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CUTICLIN GENES OF NEMATODES

LEWIS E.*, SEBASTIANO M.*, NOLA M.*, ZEI F.*, LASSANDRO F.*, RISTORATORE F.*, CERMOLA M.*, FAVRE R.* AND BAZZICALUPO P.*

KEYWORDS : parasitic nematodes, cuticle, cuticlin, dityrosine, cross-linking.

SUMMARY

Two genes coding for cuticlin components of *Caenorhabditis elegans* have been cloned and their structure is described. Recombinant proteins have been produced in *E. coli* and antibodies raised against them. Nucleic acid and specific antibodies are being used to isolate the homologues from the parasitic species *Ascaris lumbricoides* and *Brugia pahangi*.

The nematode cuticle protects the animal, serves as an exoskeleton and provides the surface over which interactions with the external environment occur. In the case of parasitic nematodes the external environment is the host and, as such, antigens expressed on/in the cuticle are within reach of both the humoral and cellular components of the immune system. The cuticle is a layered structure, the components of which are classified according to their solubility : lipids, some proteins and other readily soluble, non-structural components are mostly localized on the surface but are also distributed to a lesser extent throughout the lower layers of the cuticle ; the collagens make up the structural bulk of the cuticle, are coded for by a large and relatively well-characterized gene family, are not exposed on the cuticle surface and can be solubilized with SDS and mercaptoethanol ; and finally there is a highly cross-linked, insoluble and complex mixture of proteins present throughout the cuticle and known as the cuticlins. Up until now it has been virtually impossible to determine the roles and importance of the cuticlins because their insolubility does not allow either molecular or biochemical analysis of individual proteins.

* International Institute of Genetics and Biophysics, CNR, via G. Marconi 10, 80125 Napoli, Italy.

However, two cuticlin genes, *cut-1* and *cut-2*, have now been isolated from *C. elegans* in this laboratory, using a *D. melanogaster* probe coding for a component of the vitelline membrane of the egg. *cut-1* mRNA is 1422 nt long, has four exons coding for 423 amino acids and is transcribed to SL1, the spliced leader present at the 5' end of many mRNA's in most nematodes; *cut-2* mRNA is 847 nt long, contains only two exons coding for 237 amino acids, and is not transcribed. Northern analysis indicates that while *cut-1* is transcribed stage-specifically by worms entering the dauer larval stage, *cut-2* mRNA is transcribed during cuticle synthesis, immediately before each moult.

Parts of both genes have been expressed as fusion proteins in *E. coli* and have been used to raise specific antibodies. These have been used to study the expression pattern of the two genes by western blot, and to localize the products within the cuticles of worms at different stages by immunofluorescence and immuno-electron microscopy. The results obtained confirm the partial stage specificity of CUT-1, and the fact that CUT-2 is a component of the cuticle at all stages. Both proteins are localized on cuticle residues after treatment with strong reducing agents, showing them to be definitively members of the cuticlin residue.

The proteins deriving from the conceptual translation of the genes differ substantially, although they both begin with signal peptides and share a short motif repeated 5 times in CUT-1 and 12 times in CUT-2. Each repetition is characterized by the presence of the amino acid sequence AAPA. This same motif can also be found in some of the vitelline membrane proteins of *Drosophila* and in the proteins which make up the cuticle of *Locusta migratoria*. These proteins are all involved in the formation of insoluble, protective extracellular layers, implying that the conserved domains may have an important functional role.

Interestingly, the amino acid sequence of CUT-2 shows tyrosine residues that could participate in dityrosine bridge formation (dityrosine is present in the insoluble residue of parasitic nematode cuticles). We have shown that soluble recombinant CUT-2, produced in *E. coli*, can be polymerized *in vitro* into high molecular weight species by the action of HR peroxidase in the presence of H₂O₂. The products of the reaction become insoluble, contain tyrosine and the reaction is inhibited by the presence of free tyrosine. This clearly begs the question whether CUT-2 is responsible for the insolubility of the cuticle.

A second gene showing significant homology (> 80 %) to *cut-1* has been isolated for *C. elegans*, confirming the possible existence of a cuticlin gene family. A *cut-1* homologue has also been isolated from the plant parasitic nematode, *Meloidogyne artiella*, demonstrating the strongly conserved nature of the sequence amongst nematodes.

An insoluble cuticlin residue is present in the cuticles of all nematodes studied so far. This fact, plus the apparently conserved nature of the gene and the protein for which it codes, has prompted the search for genes homologous to *cut-1* and *cut-2* in two parasitic nematodes, *Ascaris lumbricoides* and *Brugia pahangi*.

Two parallel approaches have been used: the first involves screening a genomic library with labelled DNA probes made from *C. elegans* cuticlin genes; the second involves screening a parasite cDNA expression library with the spe-

cific antibodies raised against the recombinant CUT-1 and CUT-2 purified proteins. The positive clones have been sub-cloned into the pBluescript phagemid system and are at present being sequenced. The sequences will be checked for homology against the *C. elegans* cuticlin genes and the Sequences Data Base.

Once cuticlin gene homologues have been found the approach to their study will follow two broad pathways. Firstly transcription patterns in the parasite life-cycles will be studied using reverse transcriptase and PCR. And secondly the inserts will be expressed and the resultant recombinant proteins used in biochemical and immunological studies. Antibodies could also be raised against these proteins for use in localization and time-course experiments, and hopefully to screen the cDNA libraries of other parasitic nematodes.

Once parasitic nematode homologues of *cut-1* and *cut-2* have been fully characterized, the roles and functions of these clearly important proteins will be better understood and hopefully the knowledge can in some way be used in the control of the diseases caused by these parasitic nematodes.

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NEURONS AND GENES INVOLVED IN CHEMICAL SENSITIVITY IN NEMATODES

BAZZICALUPO P.*, HILLIARD M., LEWIS E.*, DE RISO L., SEBASTIANO M.*, RISTORATORE F.*

KEYWORDS: chemoreception, nematodes, behavior, avoidance, amphid.

SUMMARY

Organelles and neurons of nematodes involved in sensing chemical signals present in the environment are described. Laser ablation of neurons has helped assign them a specific function. Genetic mutational analysis has led to the identification of genes controlling the behaviour of the worms and/or some cellular properties of the chemosensory neurons. Some conclusions on the general organization and functioning of chemoreception in nematodes can be drawn.

Chemical signals from the environment are the most important sensory inputs for nematodes. The ability to receive and interpret chemical signals from the environment is essential for parasitic nematodes to find the host, complete their life cycle and reproduce. If better under-

* International Institute of Genetics and Biophysics, CNR, via G. Marconi 10, 80125 Napoli, Italy.