Use of the *C. elegans* model system to understand parasite gene function

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Introduction In the 1960s and 70s, a small group of scientists chose a previously unheralded organism, the free living nematode *Caenorhabditis elegans*, as a model system for the study of genetics, behaviour and developmental biology. Their reasons for doing so were that it had many of the features desirable for an experimental system: it had a rapid (3.5 day) life cycle, could be easily cultivated on plates of bacteria, it was small (1.5mm in the adult stage) and composed of sufficiently few cells (1000) that determination of the lineage was feasible. Furthermore, the nematode was translucent (allowing simple microscopical observations of internal morphology) and had a mode of reproduction that was convenient for genetic analysis. As more workers began to appreciate the benefits of *C. elegans,* other techniques were developed which further enhanced its utility as a model system. Systems for genetic transformation, gene knockouts, synchronous culture and large-scale culture were developed. By the end of 1998, the entire 100 million base pair genome of this organism had been sequenced. This resource, coupled with the other achievements of the *C. elegans* project which included the determination of the entire cell lineage, a full understanding of the anatomy (including the nervous system and all its synaptic connections) at the electron microscope level, a library of over 1600 mapped mutations in genes and an integrated physical and genetic map, meant that *C. elegans*, a small soil dwelling nematode, was the most completely understood metazoan on the face of the planet.

Contrast this with the situation faced by scientists working with parasitic nematodes. No *in vitro* culture systems for these organisms exist and, as a consequence, many developmental stages occur within a host and are inaccessible for study. Obtaining sufficient material for analysis is almost always difficult and most parasites are intractable to standard genetic analysis. Since many of these parasites have a devastating medical or economic impact on the activities of man, it is important that the information gained about nematode biology and the techniques developed during the *C. elegans* project are exploited by scientists studying nematode parasites. This article sets out to explain how *C. elegans* has been used in our laboratory in order to enable us to understand the function of genes identified from plant parasitic nematodes far more fully than would otherwise have been possible.

Chitin synthase Chitin metabolism is considered an important target for control strategies, particularly in fungi: chitin synthases are targets for several important groups of fungicides and insecticides and some evidence suggests that chitin synthase inhibitors have potential for control of plant parasitic nematodes. Little is known about the functional role of chitin in nematodes, although this polymer has been found in eggshells of many nematodes and other, less thorough, studies have suggested that chitin may be present in the feeding apparatus or body wall of some nematodes. Collaborative studies with a group in Italy led to the cloning of a gene encoding a potential chitin synthase from a plant parasitic nematode *Meloidogyne artiellia*. Database similarity searches led to the discovery that two homologues exist in *C. elegans*. Given the problems of examining gene function in plant parasitic nematodes, we used *C. elegans* for functional studies of the chitin synthase gene.

A full-length genomic sequence was obtained for the plant parasite chitin synthase gene and very basic experiments examining gene expression were possible. These showed that the gene is expressed in adult females – the nematode stage containing developing eggs. These results are consistent with a role for the chitin synthase protein in synthesising the chitin of the eggshells. Considerably more detailed experiments were possible with the *C. elegans* genes. RT-PCR, using cDNA extracted from synchronous cultures sampled at every 2 hours throughout the life cycle, was used to investigate temporal expression patterns of the genes. Constructs, in which the promoters of each the *C. elegans* genes were fused to GUS/GFP reporter proteins, were used to generate transgenic animals. Examination of the localisation of the reporter proteins was used to determine the spatial expression patterns of the chitin synthase genes.

The two *C. elegans* genes showed different temporal expression patterns as indicated by RT-PCR. One gene (T25G3.2) was expressed in adult hermaphro-

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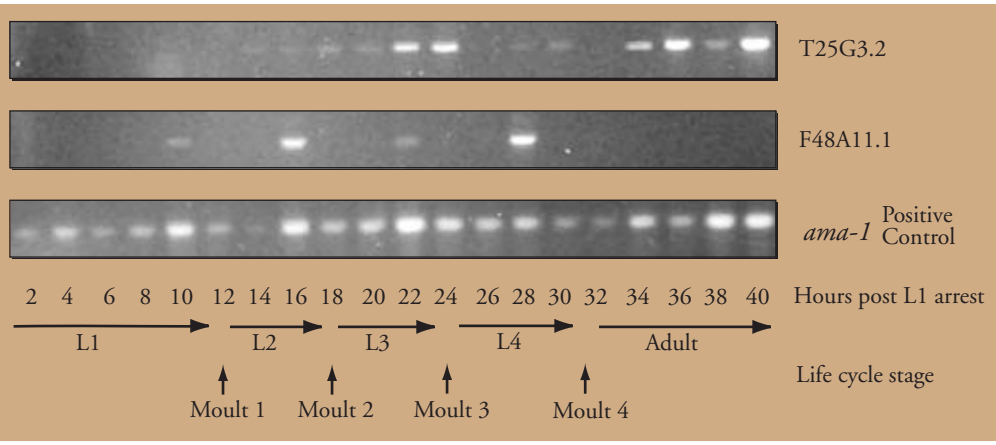

Figure 1 RT-PCR analysis of the temporal expression patterns of the *C. elegans* chitin synthases. Each lane shows the results of an RT-PCR experiment conducted using cDNA extracted from a synchronous *C. elegans* culture. The presence of a band indicates that the gene is expressed at this time in the life cycle. One gene (T25G3.2) is expressed in later larval stages and the adult hermaphrodite. The other gene (F48A11.1) is expressed only in a short period before each moult with no evidence for expression in the adult hermaphrodite.

dites and later larval stages of the nematode (Fig. 1). Chitin is known to be present in eggshells of many nematodes and since the T25G3.2 gene seems to be expressed at a time when eggshells are being synthesised, it seems reasonable to suggest that the product of this gene is responsible for synthesis of chitin in the eggshell. The RT-PCR data from the other gene (F48A11.1) suggested an entirely different role for this protein. This gene was expressed specifically in the period immediately preceding each moult (Fig 1). Eggshells are not being synthesised at this point of the life cycle, so what is the chitin being made by this protein used for? Transgenic animals containing the construct in which the 5' region of the F48A11.1 gene was cloned upstream of the reporter had GFP present in the cells of the pharynx (Fig. 2) – the structure which forms the feeding apparatus.

The feeding apparatus of many nematodes, including *C. elegans,* is replaced during each moult. The spatial and temporal expression patterns of the F48A11.1 gene therefore suggest that the chitin that it synthesises is used in the feeding apparatus. One part of the pharynx of *C. elegans* (the terminal bulb) bears a thick, ridged cuticle on its inner surface. This grinder is responsible for physically breaking bacteria ingested by the nematode prior to digestion and absorption of nutrients. It is possible that this grinder contains chitin to provide structural rigidity. Our data suggests that this is indeed the case, with the product of the F48A11.1 gene responsible for the synthesis of this chitin.

The use of *C. elegans* as a model to study gene function has therefore allowed us to discover that chitin

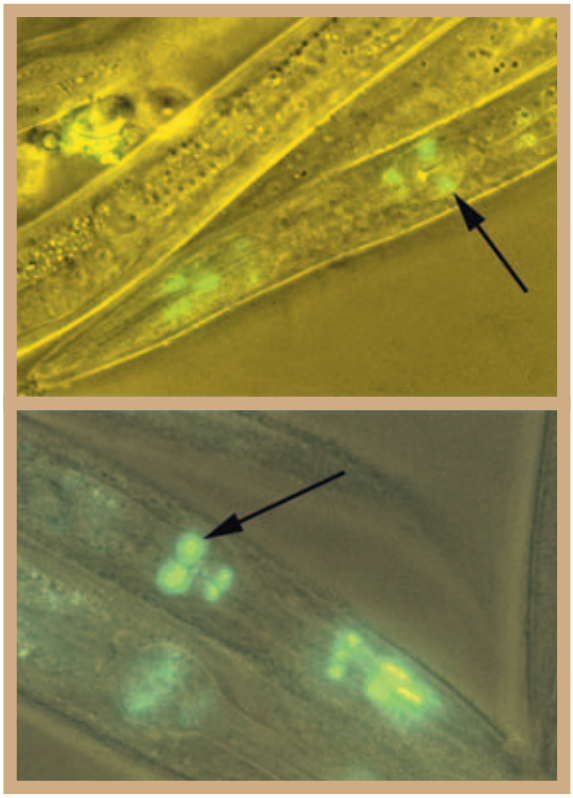

Figure 2 Spatial expression pattern of the F48A11.1 gene. The image shows transgenic animals carrying a plasmid in which the 5' upstream region (promoter) of the F48A11.1 gene is fused to a GFP reporter with a nuclear localisation signal. The presence of GFP in a cell indicates that the gene is normally expressed in that cell. GFP is observed solely in the epithelial and glandular cells of the pharynx.

synthase proteins have two roles in *C. elegans*. Having uncovered a second function for this protein, which our studies on the plant parasite did not reveal, it is now possible to examine the plant parasite in more detail in order to determine whether this second function also operates in this nematode.

These studies show that chitin plays an important role in several aspects of nematode biology. Since chitin is not present in vertebrates or plants, chitin biosynthesis may provide an excellent target for novel control methods against a variety of parasitic nematodes. The use of *C. elegans* has allowed functional studies on this protein to progress quickly and will also allow assessment of the suitability of these proteins as control targets.

Collagens All nematodes are covered by an outer layer or cuticle. The cuticle provides a flexible exoskeleton which allows directed movement while forming a barrier between the organism and its external environment preventing damage, desiccation and, in parasitic species, attack from host defences. The predominant protein constituent of the cuticle is collagen.

The sequencing of the *C. elegans* genome has allowed the identification of the entire complement of genes encoding cuticular collagens. A total of 154 collagen genes were identified which have been subdivided into families on the basis of the number and position of conserved cysteine and tyrosine residues which are thought to be involved in forming bonds between individual collagen polypeptide chains. Other species of nematode, including the animal parasites *Ascaris suum* and *Brugia malayi* and the plant parasites *Globodera pallida* and *M. incognita,* contain collagen genes which share the same basic structure as *C. elegans* collagens. The collagen families in these species have not been described in such detail but they appear to be of a similar size to those observed in *C. elegans*. Genes that are closely related to each of the six families defined in *C. elegans* can be identified. Thus, the characterisation of collagen gene function in *C. elegans* may have wider implications in predicting the function of gene homologues throughout the Phylum Nematoda.

Previous studies have shown that the most abundant collagen protein in adult plant parasitic nematodes falls into the group 1a family – of which almost nothing is known in *C. elegans*. Therefore, we characterised this family of genes both in PCN and *C. elegans*. The *C. elegans* group 1a collagen family contains 12 gene members. These have some unusual properties in that they are clustered in the genome – four family members are arranged as repeats and a further three are grouped in a cluster of three. We used RT-PCR to examine the expression patterns of some of the group 1a family members (Fig. 3). Like other

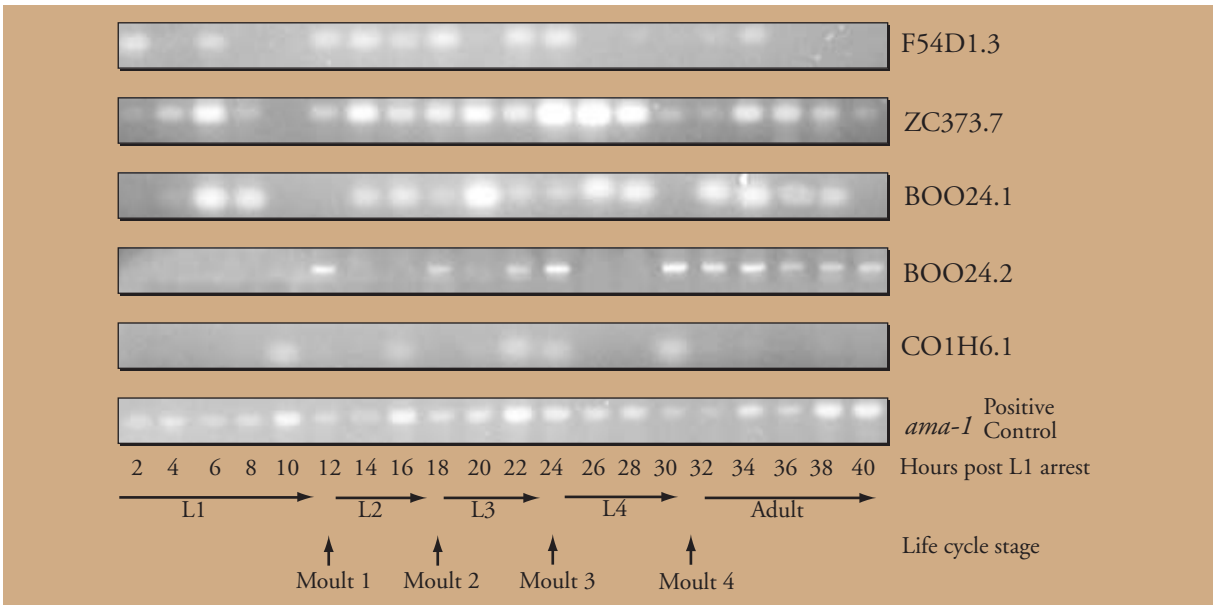

Figure 3 RT-PCR analysis of expression of some of the *C. elegans* group 1a collagen genes during development from L1 larvae to adulthood. Each lane shows the results of an RT-PCR experiment conducted using cDNA extracted from a synchronous *C. elegans* culture. The presence of a band indicates that the gene is expressed at this time in the life cycle. Expression of some genes is linked to the moulting cycle. Several of the genes are expressed in the adult stages.

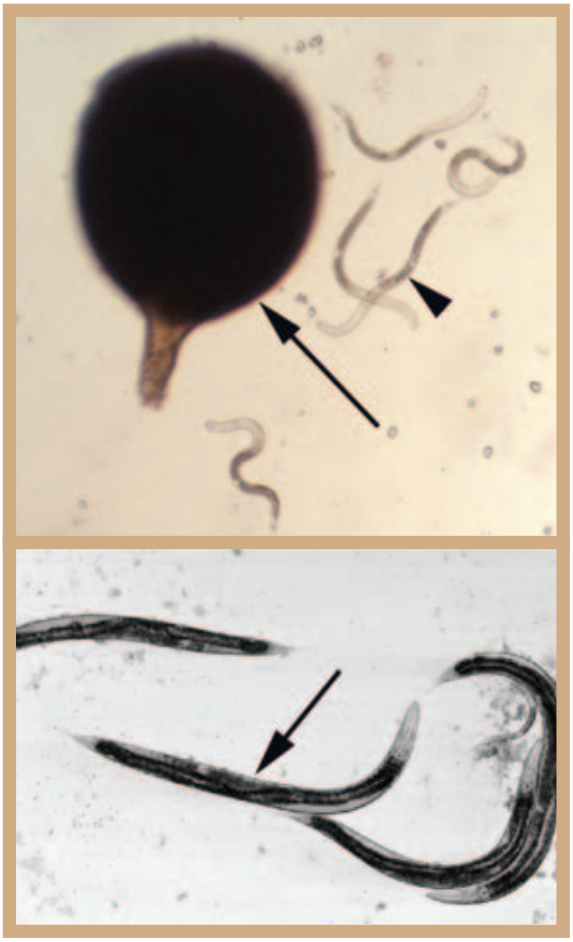

Figure 4 Comparison of the adult stages (arrows) of *Globodera pallida* (A) and *Caenorhabditis elegans* (B). The adult female of *G. pallida* is spherical and opaque, whereas the adult of *C. elegans* retains the worm-like morphology and is translucent. Note also the difference between the *G. pallida* female (arrow) and juvenile stage (arrowhead).

collagen genes in *C. elegans,* the expression of several of the group 1a genes is linked to the moulting cycle. Two of the genes (C01H6.1 and B0024.2) are expressed at, or immediately before each of the four moults that occur from L1 to adulthood while expression of another two (B0024.1 and ZC373.1) looks to be increased around the middle of each larval stage (Fig. 3). The most notable feature of these experiments was the finding that several of the *C. elegans* group 1a collagen genes (ZC373,7, BOO24.1, BOO24.2 and F54D1.3) are expressed in the adult nematode (Fig. 3). Previous observations suggest that a restricted set of collagens is expressed in the adult animal and it is therefore possible that one of the roles of the group 1a family of collagens is to provide material for the adult cuticle.

If the adult stages of *C. elegans* and plant parasites are compared (Fig. 4), the differences in morphology are striking. The *C. elegans* adult retains the transparent, elongated, cylindrical form seen in its earlier life-cycle stages, whereas the *G. pallida* adult female is roughly spherical and opaque. Is it possible that the function of the group 1a collagens is conserved in such diverged nematodes? Our studies suggest that this is indeed the case. Although it was not possible to perform analysis with PCN in the same detail as for *C. elegans*, RT-PCR experiments suggested that the PCN

group 1a genes are also expressed in the adult stages (Fig. 5). Database searches also suggest that similar genes are present in adult stages of other nematodes, including animal parasitic forms. Therefore, it seems that this collagen subfamily has a conserved functional role, in formation of the adult cuticle, throughout the Nematoda.

Although parasitic nematodes have evolved specializations in order to acquire a successful parasitic behaviour, these adaptations have been built on a framework of basic nematode anatomy and physiology. The availability of the information from the *C. elegans* project therefore represents a remarkable resource for understanding the biology of other nematodes. Our own studies, summarised here, show that in spite of the vast differences between *C. elegans* and plant parasitic nematodes in terms of their morphology and life cycles, plant nematology can benefit enormously from the *C. elegans* research effort.

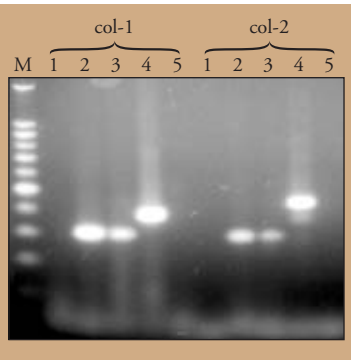

Figure 5 RT-PCR analysis of expression of two of the *G. pallida* group 1a collagen genes in J2s (1); virgin females (2) and gravid females (3) using primers specific for each gene. A positive control using a clone of each collagen gene (4) was performed. A negative control (5) in which water replaced template DNA was carried out for each set of primers. The presence of a band indicates that the gene is expressed at this time in the life cycle. The *G. pallida* genes are both expressed in the adult female stages.