylem [e.g., see Fig. 26 in Jones and Payne, 1978]. However, giant cells and syncytia obviously are not metaxylem elements. Never-the-less, it is not inconceivable that the normal route for their formation might involve large parts of the developmental pathway followed by differentiating metaxylem

elements. If this is the case, it seems likely that the nematode cues used to initiate feeding site formation might closely resemble (or modulate) normal plant effectors, and might work in concert with endogenous host signals.

GIANT CELL GENE EXPRESSION

Although it seems reasonable to suppose that giant cells share features with other plant cells, notably developing metaxylem, they are a novel cell type and presumably arise by a pattern of gene expression different from that in other plant cells. To identify such genes, a strategy was devised to clone transcripts either uniquely expressed in giant cells (compared to healthy, mature root cells) or with elevated expression levels (Wilson et al., 1994). For convenience, such genes will be termed “giant-cell specific,” although it is emphasized that, as previously speculated (Bird, 1992), these genes include functions normally expressed at different developmental times (heterochronic expression) or in different cell types (homeotic expression).

Briefly, RNA was isolated from 151, developmentally synchronized giant cells that had been individually hand-dissected from cultured tomato root explants infected with M. incognita. A cDNA library (2.2 X 106 primary recombinants) was constructed, and subjected to a rigorous subtraction against uninfected, mature-root cDNA. This step resulted in a 4,860-fold enrichment of giant cell sequences, and yielded a pool of clones that define 220, different giant-cell specific genes (Bird and Wilson, 1994a; Bird and Wilson, 1994b; Wilson et al., 1994; unpublished data). Two technical features of our strategy (Wilson et al., 1994) are worth noting. By performing the cloning step prior to subtraction, (a) only giant cell transcripts were exposed to a replicon, thereby minimizing the chance of artifactually cloning non-giant-cell-expressed sequences, and (b) the small amount of material remaining after subtraction was already cloned, permitting cDNAs representing rare transcripts to be recovered.

A comprehensive analysis of these clones has been undertaken. Most of the cDNAs have been individually labeled and used as probes to blots of tomato and *M. incognita* genomic DNA, confirming that the clones were of tomato origin, and encoded unique or low copy number genes (Wilson et al., 1994; unpublished data). Each probe was also used to challenge dot-blots of RNA from a range of healthy and nematode-infected tomato tissues (including root-knot galls, roots minus tips, root tips, cotyledon plus seedling apex, hypocotyl, mature leaf). Signals (with a wide range of intensities) were obtained for 124 probes (Bird and Wilson, 1994a; unpublished data). Although these experiments should be considered qualitative, two important conclusions can be made. Firstly, giant cells express many functions not normally expressed in mature root. Of the 124 positive results obtained, only one clone corresponded to a gene expressed at a detectable level in this organ. The remainder were expressed in a wide range of other tissues, at a wide range of relative abundance. Second, there are relatively few genes expressed in giant cells but not at a detectable level elsewhere in healthy plants. Only one of the clones defines such a gene.

Each of the cDNAs has been at least partially sequenced (Wilson et al., 1994; unpublished data), and subjected to a variety of computer and manual analyses. Based on these studies, function can unambiguously assigned to 56 clones; some examples are discussed below. Of the remaining clones, some have motifs indicative of function (e.g., DB#215 has a Zn-finger domain) and others have intriguing expression patterns (e.g., DB#165 is strongly expressed in mature leaf). However, approximately half the clones remain unidentified, either because insufficient sequence was obtained to permit overlap with other partially sequenced genes, or because they define genes encoding novel functions; these genes are termed “pioneers” (Bird and Wilson, 1994a; unpublished data). One approach to understanding the function of the pioneers will be to determine their role both in normal plant development and also in mediating the host interaction with other micro-organisms. Preliminary data (Rosewarne, Barker and Bird, unpublished) suggests that a subset of the pioneers respond to, and may be required for, invasion of mycorrhizal fungi. Elucidating the specific role of these genes in establishment of symbiotic and/or pathogenic interactions will await functional analysis.

Giant cell-specific genes

Although all the genes specifically expressed in giant cells are potentially interesting, those with deduced function consistent with the known morphology and function of giant cells are obvious candidates for detailed analysis. Such genes can be divided into three broad categories, viz., (1) wound/defense responses, (2) differentiated giant cell function, and (3) signal reception/transduction. Rather than present an exhaustive catalogue, selected examples from each class are discussed, in the context of giant cell biology.

Wound/defense responses: The gene defined by DB#268 corresponds a previously identified, tomato wound-responsive gene named pT53 (Parsons and Mattoo, 1991). In healthy plants, pT53 is constituitively expressed in several organs, including root, stem and unripe fruit. In situ analysis of fruit and stem revealed that the pT53 transcript is localized to vascular bundle cells, presumed to be protoxylem (Parsons and Mattoo, 1994); roots were not examined. Unlike many wound-responsive genes, expression of pT53 is repressed following wounding (Parsons and Mattoo, 1991). By contrast, expression of pT53 appears to be up-regulated in giant cells (unpublished data).

A striking feature of the compatible root-knot nematode-host interaction is the apparent absence of host wound or defense responses. Recent evidence (Hansen et al., 1996) suggests that cells surrounding the pathogen and feeding site express the *wun1* promoter (a potato gene that responds to invasion by the fungal pathogen *Phytophthora infestans*), but this occurs late in the infection process, and is presumably in response to mechanical forces exerted by the swelling giant cells and female nematode. Even though the relatively large (400 X 15 µm) L2 exerts sufficient force on cells in its path to separate the middle lamellae (Endo and Wergin, 1977), *wun1* appears not to be induced during the migratory phase (Hansen et al., 1996). These cells are not killed, although cells along the migration path transiently exhibit increased cytoplasmic density (Jones and Payne, 1978). It is conceivable that components of the material derived from parasite’s subventral pharyngeal glands and copiously secreted from the L2 stylet during migration (Wyss et al., 1992) actively suppress host responses. Suppression (or specific failure to elicit) wound/defense responses remains a feature of the susceptible interaction throughout the life of the nematode, and might explain the increased susceptibility of nematode galls to invasion by fungal and bacterial pathogens.

Whether or not up-regulation of DB#268 is causally related to nematode-mediated suppression of host defenses, or is merely an effect of such suppression is yet to be determined. The function of pT53 in the wound response has not been experimentally demonstrated, but it seems likely that the pT53 product is an RNA-binding protein (Parsons and Mattoo, 1994; unpublished data). Several other giant cell specific genes are postulated to encode RNA-binding proteins (unpublished data), although their roles also are arcane.

In addition to responding to wounding, plants also possess R genes (such as the tomato *Mi* gene, discussed above) that permit a specific and effective defense response to be mounted. In the case of *Mi* (and many other pathogen-resistance genes) the response involves a localized necrosis, termed the hypersensitive response (HR). A cell-autonomous HR is effective against root-knot nematodes because not only are the giant cells the sole nutritive source for the parasite, but the feeding nematode becomes sedentary and so is unable to establish a new feeding site. Because the HR is initiated in proto-giant cells it is possible that components of the signal transduction pathway that lead to an HR in a resistant host might be expressed (even required) in a compatible interaction, and it has been suggested that the Mi gene may be an allele of a component required for giant cell formation (Bird, 1992), although there is no direct evidence for this. Genes with expression restricted to giant cells (i.e., absent in healthy tissues) are candidates for this type of function, and one gene, DB#131, appears to fit this criterion.

Blast analysis implicates DB#131 as encoding a serine/threonine receptor kinase. Significantly, the highest homology was to the tomato gene *Pto* which confers resistance to the bacterial pathogen *Pseudomonas syringae pv tomato*. *Pto* specifically phosphorylates another serine/threonine kinase (*Pti*), suggesting that *Pti* is downstream of *Pto* in a kinase cascade (Zhou et al., 1995). Another, as yet uncloned gene (*Prf*) is required for *Pto* function (and also for *Fen*, which encodes resistance to the herbicide fenthion); whether it acts up- or down-stream is unclear. Because *Pti* was apparently not able to be uncovered genetically (it was identified in a yeast, 2-hybrid screen), it has been argued that it has essential functions (which tentatively include interacting with transcription factors) in addition to being pathogen responsive (Zhou et al., 1995). Like DB#131, *Pti* also is expressed in the compatible host. It is an intriguing possibility that the DB#131 gene is necessary for both the compatible and resistant responses, perhaps by encoding a kinase down-stream in the *Mi* cascade.

Differentiated giant cell function: The principal function of giant cells is to unload downwardly translocating photosynthate from the phloem, and to present it in a form accessible to the parasite. Ulturastructural examination (Jones and Dropkin, 1976) and dye-injection experiments (Grundler et al., 1994) imply that giant cells are symplastically isolated from other cells (including the phloem), suggesting that phloem unloading might be via the apoplast, presumably involving pump-driven, transmembrane channels. Measurements of transmembrane potentials (Jones et al., 1975) and in vivo fluorescence assays (Dorhout et al., 1992) revealed the presence of proton efflux pumps in giant cells. DB#226 encodes a plasmalemma, proton-ATPase, different from the two previously cloned tomato isoforms (Ewing et al., 1990), and is an obvious candidate for encoding the demonstrated pump activity. Knowing the precise location of the DB#226 product in giant cells will likely be informative, particularly if it were found, for example, to be in the plasmalemma adjacent to phloem elements.

Direct measurements indicate that cell turgor is elevated in syncytia (Grundler et al., 1994); the same is likely to be true for giant cells, and several clones, including DB#132, 153, 181, 249, 356 encode functions postulated to play a role in regulating (or responding to) cell turgor. Our identification of the gene defined by the DB#249 clone is particularly significant, because this encodes the tomato homologue of the tobacco, *TobRB7* gene, which encodes a trans-plasmalemma water channel (C. Li, D. Sarawitz and M. A. Conkling, pers. comm.). Conkling's group has undertaken an extensive analysis of the *TobRB7* promoter (Conkling et al., 1990; Yamamoto et al., 1990; Yamamoto et al., 1991). Fusions were made between a deletion series of the *TobRB7* 5'-flanking region and the bacterial reporter gene, β-glucuronidase (GUS) in order to identify sequences controlling the root-specific expression patterns. The deletion series experiments revealed that *cis*-acting elements necessary for root-specific expression of *TobRB7* are located between 636 and 299 nucleotides 5' of the transcription initiation site (Yamamoto et al., 1991). In healthy plants *TobRB7* expression was lost when the promoter was deleted to -299 (Δ0.3). However, infection with root-knot nematodes significantly alters this pattern (Opperman et al., 1994). In those plants, gus accumulation was limited to the developing giant cells and appeared to be regulated by the nematode infection. The most significant aspect of these findings is that the nematode-responsive element of the *TobRB7* promoter is not the same as the root-specific element, and can be uncoupled. Based on the novel morphology of giant cells, and reinforced by the patterns of giant cell gene expression shown globally by RNA blot experiments, and specifically by DNA sequence analysis (Bird and Wilson, 1994a), there has been an implicit assumption (Bird, 1992) that profiles of transcription regulators in giant cells are qualitatively or quantitatively different from those in other host cells; the results with the *TobRB7* promoter (Opperman et al., 1994) prove this point. Understanding those differences in transcription regulator profiles will be an important step in dissecting the mechanism of giant cell induction.

Signal transduction: A significant number of clones encode nucleic acid-associated proteins, including splicing factors (sc35, srp20 and srp75) and transcription regulators (hsf30 and *Myb*). The DB#280 *Myb* is particularly interesting because in plants *Mybs* are known to regulate a range of functions pertinent to giant cells. For example, the *Arabidopsis* GL1 *Myb* regulates an endoreduplication event associated with trichome initiation (Marks et al., 1991), and mutation of the *Antirrhinum* mixta locus, which encodes a *Myb*, results in cells with altered walls (Noda et al., 1994). Using in situ hybridization it was found that the *myb* homologues cloned from barley were expressed primarily in meristematic tissues, particularly those tissues associated with developing vasculature (Wissenbach et al., 1993). Examination of the deduced amino acid sequence of the α-helix responsible for template sequence-recognition by the DB#280 *Myb* (Bird and Wilson, 1994a) revealed it to be distinct from all previously characterized *Mybs* (except for an essential tryptophan), whereas the two other α-helices, required for non-specific DNA-binding, are highly conserved with other *Mybs* (Avila et al., 1993). Of particular interest will be to identify the

targets of the DB#280 *Myb*, and the role it might play in giant cell induction and/or function.

A second class of signal transducing molecules defined within the giant cell bank include a number of ubiquitin conjugating (E2) enzymes; the E2 defined by DB#103 (*Leubc4*), is the most abundantly expressed of the cloned giant cell transcripts, and not surprisingly, is represented multiple times in the giant cell cDNA bank. E2s play a primary role in determining the target specificity for protein ubiquitination (and subsequent proteolysis), and a specific role for ubiquitination in tobacco vascular differentiation has been demonstrated (Bachmair et al., 1990). In situ localization experiments (unpublished data) showed *Leubc4* transcripts to be restricted primarily to meristematic tissue (including lateral roots) and vascular procambium in healthy plants, and confirmed abundant *Leubc4* expression in giant cells. Antibodies raised to two, distinct synthetic peptides deduced from the *Leubc4* sequence specifically detect a correctly sized band (16.5 kDa) in isopropyl β-D-thiogalactoside-induced protein extracts from *Escherichia coli*-expressed *Leubc4*, and a protein of identical size was detected in tomato galls induced by *M. incognita*; no signal was detected in healthy root (unpublished data). The antibody signal to gall proteins and *E. coli*-expressed protein is abolished by competition with specific synthetic peptide. In collaboration with M. A. McClure, we have localized the *LeUBC4* protein in giant cells at the ultrastructural level. Strikingly, *Leubc4* encodes a nuclear protein. Several yeast E2s (e.g., rad6 and cdc34) are presumed to be nuclear proteins, and have specific regulatory functions in DNA synthesis and cell cycle progression (Seufert et al., 1990). Identifying the *LeUBC4*-target(s) in giant cells is an obvious priority.

GIANT CELL INDUCTION

The mechanisms by which root-knot nematodes establish giant cells remains elusive. However, I propose a model, which although not directly supported by experimental evidence, is consistent both with previously published observations of giant cell anatomy and also with our analyses of giant cell gene expression, particularly in situ localization of giant cell transcripts in healthy tissues (unpublished data). This model makes certain predictions that we plan to test.

Giant cell induction: a two-step process

Step one - a developmental switch: As discussed above, giant cells (and also syncytia) arise primarily from cells with the developmental potential to become xylem, and developing giant cells share many features with developing xylem. Hence it seem reasonable to propose that the giant cell precursors (initials) receive a developmental cue to initiate xylem differentiation, although because giant cells do not become mature xylem, either the inductive signal is incomplete, or the developmental pathway is subsequently suspended. The xylem-inductive cue may be active in the apoplast (i.e., extracellular), where it might be presented (perhaps as a ligand) to the cell surface, or it might be active symplastically.

Conceptually, such a cue might be provided directly by the parasite as a functional homologue of a normal plant growth regulator (e.g., a hormone, or a molecule active up- or down-stream in a hormone pathway). Several workers have reported identifying nematode-synthesized plant hormones, including cytokinin-like molecules (Bird and Loveys, 1980), although the biological significance of these activities remain to be demonstrated in planta. It is not inconceivable that novel classes of plant signaling molecule might be produced by parasitic nematodes. Nitrogen fixing bacteria (rhizobia), for example, produce a lipochitooligosaccharide ("Nod factor") which is necessary and sufficient to elicit the root cortical cell divisions that culminate in root nodules. Because chitin is a component of the nematode egg shell, these parasites presumably possess the enzymatic machinery necessary to synthesize the chitinous backbone of Nod factor; whether or not biologically-active Nod factor is produced is unknown. At least one of the host genes induced by rhizobial Nod factor (ENOD40) appears to be expressed in tomato giant cells (Hirsch and Bird, unpublished data). However, *M. incognita* remains able to induce giant cells on *Melilotus alba* plants mutant in genes required for rhizobial nodulation (Hirsch and Bird, unpublished data), suggesting that if a nematode-encoded Nod factor is involved, then subsequent steps for rhizobial nodule and giant cell formation are diverged.

Alternatively, the parasite might actively modify a pre-existing, host growth regulator. Using an antibody raised against the conserved hormone binding domain of the maize auxin binding protein, a 45kDa protein has been identified in the potato cyst nematode (*Globodera pallida*), suggesting that this nematode might possess a phytohormone receptor (W.M. Robertson, L.H. Duncan and J.R. Kusel, pers. comm.). Whether or not this putative receptor plays a role in modifying or modulating auxin levels is not known.

As discussed above, the feeding sites induced by root-knot and cyst nematodes appear to have different ontogenic routes, with the cyst nematodes inducing later facets of xylem differentiation (particularly wall dissolution). The more restricted host range of cyst nematodes (compared to *Meloidogyne* spp.) is consistent with the notion that these parasites interact more downstream in the xylem differentiation process, where the machinery is likely to be more host-specific.

Step two - a physiological switch: Giant cells (and syncytia) clearly possess the morphological, biochemical and functional attributes of transfer cells, and have previously been described as being such (Jones and Northcote, 1972). Transfer cells are a functionally defined class of cell (Gunning et al., 1968) that arise in plant tissues in response to the physiological demand for intensive, short distance solute flux. Thus, merely by feeding, the parasite provides the second cue to form what can be considered to be a chimeric cell type (i.e., with attributes of developing xylem and transfer cells). It seems plausible that expression of genes with functions consistent with transfer cell function, including the DB#226 proton-ATPase and the DB#249 transmembrane water channel, might be expressed in response to the mass action cue of feeding.

Giant cell death: Removal of the parasite (e.g., by killing it) results in breakdown of the giant cells (Bird, 1962), and implies that a continuous source of stimulation from the nematode is required for giant cell maintenance. Although the mechanism of giant cell breakdown is not known, it is an intriguing possibility that once the mass action signal (i.e., the feeding nematode) is removed, the final stages of the xylem differentiation pathway are permitted to continue. A testable prediction of this is that giant cell death will be apoptotic (as occurs in xylem tracheid formation) rather than necrotic.

RATIONALLY DESIGNED NEMATODE CONTROL

In addition to providing clues to the mechanism of the host-parasite interaction, genes expressed at the feeding site represent a pool of functions that might be manipulated to effect nematode control. Two general strategies can be envisioned, both based on the fact that giant cells are the obligate nutritive source for the developing nematode. In one scenario, giant cells might be exploited to present specific compounds to the feeding nematode, including toxins or other bioactive molecules. A second approach might be to mimic the *R*-gene-mediated necrosis (HR) of proto-giant cells.

Giant cells as a delivery system

Conceptually, the simplest strategy is to express (in a transgenic plant) a nematode-toxic protein under the control of a constitutive promoter. Although protein toxins that act specifically upon plant parasitic nematodes might be the best choice, this is not a strict requirement. One approach, in which the plant, proteinase-inhibitor, oryzacystatin-1 was constituitively expressed in tomato roots appeared to restrict growth of the potato cyst nematode, *G. pallida*, presumably by acting as an anti-feedant (Urwin et al., 1995). Other types of protein can be conceived. For example, transgenic plant expression of *Bacillus thuringiensis* (BT) toxins has been demonstrated to confer resistant to insect pests (Barton et al., 1987); perhaps BT also is nematicidal. A related, but distinct approach would be the expression of antibodies (plantibodies) directed against some parasite function; initial approaches (e.g., Baum et al., 1996) are yet to be successful, perhaps because there is no a priori way of knowing which nematode antigens are essential for the parasitic interaction.

However, constitutive expression of "toxin" genes (including anti-feedants and plantibodies) may place very strong selective pressure for resistance on the sensitive nematode population, rendering the durability of this type of defense in doubt. In addition, the global, constitutive expression of toxin genes guarantees that non-target species, including humans, will be exposed to the protein products. A refinement of this approach would be to place a nematode-specific toxin gene under the control of a tissue-specific, or nematode-inducible promoter such as the *TobRB7* Δ0.3 promoter discussed above, but the selective pressures for toxin-resistant nematodes remain.

As more is known about parasite biology, it might be possible to identify nematode products, such as peptide hormones, that could be provided exogenously by the host to modulate parasite biology or development.

Feeding site disruption

An approach, based on the *TobRB7* Δ0.3 promoter (see above) driving expression of the cell-autonomous, cytotoxic ribonuclease, Barnase, has been developed (Opperman et al., 1994). In the absence of nematodes, plants transgenic for this construct have no observable phenotype. In one transgenic line, no galls and 2 small egg masses were detected, in contrast to the heavy galling and large numbers (>300) of egg masses recovered on control plants. Histochemical examination of the roots revealed that many second-stage juveniles had penetrated the Δ0.3 promoter::Barnase roots, but had arrested in development after 4-6 days. However, although experiments with the Δ0.3 promoter::Barnase plants have proven that this approach to engineering nematode-resistant plants works, the extremely toxic nature of Barnase presents several actual and potential problems, including inactivation by "gene silencing."

An alternative to expressing a cytotoxin per se in giant cells is to interfere with the plant gene expression patterns in the developing giant cells, thereby disrupting the proper formation and function of the feeding site. Obviously the genes my group identified represent a large pool of potential targets for inactivation, and also a pool of potentially useful promoters, particularly for root-specific genes such as that defined by DB#113 (Bird and Wilson, 1994a). Several inactivation strategies can be envisioned, including ribozymes or antisense constructs to target specific transcripts, or the design of dominant-negative mutations of genes to target proteins. A nematode attempting to feed on cells carrying a nematode-inducible promoter fused to a gene encoding one of these molecules would initiate gene expression resulting in the degradation of the feeding site. This type of engineered resistance is conceptually similar to, although mechanistically distinct from, the natural HR to nematode parasites in resistant plants, and has the added advantage of using naturally occurring plant genes as opposed to foreign sequences. It just might be that the combination of promoter and target gene that provides the most durable and effective control might resemble something that exists in nature.

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