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 transpliced 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 transpliced. Northern analysis indicates that while *cut*-1 is transcribed stage-specifically by worms entering the dauer larvae stage, *cut*-2 mRNA is transcribed during cuticle synthesis, immediatly 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 specifity 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 H_2O_2. 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 specific 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

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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-

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stood at the cellular and molecular level, chemoreception would have enormous potential as a target for chemotherapy and possibly immunoprotection.

Unfortunately, little is known about chemoreception in parasitic nematodes and its study in these organisms presents many difficulties. However some information is available about chemoreception in the free living nematode *C. elegans*, and more importantly a variety of experimental approaches are applicable in the study of this model organism. Although nematodes differ from each other in size, life cycle, and other important aspects, the general plan according to which they are constructed is very conserved. Thus a realistic strategy to study chemoreception in parasitic nematodes is to start with *C. elegans*, with the aim of eventually using the knowledge and the molecular reagents and probes derived from this study, to identify the homologous components from parasitic species.

The main chemoreceptor organs of nematodes are the Amphids, one on each side of the head. A chemoreception function is likely also for the two Phasmids and the six Inner Labial Neurons. As in other nematodes, sensory organs in *C. elegans* are each made of two non neuronal support cells, the sheath cell and the socket cell that form a channel around a bundle of ciliated neuronal endings ; in the case of chemosensory neurons, the endings reach the exterior through a gap in the cuticle. In *C. elegans* the cellular anatomy of these organs and the precise ancestry of the cells contributing to them have been described.

Caenorhabditis elegans behaviours influenced by chemical stimuli present in the environment include : orientation in gradients of a number of chemicals acting as attractants or repellents ; avoidance of some chemicals acting as a repellent ; entry and exit from the dauer larvae developmental pathway ; egg laying ; attraction of males but not hermaphrodites to hermaphrodites. We have concentrated our studies on the avoidance response. When it encounters a repellent chemical, *C. elegans* stops and inverts its movement, backing up. This response is relatively simple and, at least in principle, does not require the integration of information from different signals or from the same signal varying in time or space. As for other chemoreception dependent behaviour, laser ablation experiments have led to the identification of the main sensory neurons involved in this response ; they are two neuron pairs, ASH and ADL, which send their ciliated sensory termination to the amphid channel. ASH and ADL are also the only two amphid neurons synapsing directly onto AVA, the main interneuron driving backward movement. It is possible therefore that the avoidance response is mediated by a relatively simple three element circuit.

Genetic mutational analysis is, in *C. elegans*, extremely powerful and is the staring point from which to arrive at the molecular cloning of genes ; it has therefore already been used in the study of chemoreception. The mutants that have been isolated so far can be divided into two main groups :

i) mutants isolated on the basis of alterations in behaviour that depend on the reception of chemical signals. These include mutants abnormal for chemotaxis, *che*, osmotic avoidance, *osm*, dauer formation, *daf*, and volatile chemical sensitively *odr*.

ii) Dyf mutants. Chemosensory neurons of live wild type *C. elegans* take up Fluoresceine Isothiocyanate (FITC) from the medium. For wild type worms, conditions have been found under which only six neurons of each amphid and the two neurons of each phasmid take up the dye, transport it retrogradely and become fluorescent. Mutants with alterations in this staining pattern have been isolated (*dyf*, for dye filling alteration) and have been shown to have various chemosensory defects. The main advantages of this phenotype are that it can be detected on single live worms, making genetic analysis of mutants faster ; and that the phenotype is largely dependent on specific properties of chemosensory neurons and thus can generate chemoreception specific mutants. Mutations in many of the behavioural mutants also alter FITC uptake, resulting in unstained or poorly stained animals.

We have isolated 19 Dyf mutants and have studied some basic aspects of their behaviour. The sensitivity most often lost in these mutants is towards the presence of high osmotic strength in the medium. All the mutants show normal responses to touch, indicating that they are mutated in genes specifically required for chemoreception and are not general mutants of the nervous system.

We have also studied in more detail *dyf-1* a new gene on *LGI*. Besides the lack of staining of chemosensory cells, *mn335*, the *dyf-1* allele we have studied, responds very poorly to high osmotic strength, SDS and Cu^{++}. However its ability to avoid garlic and a repellent present in worm extracts seems unaltered, as is its capacity to respond to light touch. Ultrastructurally the main defect of *mn335* is shorter sensory cilia and a collapsed amphidial channel. We have mapped the gene to Linkage Group *I*, near *unc-38* and have started a project to clone it molecularly.

Dyf mutants, including *dyf-1* , identify genes involved in the general physiology of the chemoreceptor neurons and/or of the support cells ; their products are necessary for the uptake of the dye from the medium and the retrograde transport to the cell body. Genes coding for the specific receptor molecules located on the neuronal endings, and for the components of the chemo-sensory transduction pathway remain largely undetected in this type of screen. In order to identify these types of gene we have recently started to isolate behavioural mutants with the aim of finding those specifically unable to sense and avoid certain chemicals but still capable of sensing others. We have isolated several mutants and are characterizing them genetically and behaviorally. We have used stringent behavioral assays in the screening stage and it is interesting that most of the mutants isolated do not show a Dyf phenotype. This result indicates that it is possible to isolate mutants in a different set of genes which could include the receptors and the chemosensory pathway.

The anatomical studies, the laser ablation experiments and the behavioural and ultrastructural analysis of the mutants isolated are providing important informations regarding some basic features of chemoreception in nematodes :

1. Each chemosensory neuron bears receptors for more than one chemical.
2. Receptors for chemoattractant and for dauer formation are present on more than one chemosensory neuron.
3. Most behavioral mutants, although isolated on the basis

of a single altered behaviour, are pleiotropic and therefore defective in other chemical sensitivities.
4. The Dyf phenotype is associated with the loss of sensitivity to many different chemicals. Alterations in the shape or matrix content of the amphidial channel also result in a Dyf phenotype.
5. While water soluble chemicals seem to have receptors on the neurons whose cilia are exposed to the outside (ASH and ADL), attraction to and repulsion by volatile chemicals are mediated by receptors present on the amphidial wing cells AWA, AWB and AWC.

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N-ACETYLATION OF POLYAMINES AND BIOGENIC AMINES IN PARASITIC NEMATODES

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KEYWORDS : Polyamines. biogenic amines. N-acetylase. nematodes.

The previously described polyamine N-acetylase from *Fasciola hepatica* has been observed to have an additional function, the acetylation of biogenic amines (Aisien and Walter, 1992, 1993). It was concluded that N-acetylation plays a major role in the amine metabolism of trematodes. In continuation of these ongoing studies on the process of N-acetylation in parasitic helminths, we have detected biogenic amine acetylation in the tissue dwelling filaria *Onchocerca volvulus* and the intestinal parasite *Ascaris suum*. The aim of our study was to ascertain if our previous finding that a single enzyme is responsible for diamine, polyamine and biogenic amine acetylation in trematodes, is a feature common to all helminths. Results from investigation using *A. suum* indicate that two independent enzymes are respectively responsible for polyamine and biogenic amine acetylation. Chromatography of the 100,000 g supernant on DEAE-cellulose revealed two enzyme activity peaks, both of which have activity for histamine. The first peak was observed to catalyse only biogenic amine acetylation while the second acetylated putrescine and other diamines. On the basis of the K_m-values obtained with both enzymes for histamine, peak II seems to be more specific for this substrate with a K_m-value of 37 µM compared to peak I which had a K_m-value of 500µM. In contrast the K_m-values obtained for biogenic amines with peak I were as follows : Tyramine, 0.9 µM, tryptamine, 1.7 µM, octopamine, 29 µM, serotonin, 9.5 µM and ß-phenylethylamine, 1.5 µM. Epinephrine, norepinephrine and dopamine were not physiological substrates for the N-acetylase. The enzyme has a molecular mass of approximately 30 kDa and was slightly inhibited by coenzyme A, a product of the acetylation process. The specificity of peak II for diamines and histamine and its lack of activity for polyamines indicates that this enzyme is most probably the novel putrescine acetylase previously reported by Wittich and Walter (1990, 1991) from *O. volvulus* and *A. suum*.

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ASSESSMENT OF THE POLYAMINE METABOLISM OF FILARIAL WORMS AS A TARGET FOR CHEMOTHERAPY

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KEYWORDS : Polyamines. metabolism. chemotherapy. filaria. N-acetyltransferase. polyamine oxidase. S-adenosylmethionine decarboxylase.

Polyamines are essential for the proliferation and differentiation of cells and organisms. The presence of polyamines has been demonstrated in *Onchocerca volvulus* and allied parasites and the investigation of the polyamine metabolism has identified unusual pathways which are potential chemotherapeutic targets. These pathways are crucial for parasite survival and differ in some biochemical aspects from the host counterparts, thus allowing for the rational design of drugs for a parasite-specific chemotherapeutic attack. A proposed scheme of the polyamine metabolism in filarial worms is shown in Figure 1.
Since the presence of ornithine decarboxylase (ODC) activity is questionable, the initial step in the biosynthesis of polyamines appears to be lacking and thus the parasite depends on uptake from the host for its polyamine supply. An interconversion pathway for polyamines has been demonstrated which does not involve the polyamine N-acetyltransferase, the rate limiting step in the interconversion of polyamines in mammals. A novel type of polyamine oxidase has been identified which is solely responsible for the interconversion and degradation of polyamines. In addition, a parasite-specific N-acetyltransferase for putrescine was found which is involved in the degradation and excretion of excess polyamines.

Depletion of polyamine levels in filarial worms is thought to have cytostatic if not cytotoxic effects, as has been shown previously in the chemotherapy of cancer and protozoan infections by DL-α-difluoromethylornithine (DFMO). Filarial worms and other helminths lack ornithine decarboxylase, the target for DFMO. Thus, as the parasite depends on the uptake of polyamines from the host tissues, inhibition of transport mechanisms resulting in rapid depletion of polyamines should be exploitable for chemotherapy.

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