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BARD

FINAL REPORT

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**Functional Analysis of Root-Knot Nematode
Surface Coat Proteins to Develop Rational
Targets for Plantibodies**

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Cover Page

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged		6		6
Submitted, in review, in preparation			1	1
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts	1			1
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant. **Dr. Guanggan Hu. SS # 600-71-0472**

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	2		3
Longer Visits (Sabbaticals)				

Description Cooperation:

Planning, strategy, and coordination meetings in both Israel and USA. Constant correspondence and collaboration via the internet.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

The goal of this research was to provide a better understanding of the interface between root-knot nematodes, *Meloidogyne* spp., and their host in order to develop rational targets for plantibodies and other novel methods of nematode control directed against the nematode surface coat (SC). Specific objectives were: 1. To produce additional monoclonal SC antibodies for use in Objectives 2, 3, and 4 and as candidates for development of plantibodies. 2. To determine the production and distribution of SC proteins during the infection process. 3. To use biochemical and immunological methods to perturbate the root-knot nematode SC in order to identify SC components that will serve as targets for rationally designed plantibodies. 4. To develop SC-mutant nematodes as additional tools for defining the role of the SC during infection.

The external cuticular layer of nematodes is the epicuticle. In many nematodes, it is covered by a fuzzy material termed "surface coat" (SC). Since the SC is the outermost layer, it may play a role in the interaction between the nematode and its surroundings during all life stages in soil and during pathogenesis. The SC is composed mainly of proteins, carbohydrates (which can be part of glycoproteins), and lipids. SC proteins and glycoproteins have been labeled and extracted from preparasitic second-stage juveniles and adult females of *Meloidogyne* and specific antibodies have been raised against surface antigens. Antibodies can be used to gain more information about surface function and to isolate genes encoding for surface antigens. Characterization of surface antigens and their roles in different life-stages may be an important step towards the development of alternative control. Nevertheless, the role of the plant-parasitic nematode's surface in plant-nematode interaction is still not understood. Carbohydrates or carbohydrate-recognition domains (CRDs) on the nematode surface may interact with CRDs or carbohydrate molecules, on root surfaces or exudates, or be active after the nematode has penetrated into the root. Surface antigens undoubtedly play an important role in interactions with microorganisms that adhere to the nematodes.

Polyclonal (PC) and monoclonal (MC) antibodies raised against *Meloidogyne javanica*, *M. incognita* and other plant-parasitic nematodes, were used to characterize the surface coat and secreted-excreted products of *M. javanica* and *M. incognita*. Some of the MC and PC antibodies raised against *M. incognita* showed cross-reactivity with the surface coat of *M. javanica*. Further characterization, *in planta*, of the epitopes recognized by the antibodies, showed that they were present in the parasitic juvenile stages and that the surface coat is shed during root penetration by the nematode and its migration between root cells.

At the molecular level, we have followed two lines of experimentation. The first has been to identify genes encoding surface coat (SC) molecules, and we have isolated and characterized a small family of mucin genes from *M. incognita*. Our second approach has been to study host genes that respond to the nematode, and in particular, to the SC. Our previous work has identified a large suite of genes expressed in *Lycopersicon esculentum* giant cells, including the partial cDNA clone DB#131, which encodes a serine/threonine protein kinase. Isolation and predicted translation of the mature cDNA revealed a frame shift mutation in the translated region of nematode sensitive plants. By using primers homologous to conserved region of DB#131 we have identified the orthologues from three (nematode-resistant) *Lycopersicon peruvianum* strains and found that these plants lacked the mutation.

Achievements

We have produced additional mono- and polyclonal antibodies to study the SC biological role in host-parasite interaction and in the host-recognition process which takes place either before the pre-parasitic second-stage juvenile (J2) of *Meloidogyne* locates the root, or after the J2 enters the root. With these antibodies, we have localized the origins of at least two different SC antigens and we have developed a simple and elegant bioassay to evaluate the role of the SC in the host-finding process. This method is based on continuous exposure of the nematodes to the SC-originated antibodies, while they are making their way to the host roots. The method overcame the difficulties due to the dynamic nature of the nematode SC. We were able to trace the SC 'printings' *in planta*, as a result of the SC shedding, after the J2 penetration and migration in the roots. We demonstrated that the movement pattern of *M. javanica* J2 toward the host root was affected by all the antibodies bound to the surface coat of the pre-parasitic J2. Continuous binding of the antibodies to the *M. javanica* surface inhibited the infection of *Arabidopsis thaliana* roots on agar plates. This important finding suggests that the SC may play a pivotal role in pathogenesis, thereby, disruption or modification of the SC will effect nematode pathogenesis.

The hypodermis of the infective juveniles was labeled by one of the PC and one of the MC antibody, suggesting that the hypodermis is the site of production of these surface antigens. SC epitope(s) also originated in the rectal gland dilation of the J2 and were distributed via the anus to the J2 surface. This epitope was shed by the J2 during penetration and migration within the plant root and it accumulated in intercellular spaces around the nematode where it bound to host cell walls at the feeding site. Nematode behavior was affected by all the antibodies binding to the surface coat of the pre-parasitic J2 and we demonstrated that the movement pattern of the *M. javanica* J2 was affected by these antibodies. We now have a panel of antibodies that will insure a wide range of reactivity with one or more components of the SC and that will enhance our ability to define critical targets for plantibodies.

The mucin genes that we have isolated and characterized appear to be expressed in the excretory gland, strongly suggesting that they encode a SC component. Initial evidence suggest that these genes exhibit alternative splicing, possibly in response to alterations in the environment (such as host defense responses); we will investigate this in the future. Our second approach has been to study host genes that respond to the nematode, and in particular, to the SC. Our previous work has identified a large suite of genes expressed in *Lycopersicon esculentum* giant

cells, including the partial cDNA clone DB#131, which encodes a serine/threonine protein kinase. Isolation and predicted translation of the mature cDNA revealed a frame shift mutation in the translated region of the gene in these nematode-sensitive plants. By using primers homologous to conserved region of DB#131 we have identified the orthologues from three (nematode-resistant) *Lycopersicon peruvianum* strains. All of them possess intact open reading frame, DB#131 and orthologues are homologous (55% sequence similarity, 46% identity) to the R-locus encoded by the rice Xa-21, which confers resistance to the bacterial leaf pathogen *Xanthomonas oryzae*. DB#131 orthologues, however, appear to lack a Xa21-like extracellular receptor domain and computer analysis of the full-length cDNAs of DB#131 revealed a putative signal peptide with a strongly predicted cleavage site, which suggests DB#131 may be secreted from the plant cell. This is the first known secreted plant kinase. Initial RNA dot-blot results suggested DB#131 is expressed only in nematode-induced giant cells. Nevertheless, using the sensitive method of quantitative RT-PCR we detected expression not only in nematode-infected roots (where it is strongly expressed) but also in healthy roots and vegetative tissues. To spatially localize DB#131 expression we used an in situ RT-PCR method. In floral buds DB#131 is expressed in the carpel and stamen of both tomato species *L. esculentum* and *L. peruvianum*, and at a lower level in all cells of the uninfected roots. Thus, this gene likely plays a role in normal plant development. We have confirmed DB#131 expression in giant cells, and, intriguingly, in plant cells that were in close proximity to the nematode body. In fact, the in situ patterns of induction correlate very well with the patterns of SC distribution in planta, and we postulate that expression of the DB#131 kinase is directly stimulated by the nematode SC.

The potential for the application of these findings to practical aspects of nematode control looks promising. For example, we suspect that the secreted kinase, DB#131, plays a pivotal role in modulating both compatible and resistance responses of the host, possibly via direct interaction with the SC. Understanding this interaction will be an important goal for the future, and will likely represent a target for development of a novel form of nematode control. Likewise, we have demonstrated that nematode pathogenesis can be delayed or inhibited by the presence of antibodies to the SC, thus directly implicating the SC in the infection process and reinforcing our hypothesis that plantibodies directed against the nematode surface coat may be an effective control strategy. Such a goal could be reached in the near future (4-6 years) with adequate funding.

Plant parasitic nematodes cause an estimated \$9 billion in annual crop losses, in the United States alone. Thus, even a small reduction in these losses, due to effective control measures, could have a significant economic impact with a favorable cost/benefit ratio. Increased efficiency of plant

nutrient uptake and utilization due to nematode control could effect further savings and reduce nitrate/phosphate pollution in agricultural water sources and run-off as well as reduce overall water requirements in arid and semiarid environments.

This project has been a cooperative effort by the laboratories of the principal investigators. Initial conferences in Arizona, with follow-up discussions at international meetings and workshops, have enabled us to proceed in our own laboratories without duplication of effort and with full awareness of each others activities and findings. Frequent communications via the internet have sustained this cooperation.

Publications:

Bird, D. McK and H. Koltai. 2000. Plant parasitic nematodes: Habitats, hormones and horizontally-acquired genes. *Journal of Plant Growth Regulation* 19: 183-194.

Hu, G.G., M.A. McClure, and M.E. Schmitt. 2000. Origin of a *Meloidogyne incognita* surface coat antigen. *Journal of Nematology*. 32(2): 174-182.

Koltai, H., Dhandaydham, M., Opperman, C., Thomas, J. and D. McK. Bird. 2001. Overlapping plant signal transduction pathways induced by a parasitic-nematode and a rhizobial endosymbiont. *Molecular Plant-Microbe Interactions* in press.

Koltai, H. and D. McK. Bird. 2000. High throughput cellular localization of specific plant mRNAs by liquid-phase *in situ* RT-PCR of tissue sections. *Plant Physiology* 123: 1203-1212.

Koltai, H. and D. McK. Bird. 2000. Epistatic repression of *PHANTASTICA* and class 1 *KNOTTED* genes is uncoupled in tomato. *The Plant Journal* 22: 455-459.

E. Sharon, Y. Spiegel, R. Salomon, and R. H. C. Curtis. Characterization of *Meloidogyne javanica* surface coat using antibodies and their effect on nematode behavior. *Parasitology* (submitted).

Thiery, M. G., Greene, A. E. and D. McK. Bird. 1999. The *Lycopersicon esculentum* ortholog of *PHANTASTICA/rough sheath2* (Accession No. AF148934) is expressed in feeding sites induced by root-knot nematodes. *Plant Physiology* 120: 934.

APPENDIX

Published Papers

Hu, G.G., M.A. McClure, and M.E. Schmitt. 2000. Origin of a *Meloidogyne incognita* surface coat antigen. *Journal of Nematology*. 32(2): 174-182. (Three copies enclosed).

Gravato-Nobre, M. J., M.A. McClure, D. Calder, K.Davies, B. Mulligan, K. Evans, and N. von Mende. 1999. *Meloidogyne incognita* surface antigen epitopes in infected *Arabidopsis* roots. *J. Nematol.* 31:212-223. (Three copies enclosed).

Esnard, J., M.A. McClure, D.W. Dickson, T.E. Hewlett, and B.M. Zuckerman. 1997. Effects of monoclonal antibodies, cationized ferritin, and other organic molecules on adhesion of *Pasteuria penetrans* endospores to *Meloidogyne incognita*. *Journal of Nematology* 29(4): 556-564. (Three copies enclosed).

Reprints of other papers listed above will be posted separately.

Submitted Papers

E. Sharon, Y. Spiegel, R. Salomon, and R. H. C. Curtis. Characterization of *Meloidogyne javanica* surface coat using antibodies and their effect on nematode behavior. *Parasitology* (submitted). (One copy enclosed).