

The secret(ion) life of worms

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Abstract

Tandem mass spectrographic analysis of the secreted proteins of plant- and human-parasitic nematodes reveals molecular similarities that reflect the shared need to counter host defenses.

Nematodes are the most important parasites of plants and animals worldwide, contributing to human misery both through direct infection and through losses to livestock and agricultural crops. Driven largely by genome projects, several parasitic nematode species have emerged as model organisms. These include *Brugia malayi* [1], a filarial nematode parasitizing the lymphatic system and causing elephantiasis in humans, and root-knot nematodes of the genus *Meloidogyne* [2,3], which attack essentially all crop plants. How these parasites are able to evade host defense responses and establish the intimate association within the host necessary for feeding and subsequent reproduction has been a key question over the past 50 years [4].

It is believed that secretions from the nematode are crucial in invasion and establishment in the host, and the advent of molecular and genomic technologies has allowed researchers to catalog and describe a large set of secreted proteins from plant-parasitic and human/animal-parasitic nematodes. Recently, the power of tandem mass spectrometry coupled with liquid chromatography (LC-MS-MS) has been used to experimentally identify peptides defining the parasites' secreted proteins (the secretome), and two recent publications demonstrate the tremendous progress that has been made. Bellaïfiore *et al.* [5] describe the secreted proteome of the root-knot nematode *Meloidogyne incognita* in larvae poised for infection, and Moreno and Geary [6] compare the secreted proteins from three life stages of *B. malayi*. The results paint a fascinating picture of the secretomes of these two important parasites and reveal a commonality of molecular strategies unexpected in nematodes with an evolutionary divergence that may approach a billion years [7].

The secretome of *M. incognita*

Bellaïfiore *et al.* [5] exposed *M. incognita* larvae to root exudates followed by treatment with resorcinol, an agent previously shown to induce copious expulsion of protein from the pharyngeal glands via the feeding stylet. Following this treatment, 486 secreted proteins were identified by LC-MS-MS. These findings are significant for a number of reasons, including the simple point that they represent the largest set of secreted proteins yet identified from a root-knot nematode. Not surprisingly, many of the identified proteins have significant matches in the reference datasets, which include published nematode and plant proteins and unpublished *M. incognita* expressed sequence tags (ESTs), but, curiously, the authors did not examine the draft genome of *M. incognita* itself [2].

One remarkable finding is that a number of the secreted proteins have matches to plant proteins. It is suggested that these proteins may be secreted by the nematode to mimic normal plant proteins involved in control of the plant cell cycle and cellular growth and development. Furthermore, a number of proteins previously postulated to have been acquired from bacteria by ancestral nematodes via horizontal gene transfer were identified in the MS analysis. The cooption of bacterial functions has been proposed as a major driving force in the evolution of parasitism [8].

One telling example is the nematode homolog of the rhizobial *NodL* gene, which in nitrogen-fixing rhizobacteria encodes an enzyme involved in the biosynthesis of the principal bacterium-plant signaling molecule (a lipochito-oligosaccharide termed Nod factor). On the basis of

bioinformatics analysis, this enzyme was predicted to be cytosolic in the nematode [9], but the LC-MS-MS analysis [5] places it firmly in the secretome. This adds support to previous hypotheses that root-knot nematodes and rhizobial symbionts use similar strategies and tactics to establish themselves in the host [10], and also implies that this nematode-encoded enzyme might act on a plant substrate. The failure of standard informatic tools to predict particular proteins as being secreted was also noted in the *Brugia* study [6] and should be considered a cautionary tale. It also suggests that we have a way to go before we fully understand the factors involved in the release of these proteins from nematodes.

The *M. incognita* secretome contains a number of proteins thought to interact directly with plant transcription factors, although the evidence is circumstantial. For example, 26 of the proteins have a nuclear localization signal, and 40 have predicted nucleotide-binding ability. Although these data are compelling, the potential role of these proteins in parasitism remains obscure. Not surprisingly, a large number of secreted proteins involved in cell-wall modification were found, confirming previous reports that these might be secreted during migration of the infective larvae as well as during establishment of the feeding site [11]. The analysis also revealed a number of proteins thought to be involved in the detoxification of plant defense responses such as reactive oxygen species (ROS). It has been reported previously that enzymes protecting against ROS are important in symbiotic relationships in general, and this finding of Bellaïfiore *et al.* seems to indicate that nematodes also use this mechanism.

In addition to the above findings, several proteins were identified that are thought to be involved in the regulation of the cell cycle in the host, including a CDC48-like protein. Intriguingly, this protein is secreted by the nematode amphids, a pair of sensory organs at the worm's anterior. Although the amphids have long been known to be involved in chemoreception, this is the first suggestion of a direct role in plant parasitism. Taken as a whole, these data reveal a nematode that attacks by stealth and deception, subverting host defenses, and which carries a highly sophisticated array of weapons.

The secretome of a human parasitic nematode

Also using an LC-MS-MS approach, Moreno and Geary [6] analyzed the secreted proteins from three life stages of *B. malayi*: adult male and female worms, as well as the infective microfilariae - minute threadlike larvae (Figure 1). Their results revealed a set of 228 secreted proteins, many of which are thought to be involved in regulation of the host's immune response. A testament to the power of the proteomic approach was the finding that all but two of the previously identified secreted proteins from *Brugia* were found in this study, and proteins known to be secreted by other filarial nematodes were all also identified. As found for *M. incognita*,

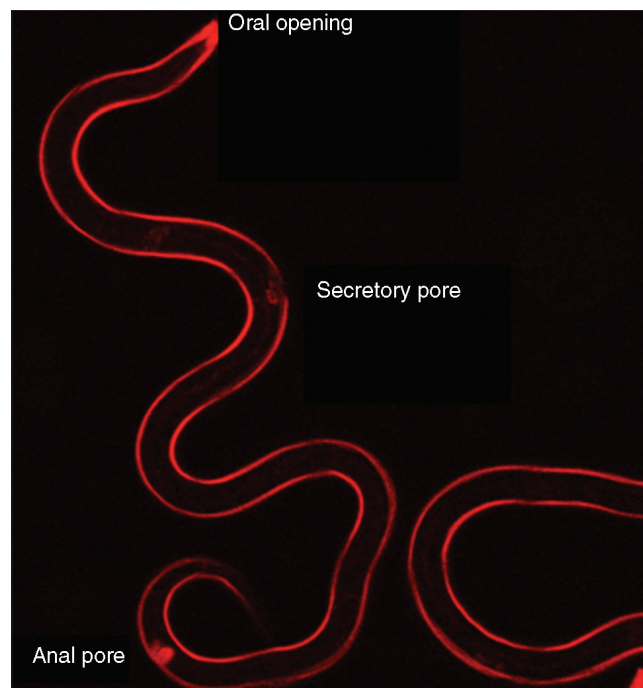


Figure 1
Brugia malayi microfilaria stained with rhodamine-conjugated phalloidin to show the three openings through which proteins are thought to be secreted into the host: the oral opening, the secretory pore and the anal pore. Image courtesy of J Solomon, Y Moreno and T Geary.

a large percentage of these have unknown functions. Strikingly, only approximately 14% of the identified proteins were present in all three stages, and the authors speculate that these may be involved in key functions for avoiding host immune responses. However, a large percentage of these proteins have no assigned function or Gene Ontology term to help reveal their role in the nematode's life cycle. The proteins identified include glycolytic enzymes and potential immunomodulatory proteins.

That the different life stages have very different secretion profiles indicates that parasitism by *Brugia* is a dynamic process that is tightly linked to the life cycle. Many more secreted proteins were identified from adult females than from either males or microfilariae. The secretome of microfilariae differs greatly from that of both adult female and adult male worms, sharing only 3.2% with the female and 0.9% with the male. Microfilariae are found in the blood stream and have to face a myriad of different challenges compared with adult worms resident in lymphatic tissue. An intriguing discovery is that an endochitinase secreted by microfilariae appears to be essential for its life stage, but is not necessary for adult worms. Moreno and Geary suggest that this enzyme may be involved in the molting that occurs during microfilarial development.

Differences between microfilariae and adults are also reflected in relative protein abundances. While most of the proteins found in the microfilariae were not found in adults, the few that were are of low abundance. Differences between male and female nematodes were also observed, including the presence of major sperm protein in males and the presence of a macrophage migration inhibitory factor in females. It is also interesting that none of the proteins from any stage could be assigned to the apparently obligate *Walbachia* endosymbiont carried by *Brugia* [12].

As observed in *M. incognita*, *Brugia* secretes enzymes to detoxify ROS derivatives produced by host defense mechanisms. This is one of several mechanisms that appear to be conserved between plant- and animal-parasitic nematodes. Taken as a whole, these results indicate that although animal- and plant-parasitic nematodes diverged long ago on the evolutionary time scale [7], there is a measure of universality in regard to the mechanisms necessary to successfully parasitize a host. All parasites must evade or suppress host defense responses, as well as time their development to coincide with establishment in the host. The recent progress in sequencing nematode genomes suggests that these are just the first steps towards a deeper understanding of parasitic abilities and the development of novel and sustainable management strategies for these devastating parasites.

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