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Molecular Analysis of the Interactions between Cyst Nematodes and Their Hosts[1]

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Abstract: In order to complete its life cycle, a cyst nematode must stimulate the production of a specialized syncytial feeding site within host root tissues. This process is characterized by major changes in local root morphology, including enlargement of affected nuclei and nucleoli, cell wall degradation, and proliferation of subcellular organelles. At the molecular level very little is known about the processes involved in this host response, but recent evidence suggests that cyst nematodes are able to regulate specific host genes. The host–parasite model system provided by *Arabidopsis thaliana* and *Heterodera schachtii* will be fundamental to our future understanding of the formation of syncytia. Molecular biology now offers us the opportunity to study this complex host–parasite interaction in great detail. A better understanding of the host genes regulated by cyst nematodes and the mechanisms by which this regulation is achieved will facilitate the engineering of crop cultivars that possess novel forms of resistance to these adept parasites.

Key words: *Arabidopsis*, cDNA, cyst nematode, nematode, polymerase chain reaction, syncytium, transgenic plant.

With very few exceptions, the diverse feeding strategies utilized by plant-parasitic nematodes can be divided into two broad groups, depending on whether the nematodes have adopted a migratory or a sedentary feeding habit. Most species of phytophagous nematodes are migratory ectoparasitic or endoparasitic browsers that frequently kill the cells on which they feed before moving on to another cell. This simple feeding strategy is in marked contrast to that of the largely endoparasitic sedentary nematodes, which have evolved complex and intimate relationships with their hosts. Successful sedentary plant-parasitic nematodes must possess the biochemical finesse to induce and maintain a specialized feeding site within their host plants; for if the cells on which they feed die, their sedentary habit prevents them moving to an alternative site.

Sedentary endoparasitism has a number of evolutionary advantages (16). In many cases the plant tissues that enclose the nematodes provide protection from predation and parasitism and partially insulate the nematodes from fluctuating environmental conditions. The maintenance of a feeding site requires less energy than migration from site to site; and this fact coupled with development of the enlarged females that are typical of sedentary species, contributes to egg production and thus increases fecundity. Members of the family Heteroderidae, most notably the root-knot nematodes (*Meloidogyne* sp.) and two genera of cyst nematodes (*Heterodera* and *Globodera*), are some of the most advanced and specialized sedentary endoparasites, and it is no coincidence that these genera contain the agriculturally most damaging and widespread species (4,11).

To complete their life cycles, the invasive second-stage juveniles of root-knot and cyst nematodes must enter the roots of a susceptible host plant and there initiate localized reorganization of the host's morphology and physiology, resulting in the formation of specialized feeding sites (8). Although the feeding sites of *Meloidogyne* spp. (giant cell complexes) differ in almost every respect from the feeding sites induced by the cyst nematodes (syncytia), the end function is the same, i.e., to mediate the efficient transfer of nutrients from the plant's vascular tissue to a feeding nematode as it grows and molts to an adult.

Due to the economic importance of these obligate parasites and with a view to novel control strategies, much effort has been directed towards establishing the pri-

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mary mechanisms responsible for the production of giant cells and syncytia. This is especially so for cyst nematodes, where the initiation, development, and maintenance of their syncytial feeding sites is currently the subject of a great deal of research interest.

Host Genes are Involved in the Formation of Syncytia

Observational studies using light and electron microscopy (5,6,14) have yielded much information concerning the timing, sequence, and histology of the events that lead to the production of syncytia, but practically nothing is known at the molecular level about this essential phase.

The active involvement of the host's own genes in the production of syncytial feeding sites has been proposed previously (3,8) but irrefutable evidence for this has been hard to find. Strong circumstantial evidence for the probable central role played by the host's genes in syncytial formation is provided by objective interpretation of the morphological changes observed in developing syncytial and in affected plant cells before their incorporation into these feeding sites. For example, when serial sections of infected roots are observed with the light microscope, developing syncytia have a dense granular cytoplasm, often in marked contrast to the highly vacuolated, unaffected adjacent cells. The turbid appearance of syncytia is caused by a proliferation of subcellular organelles, principally endoplasmic reticulum, mitochondria, and ribosomes (6). The host genes necessary for the production of these organelles must therefore be turned on or up-regulated, and this process is consistent with the apparent general metabolic stimulation within syncytial feeding sites. Likewise, the nuclei of affected cells enlarge and become amoeboid in cross-section, with prominent nucleoli. A large irregular nucleus may facilitate increased nuclear–cytoplasmic exchange (8), whereas an enlarged nucleolus is symptomatic of increased transcription of ribosomal RNA genes. The metabolic stimulation, the proliferation of subcellular organelles, and the changes in affected nuclei suggest a significant increase in transcription and translation within syncytia.

Further support for the direct involvement of host genes in the production of cyst nematode feeding sites results from the observation that the development of syncytia depends on the breakdown of host cell walls between neighboring affected cells. Cell wall degradation, which is first evident 24 hours after syncytial induction, is the principal mechanism for the growth and expansion of syncytia. The breakdown of host cell walls appears to be a very directed and controlled process that is difficult to explain in terms of the simple injection of hydrolytic enzymes by the nematodes. It seems likely that cyst nematodes are able to influence, either directly or indirectly, expression of the host's own cell wall degrading machinery. Similarly, cell wall turnover, but this time the process of deposition, is important as syncytia approach maturity and begin to develop plasmalemma-lined cell wall ingrowths at the interface between the syncytium and the vascular bundle (9). Ingrowths such as these are typical of transfer cells, in which ingrowths facilitate the short-distance transport of solutes across cell walls (13). During the production of these ingrowths, syncytia are often still growing actively by continued cell wall breakdown. This presents the interesting situation in which cell wall degradation and deposition are occurring simultaneously within the same cytoplasmic continuum. Such a balance would be difficult to achieve without the involvement of the host's own genes.

The many morphological and physiological changes that are seen during the production of syncytia occur also in healthy plant tissues at definite stages of normal plant growth and development. This observation, coupled with the conservative structure of the syncytia induced in many different plant families, indicates that cyst nematodes can probably regulate

normal and universal plant developmental pathways (3). Although the host pathways and genes involved are not nematode specific, the overall pattern of expression may be. The formation of syncytia seems to require concurrent expression of host genes that would not usually be expressed together in the same plant tissues or in the same developmental time frame.

Molecular Analysis of Syncytial Feeding Sites

Molecular biology now offers the opportunity to clone and characterize the host genes necessary for the induction and formation of syncytial feeding sites. The knowledge gained from such work will give a fascinating insight into the complex relationship that has evolved between cyst nematodes and their hosts. Total dependence on such a specialized host–parasite relationship may prove to be a vulnerable phase in the life cycle of these important pests. A better understanding of the key molecular events involved in the induction and development of synctia could lead to the production of novel genetic constructs that, when expressed in crop plants, prevent the initiation or normal development of syncytia.

The ability to study differential gene expression in separate cell lines and tissues or at different developmental stages is a basic requirement in molecular biology. The differential expression of genes is most frequently identified and studied using complementary DNA (cDNA) libraries. cDNA is made by the reverse transcription of messenger RNA (mRNA) into DNA, which is subsequently cloned. In principle, a representative cDNA library will contain cDNA clones of the genes that were "active" at the time of mRNA preparation. Genes that are expressed in one tissue but not in another can then be identified by differential screening of appropriate cDNA libraries. Such an approach is needed to clone and identify genes that are either up-regulated or down-regulated in infected roots compared to uninfected roots.

Amplification of cDNA with the polymerase chain reaction: Although not impossible, the construction of representative cDNA libraries from infected roots has proven prohibitively laborious due to the quantity of syncytia (or at least root fractions rich in syncytia) required to provide sufficient mRNA. A solution to this problem has been reported recently (1) that links conventional cDNA methodology to the polymerase chain reaction (PCR), which allows the amplification of minute amounts of cDNA prior to cloning. It is suggested that in this manner representative cDNA libraries can be constructed starting with the mRNA equivalent to as few as 10 cells (1). This economic approach to the construction of cDNA libraries has clear advantages when attempting to clone the genes that are expressed preferentially in syncytia. Gurr et al. (7) used a modification of this technique to make a cDNA library from potato roots infected with the potato-cyst nematode *Globodera rostochiensis*. The authors believe that one of several clones isolated from this library represents a single copy host gene that is syncytium "specific." Despite sequence analysis, the function of this gene—and hence the role it may play in the formation of syncytia—is unknown.

Work such as this clearly benefits those actively interested in transgenic plants for nematode control, but because of insufficient molecular knowledge concerning the host plant, little is revealed about the key molecular events involved in the formation of syncytia. The value of basic information such as this should not be dismissed or underestimated, as it is of much more than mere academic importance. It can be argued that in the race to commercial exploitation of nematode-resistant transgenic plants, an understanding of the underlying molecular processes of syncytial development is unnecessary. This may be so (at least in the short term), but without this basic molecular information it will be difficult to ensure that any particular strategy used to produce nematode resistance is potentially the most effective and the most

enduring in the field and at the same time minimizes disruption of the host plant. Furthermore, as with most fundamental studies, basic research into the molecular mechanisms of the formation of syncytia is likely to reveal other previously unconceived novel control targets.

The amount of time and effort required to establish the molecular basis of the induction and development of syncytia is likely to be considerable. However, the complex task of identifying, cloning, mapping, and functionally evaluating the host genes implicated in this process could be made much quicker and easier by selecting the right model host system to study. The small cruciferous weed *Arabidopsis thaliana* is an excellent candidate for such a model host.

Arabidopsis as a model host: Although of no commercial value, *A. thaliana* is currently the model plant in a worldwide research effort aimed at investigating the basic biology, biochemistry, and molecular biology of plants. The size and organization of the genome of this plant make it well suited for molecular genetics (2,10). *Arabidopsis* has one of the smallest nuclear genomes recorded for any flowering plant, only 7.0×10^7 base pairs. Most of the major gene families are organized very simply, with an almost total lack of dispersed repetitive DNA. A great deal of international work is directed towards improving the resolution of the already detailed restriction fragment length polymorphism (RFLP) and physical maps of this genome (12), and the number of characterized and mapped genes is increasing constantly. The critical axenic culture conditions necessary to establish *A. thaliana* as a host for several plant-parasitic nematodes, including cyst nematodes, have been developed recently (15). The use of *Arabidopsis* as a susceptible host in which to study the induction and formation of syncytia by cyst nematodes will benefit greatly from the established expertise in *Arabidopsis* research and from the current (and future) wealth of molecular information pertaining to this international model plant.

At Rothamsted, the unique combination of PCR generation and amplification of cDNA, coupled with the use of *Arabidopsis* as a model host, has been used in the first steps toward elucidating the key molecular events involved in the formation of syncytia. Although this work is still in its initial stages, preliminary results are extremely encouraging. A cDNA library has been constructed from *A. thaliana* (ecotype Landsberg) roots infected with *Heterodera schachtii*. The syncytia induced by *H. schachtii* in these thin transparent roots (Fig. 1) are very accessible and easy to prepare for molecular analysis at well-defined stages of development essentially free of nematodes or "untransformed" root cells. Total RNA was prepared from approximately 50 syncytia at 10 days after induction by the nematodes. The method used to produce and amplify cDNA from this

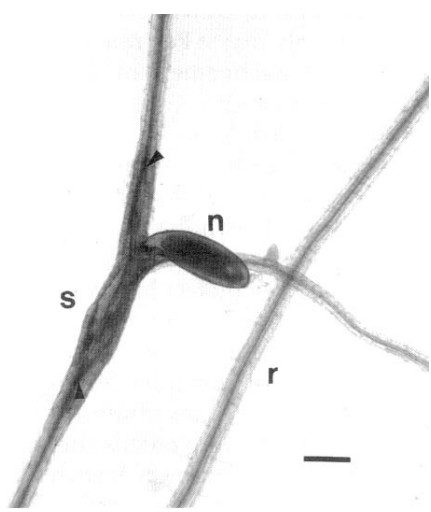

FIG. 1. A syncytium (s) in the roots of *Arabidopsis thaliana* 10 days after induction by a *Heterodera schachtii* female (n). Note the large size of the syncytium when compared to the thin transparent uninfected roots (r). The relative size of syncytia coupled with the observation that at all stages of development the nematodes often have no more than their necks buried in the roots, makes both syncytia and nematodes readily accessible for many types of analysis. The physical and molecular attributes of *Arabidopsis* as a model host of cyst nematodes are considerable. Arrows delineate the boundaries of the expanding syncytium (acropetal [lower], basipetal [upper]). Scale bar = 150 μm.

material was essentially that of Belyavsky et al. (1), except that the PCR primers were designed to give internal 5′ and 3′ sites for restriction enzymes (Eco RI and Not I respectively), thus allowing efficient directional cloning into an appropriate vector. This initial work is directed at cloning and characterizing only host genes that are turned on or up-regulated significantly during syncytial development. Screening of <5,000 recombinants has already revealed three putative infection-specific cDNAs. The final test as to whether these (and other) cDNAs are truly syncytium specific and not just associated with a more general or systematic response to the nematode will be resolved using in situ hybridization techniques. Sequencing and identifying the *Arabidopsis* genes represented by cDNAs like these will at last provide valuable insight into the molecular basis of the formation of cyst nematode feeding sites.

In conclusion, transgenic plants will almost certainly play a key role in the future control and management of obligate plant-parasitic nematodes. Cyst nematodes and other sedentary groups may be particularly vulnerable to such an approach due to the complex relationships they have evolved with their hosts.

The fundamental research necessary to establish the key molecular events involved in the formation of the syncytial feeding sites of cyst nematodes has begun. The use of *Arabidopsis* as a model host will be of great benefit and contribute significantly to our understanding of this complex process. The many common features of syncytia, irrespective of host or cyst nematode species, encourages the belief that the lessons learnt using *Arabidopsis* may be equally useful when applied to crop systems. The greater knowledge that this brings will increase the chance of engineering truly effective and enduring resistance to these important agricultural pests.

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